Cyclic dormancy, temperature and water availability control germination of *Carrichtera annua*, an invasive species in chenopod shrublands

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Abstract   We studied the germination of seeds of *Carrichtera annua* L. from a single cohort, stored in the field for up to 18 months, when retrieved at different times and subject to different combinations of temperature and water availability. Germination was affected by season of retrieval, and temperature and water availability in a complex interactive way. Germination rates were lowest when seeds were retrieved during summer or spring, but seeds germinated readily when retrieved during autumn and winter, if exposed to temperatures simulating autumn or winter conditions, and provided water equivalent to at least 50% field capacity. High temperatures and low water availability reduced germination substantially. The results indicate that this species has a combination of cyclic dormancy and germination requirements that minimizes the risk of germination during periods when the risk of prereproductive mortality is high. Given the short life of the seeds of this species, these mechanisms may be essential for the persistence of the species in the highly unpredictable arid lands of southern Australia.

Key words: annual plant, desert plant, invasive species, seasonal dormancy, temporal variability.

INTRODUCTION

Theoretical models suggest that seed dormancy has evolved to maximize persistence in a variable environment by ensuring that some seed persists over time when conditions for seed production are poor (Cohen 1966; Ellner 1985a,b). However, germination fraction varies over time, and depends greatly on environmental factors (Baskin & Baskin 1998). This suggests that in nature more subtle evolutionary forces are at play, including the potential that species germination cues are predictors of the likely environment during the growing period (Pake & Venable 1996) or reflect trait differences between species that have a role in reducing the effects of interspecific competition (Chesson *et al.* 2004). Seeds of annual plants in arid systems often have dormancy mechanisms that allow only a fraction of all seeds present to germinate at any given time, creating a long-lasting soil seed bank (Kemp 1989; Facelli *et al.* 2005). This reflects the largely unpredictable nature of deserts (Guo & Brown 1996; Pake & Venable 1996) where conditions favourable for germination may be followed by mass mortality of seedlings.

Several studies report on the ecophysiology of germination of desert annual plants (Elberse & Breman 1989; Elberse & Breman 1990; Briede & McKell 1992; Gutterman 1994). Overall, desert annuals require relatively high water availability (Mott 1972, but see Briede & McKell 1992), and have adaptations that allow them to time their germination to the most favourable part of the year (Baskin *et al.* 1993; Clauss & Venable 2000). The tuning of emergence to environmental conditions, achieved through dormancy with a physiological basis, is prevalent (Baskin *et al.* 1993; Gutterman 1994). In addition to responding to water, temperature and light, seeds of some desert annuals undergo dormancy cycles controlled by the preceding temperature conditions (Baskin *et al.* 1993). This mechanism may prevent germination during brief periods of high water availability within the seasons when post-germination mortality risk is high (Pake & Venable 1996). Adondakis and Venable (2004) and Facelli *et al.* (2005) have demonstrated substantial differences in the germination biology of different species of annuals in arid lands. These differences in specific germination requirements can provide fine temporal partitioning, as required for coexistence through the storage effect hypothesis (Chesson *et al.* 2004). The longevity of the seeds and the germination fraction of the different species are also important determinants of the system's dynamics as they determine the ability

of the species to persist through a series of unfavourable years – not an uncommon event in arid lands.

Carrichtera annua L. (Brassicacea) is an annual plant native to the drier parts of the Mediterranean basin which has become the dominant species in the annual guild of many chenopod shrublands of South Australia. Studies in its native range have identified a peculiar seed strategy (Gutterman 1994; Gat-Tilman 1995; Zaady *et al*. 1997). After the death of the mother plant at the end of spring, the seeds are retained in the fruits. Dehiscence is triggered by large rainfall events, after which usually germination follows. Otherwise, the dehydration of mucilaginous cover of the testa pushes the seed into the ground (Gutterman & Shem-Tov 1997). Its seeds are poorly represented in the soil seed bank in chenopod shrublands, even in sites where it is consistently abundant in the growing community (Meissner & Facelli 1999; Facelli & Temby 2002; Facelli *et al*. 2005). In contrast, most other species in the annual guild form large seed banks (Facelli *et al*. 2005). Detailed studies of its ability to persist in the soil demonstrated that few seed persisted beyond 2 years in the soil (Facelli *et al*. 2005), while the number of seeds of four other annual species in the guild declined by 30% or less.

