Environmental control of germination in semi-arid Mediterranean systems: the case of annuals on gypsum soils

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Abstract

Whether or not it is time for germination is a dilemma for annuals since they have only a single opportunity to reproduce successfully. This is critical for species living in stressful and unpredictable environments such as Mediterranean habitats. In order to clarify the environmental cues of germination, four annuals of different families, all of them occurring very frequently on gypsum soils, were selected and their germination observed under 14 climate scenarios, mimicking temperature and photoperiod conditions from autumn to spring, at two levels of water availability (continuous irrigation versus an initial single irrigation event followed by a progressive soil desiccation). In spring scenarios, two seed-storage conditions were compared: dry cold and room conditions. In the absence of water limitation, germination decreased from early autumn to late spring. Water scarcity always reduced germination, especially in early spring. Our results suggest a facultative winter germination behaviour and highlight the crucial role of dry cold storage in reducing spring germination. In conclusion, Mediterranean ephemerals showed a very plastic germination response that allows them to take advantage of favourable environmental conditions from autumn to spring. This environmental cueing is combined with the ability to dilute the risk through a variable rate of seed dormancy that, according to bet-hedging strategies, increases from secure autumn to riskier spring.

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Introduction

The moment when germination occurs is critical for annual plant species (Levine *et al.*, 2008, 2011; Picó, 2012; Volis, 2012). Thus, species-specific germination timing is very likely to be under strong evolutionary selection, since the species better able to adjust germination timing to environmental conditions are more prone to survive (Donohue *et al.*, 2010). In this context, it would make sense to consider that seeds are able to perceive environmental cues in order to predict appropriate environmental conditions to germinate and establish (Barua *et al.*, 2012). When the probability to germinate successfully just after dispersal is low or unpredictable, evolutionary selection and adaptation have led to a variable percentage of physiologically dormant seeds in many species (Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006). These dormant seeds enter the soil seed bank, a life stage that has been explained in terms of dispersion in time (Thompson, 2000), and as a bet-hedging strategy for population maintenance under uncertain environmental conditions (Venable, 2007).

Water thresholds are critical for germination (Facelli *et al.*, 2005; Batlla and Benech-Arnold, 2006). Several studies have dealt with the effect of water availability on seed germination of different species by exploring responses along natural precipitation gradients (Clauss and Venable, 2000; Petrů and Tielbörger, 2008; Orlovsky *et al.*, 2011; Volis, 2012), inter-annual variability in water availability (Levine *et al.*, 2008), simulated increases/reductions of rain (Freas and Kemp, 1983; Facelli *et al.*, 2005) and changes in rainfall timing (Levine *et al.*, 2011). In addition, physiological processes that promote germination are controlled by temperature (Probert, 2000; Finch-Savage and Leubner-Metzger, 2006), and temperature effects on seed germination have been broadly studied (e.g. Milberg and Andersson, 1998; Facelli *et al.*, 2005; Levine *et al.*, 2011; Hu *et al.*, 2012). Finally, photoperiod in combination with temperature and water availability also plays an important ecological

role as a predictable cue to anticipate forthcoming environmental conditions (Baskin and Baskin, 1998; Gutterman *et al.*, 2000).

In Mediterranean ecosystems the most critical period for plant survival is the summer drought (Kigel, 1995; Gutterman *et al.*, 2000). Most species germinate mainly once autumn rains appear (Espigares and Peco, 1993), and thus they maximize the time plants have to produce new seeds before next summer. Winter temperatures are, to a great extent, physiologically incompatible with germination; however, winter germination is quite common among Mediterranean species, even under continental climatic conditions (Escudero *et al.*, 1997; Herranz *et al.*, 2002). Finally, and especially for facultative winter annuals, *sensu* Baskin and Baskin (2004), spring germination also occurs (Ortega *et al.*, 1997) but at lower intensity than during autumn.

Our field experience in semi-arid Mediterranean gypsum habitats informs us that spring germination of annuals is lower than what should be expected due to mild weather conditions, even when field plots are artificially watered (Luzuriaga *et al.*, 2012). However, the experimental trials, on which our knowledge of environmental control of germination is mainly based, usually fail to reproduce field conditions in a realistic way. In other words, although the temporal pattern of germination is quite well known for Mediterranean annuals, the environmental cues that regulate germination, and how they interact, are still not completely clear. Our working hypothesis is that although water, temperature and photoperiod should be important cues for adjusting the timing of germination, they are not enough to explain the observed seasonal patterns of germination. More specifically, the low frequency of spring germination in semi-arid Mediterranean habitats should be related to the fact that some seeds enter secondary dormancy as a consequence of cold winter temperatures.

With this in mind, we set up a germination trial in which the environment was controlled to mimic conditions experienced in the field. We selected four abundant annuals from gypsum semi-arid communities in central Spain, to germinate under simulated autumn, winter and spring photoperiods and temperature conditions, combined with two contrasting water availability treatments: an initial single irrigation to simulate a downpour followed by drying out of the soil, and a permanent irrigation treatment to simulate continuous water availability. In order to be as realistic as possible, and as previous studies have documented the important effect of soil type on germination (Mikhiel *et al.*, 1992; Tlig *et al.*, 2008), the trials were conducted in pots filled with gypsum soil instead of in Petri dishes. Additionally, in order to evaluate if spring germination was controlled by previous winter temperatures, spring scenarios were set up both with cold dry stored seeds and with seeds stored under room conditions.

Our results will help to disentangle environmental control of germination of semi-arid annual communities. Besides, evidence on the response to environmental cues will help to explain the observed inter-annual community variability. Finally, if this response is species specific, we could hypothesize that germination is one of the processes behind species coexistence in this rich community through a storage effect mechanism.