While this strategy may be advantageous in a predictable environment where rainfalls are followed by conditions conducive to growth and reproduction, this may not be the case in a system where rainfall may occur at any time of the year, and hot dry conditions follow summer downpours as frequently happens in the chenopod shrublands of South Australia. Seedlings that emerge at those times are unlikely to reach the reproductive stage and therefore constitute a drain to the population. Under these conditions a large germination fraction exposes the population to local extinction. Indeed the predictability of Australian arid lands is much lower than that of the Mediterranean region. While having a predominantly winter rainfall regime, southern rangelands of Australia frequently experience large summer rainfall events. While occasionally these events are large enough to allow seed production, more often than not they are followed by long dry and hot periods which are likely to produce the death of the seedlings. One mechanism that could protect populations from extinction is cyclic dormancy. Baskin *et al*. (1993) demonstrated that in seeds of winter annuals dormancy can be controlled by temperatures, which act as a signal of the risk of post-germination mortality.

The objective of this study was to characterize the germination biology of *C. annua*. We speculated that the germination of *C. annua* is controlled not only by current temperature and water availability, but has also a seasonal component. To test this we buried seeds of this species in the field, retrieved them seasonally, and placed them under different combinations of water availability and temperature.

METHODS

Study site

The study was carried out at Middleback Field Centre, 16 km north-west from Whyalla, South Australia (32°57′S, 137°24′E). Details of the study site are described in Facelli and Temby (2002). The area is characterized by hot summers and mild winters. The average yearly rainfall is 223 mm (1923–2005), but between year variability is large (from 92 to 623 mm in the same period). The winter rains are relatively predictable Coefficient of Variation ((CV) for June–August = 85.51%), typically falling as a series of frequent small and medium sized rainfall events. Summer rainfall is far more variable (CV December–February = 145.23%) and tends to fall in few, large rainfall events.

The typical vegetation is an open woodland of *Acacia papyrocarpa* with a sparse understorey dominated by chenopod shrubs on brown calcareous soils with clay-loam texture. A large number of annual plants are found in the system (Facelli & Temby 2002), mostly growing during winter–spring in open spaces between shrubs. Currently the dominant species in the guild is *C. annua*, introduced from the Middle East (Gutterman & Shem-Tov 1997). For plant nomenclature we follow Jessop and Toelken (1986).

Seed germination trials

All seeds were collected from mature plants in October 1995 (late Spring), and promptly placed in plastic mesh bags (5×5 cm, mesh size 1 mm). Each bag was filled with sterile soil from the study site with 100 seeds mixed in. In November 1995, 80 bags containing seeds of *C. annua* were buried along a 1.5 cm deep trench in the field site. At the end of each season during 1996 and 1997 we collected 10 randomly selected bags. They were taken directly to the laboratory and their contents inspected. After carefully separating the soil from the seeds, the seed condition was assessed: all empty seeds or seeds that were crushed by gentle pressure with a pair of tweezers were discarded. The remaining, seemingly viable, seeds were counted and used to assess their germination responses to different combinations of water availability and temperature. While we expected to complete 2 years of trials, the reduction in seeds remaining in the bags (probably to a great extent because of germination, see Facelli *et al*. 2005; fig. 2) forced us to terminate the study after 18 months.

The experiment was run in a series of chambers with independent temperature control. In each chamber a 50 L water bath was connected to a temperature sensor, which relayed information to a computer. The

computer controlled cooling and heating units that produced the transition between maximum and minimum temperatures over 6 h to achieve realistic daily temperature cycles (rather than 12 h at minimum/12 h at maximum temperature). We used three temperature regimes: summer (28°C/15°C), autumn and spring (20°C/10°C), and winter (15°C/5°C). To control water availability we used a modified version of the technique proposed by Briede and McKell (1992). The water bath contained tubs with solutions of polyethyleneglycol (PEG) adjusted to produce osmotic water potentials of −50 kPa (high water availability), −225 kPa (medium water availability), and −440 kPa (low water availability). In each tub there were three plastic containers with the bottoms removed and replaced by a semi-permeable cellophane film (medical standard), so that the water potential in the soil equilibrated with that of the solution, while preventing PEG to reach the seeds (to avoid potential toxic effects). Thus the seeds were physically and chemically isolated from the PEG while still experiencing the osmotic potential that it determined. Ten seeds C. annua in each container were placed on the soil surface to simulate natural germination conditions (Gat-Tilman 1995; Gutterman & Shem-Tov 1997). In summary, there were three chambers for each temperature, in each of them one tub for each water potential treatment, each containing three containers (except for the last trial) with 10 seeds each. Germination was assessed at the end of the sixth week, as preliminary studies indicated that 90% germination occurred in the first 4 weeks.

The proportion of germinating seeds was analysed using a three-way factorial ANOVA, with temperature, water and time of retrievals as factors. Because no germination occurred in the low-water treatments, only the two highest levels of water were included in the analysis. This avoided the heterogeneity of variances produced by treatments with all values being zero. To ensure compliance with ANOVA assumptions, data were arc sine transformed. Pairwise comparisons were performed using Tukey test. Analyses were performed using JMP IN Version 4.0.3 (SAS Institute).

RESULTS

The germination of C. annua had a complex pattern, with strong interactions between season of retrieval, temperature and water availability (Table 1, Fig. 1). There was almost no germination at the lowest water availability, while with medium water availability the winter and autumn/spring temperatures elicited considerable germination. On the other hand, of the treatments with high water availability, samples subject to the summer temperatures had very low emergence rates. Within both high and medium water availability

Table 1. ANOVA on the number of seed of *Carrichtera annua* that germinated under different combinations of temperatures and water availability after being recovered at different times from mesh bags buried in the soil

Source	d.f.	SS	F	P
Water (W)	1	5162.75	88.41	0.0001
Retrieval (R)	5	4777.77	16.36	0.0001
Temperature (T)	2	8616.71	73.78	0.0001
W × R × T	10	1126.22	1.92	0.0457
W × R	5	1444.24	4.94	0.0003
R × T	10	3099.95	5.30	0.0001
W × T	2	2786.97	23.86	0.0001

treatments, winter temperatures produced overall higher germination than autumn/spring temperatures. In addition, germination was strongly affected by timing of retrieval of the samples: the highest germination rates occurred in samples retrieved from the field in autumn and winter. When retrieved in autumn, germination was higher at the winter temperature regime than at the autumn/spring regimes, but such a difference was not detected in samples retrieved from the field in winter. Germination of seeds retrieved in spring and summer was very low. In spring, there was only substantial germination at high water availability and at temperatures corresponding to winter and autumn/spring, while in the summer germination mostly happened at higher water availability and in the winter temperature regime.

DISCUSSION

The complex germination response documented for C. annua is consistent with an obligate autumn–winter germination strategy that ensures germination under favourable conditions occurring during the most predictable part of the year. First, germination was controlled by the season of retrieval. Seeds recovered in summer had the lowest germination rates, and then only at the lowest temperatures and higher water availability. Germination was also low when seeds were recovered in spring, indicating the onset of a new dormancy cycle (Baskin & Baskin 1989; Baskin et al. 1993). Seeds recovered in autumn and winter had much higher germination rates, in particular at high water availability, although some germination was also recorded at medium water availability. Winter retrieved seeds were less responsive to temperature. The fact that the decline of germination between the winter retrieval and the retrieval in the following spring and summer, reverted in the last autumn retrieval is clear indicative of a cyclic dormancy pattern. This mechanism avoids the risk of germination under transient favourable conditions at times when the environment
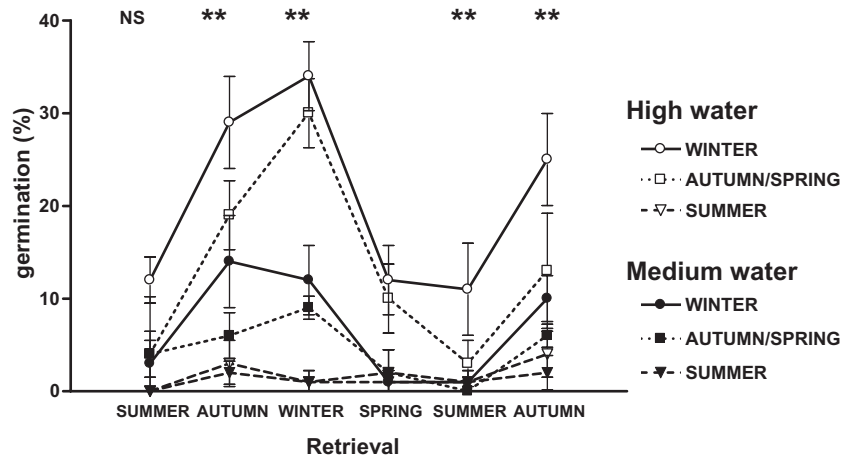
Fig. 1. Germination of seeds of *Carrichtera annua* from a single cohort, retrieved from the field at different times, and incubated at different temperature regimes and water availabilities. Asterisks indicate dates when different treatments had significantly different effects. Means and SD are shown.

is likely to limit survivorship to reproductive stage (Baskin *et al*. 1993; Gutterman 1994). Given the lack of long-term seed storage in this species, this strategy may be critical to ensure that a minimum number of seeds is produced every year. Less strict germination requirements during the winter period increases the chance of successful reproduction, maintaining low risks. It is important also to note that *C. annua* has another important mechanism to control germination: the seeds are retained in the fruit until a substantial rainfall event triggers the dehiscence and seed release (Zaady *et al*. 1997). Even when this happens during the summer months, germination would be unlikely. Only when the seeds are released after the end of the summer, and provided water availability is adequate, will substantial germination occur.

Given the low persistence in the soil seed bank, it is likely that the long-term presence of *C. annua* in this community depends strongly on the build up of the population during favourable years, and not on survival in the seed bank over years unfavourable for seed production. Large average population sizes together with large seed size probably buffer the population against local extinction. Our results suggest that local extinction could occur after just a few consecutive years of zero recruitment. It is important to point out, however, that our study does not incorporate some important components of the heterogeneity of the systems, mainly local depressions and microenvironments under shrubs. Small depression may accumulate enough water to ensure some reproductive output even in the most unfavourable years. A similar effect may be produced by facilitative effects under shrubs (Pugnaire *et al*. 1996; Facelli & Temby 2002). Thus, the species may also rely on the presence of such refugia to persist during the most unfavourable years,

re-colonizing the main areas of the system when conditions that allow positive population growth return. Such spatial heterogeneity can be an alternative to the presence of a seed bank in coexistence by the storage effect (Chesson 1990).

It must be noted that four native species (*Austrostipa nitida*, *Vittadinia cuneata*, *Daucus glochidiatus* and *Brachycome lineariloba*) treated in the same way, produced such low germination numbers that no analyses were possible (data not shown). Facelli *et al*. (2005) found that *C. annua* also differed from these four species in having much lower seed survivorship in the soil, and by being always in low numbers in the soil seed bank (see also Meissner & Facelli 1999; Facelli & Temby 2002). The findings here that *C. annua* had much higher germination than the natives is consistent with these prior studies, as a trade-off between persistence of seeds in the soils and germination fraction is expected (Ellner 1985b).

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