Materials and methods

Four annual species were selected: *Ziziphora hispanica* L. (Lamiaceae), *Plantago afra* L. (Plantaginaceae), *Alyssum simplex* Rudolphi (Asteraceae) and *Helianthemum salicifolium* (L.) Mill. (Cistaceae). These species are common in annual plant communities growing on gypsum soils (Luzuriaga *et al.*, 2012) and all of them, except *A. simplex,* form persistent seed banks (Caballero *et al.*, 2005; Olano *et al.*, 2005). Seed mass ranged from 0.0139 g/100 seeds of *H. salicifolium* to 0.0618 g/100 seeds of *P. afra* (Table 1).

Seeds were collected from up to 20 random individuals throughout May and June 2008 at the Aranjuez Experimental Station located in southern Madrid, central Spain (40°02′N, 3°37′W; 580 m above sea level) on massive gypsum soils. The climate is semi-arid Mediterranean with a mean annual rainfall of 400 mm m^{-2} yr^{-1} and mean annual temperature of 14.5°C. The vegetation is a semi-arid gypsum steppe mainly dominated by *Stipa tenacissima*, a tussock-forming grass, scattered in a matrix of biological soil crust dominated by crustose lichens and a very rich annual plant community (Luzuriaga *et al.*, 2012), in which the selected species are quite frequent and abundant. The life span of this ephemeral plant community usually extends from October, when first germinations occur, to the end of May when almost all plant species have dispersed their seeds and subsequently dried out.

Table 1. Seed mass (100 seeds) and soil seed bank properties (unpublished data). Soil samples were collected prior to autumn germination (total seed bank), and after spring germination, but prior to seed dispersal (persistent seed bank)

	Mass (g)	Seed bank (seeds m^{-2})	
		Total	Persistent
Ziziphora hispanica	0.0202	20.5	0.4
Plantago afra	0.0618	3.0	1.2
Helianthemum salicifolium	0.0139	8.2	0
Alyssum simplex	0.0384	0.7	0

Seeds were stored in paper bags at outdoor temperatures during summer months. Later on, in late September, the seeds were relocated to the laboratory at room temperature (*c*. 20°C). Germination trials were set up in early December for all the environmental scenarios, except for a subset of seeds that were stored inside paper bags in a fridge at 5°C for 4 months (dry cold storage). This treatment was deliberately chosen to avoid any germination during winter and allow studying the effect of different spring scenarios (cold and warm) on seed germination. These conditions are still far from those experienced by seeds on the soil in the Aranjuez Experimental Station, where temperatures can drop during winter to − 10°C. Additionally, *H. salicifolium* seeds were scarified with sandpaper in order to break physical dormancy prior to the germination tests (Pérez-García and González-Benito, 2006).

Seed germination test

Ten seeds of *H. salicifolium* and 20 seeds from each of the other three species, were placed in plastic pots (6.5 cm × 6.5 cm × 10 cm) filled with gypsum soil taken from the field after removing the top 3 cm of soil, in order to eliminate the soil seed bank (Russi *et al.*, 1992). Pots were located randomly inside germination chambers and they were randomly rearranged periodically inside each chamber in order to avoid confounding factors.

We designed 14 scenarios and each of them was assigned to one chamber. The experimental scenarios resulted from the combination of three photoperiods (autumn, winter and spring), two temperature ranges (cool and warm scenarios likely to occur in each season), two water availability treatments (continuous versus single irrigation) and, exclusively for spring scenarios, two seed-storage conditions (cold dry storage and room conditions storage) (Table 2). The three different photoperiods were established according to the mean number of light and dark hours in October (for the autumn photoperiod treatment), January (winter) and April (spring) in our study area. Warm scenarios were established with the average values of the mean and maximum temperatures of October, January and April for night and day hours, respectively. Cool scenarios were established with average values of mean and minimum temperatures, for day and night hours, respectively. Due to technical reasons, our germination chambers could not simulate the cool winter experimental scenario (temperatures below 5°C). Light and temperature reference values were obtained from a Spanish Meteorological Agency (AEMET) thermo-pluviometric station in the study area ($n = 29$ years). Half of the pots of each scenario were irrigated to field capacity every 2 or 3 d, during the whole germination period (continuous irrigation treatment), to imitate full availability of water. The other half of the pots were watered to field capacity only once at the beginning of the experiment (single irrigation treatment), simulating the existence of a single rain event followed by a drought period. We set up a total of 560 samples, 10 replicates per experimental scenario ($n = 14$) and species ($n = 4$). Germination was monitored every 2–3 d and seedlings were registered and removed when cotyledons emerged. Germination chambers were equipped

Table 2. Environmental conditions set in each of the 14 experimental scenarios according to photoperiod (month of reference for each season: autumn, winter and spring), temperature (cool and warm), water availability (single irrigation and continuous irrigation) and, only for spring scenarios, winter storage (cold dry storage and room storage)

Photoperiod (light/dark hours)	Temperature (light−dark °C)	Irrigation	Storage
October (11 h/13 h)	Warm autumn (20−15)	Single	–
		Continuous	–
	Cool autumn (15−9.5)	Single	–
		Continuous	–
January (9 h/15 h)	Warm winter (10−5)	Single	–
		Continuous	–
April (13 h/11 h)	Cool spring (12−6)	Single	Cold dry Room
		Continuous	Cold dry Room
	Warm spring (18−12)	Single	Cold dry Room
		Continuous	Cold dry Room

Table 3. Quasi-binomial GLMs for germination of the four studied species. Standard errors were corrected using a quasi-binomial GLM model and logit link function. Likelihood ratio test (LR) chi-squares are indicated. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. D-squared (D^2), amount of deviance accounted for each model, is also provided

	df	*Alyssum simplex*	*Helianthemum salicifolium*	*Plantago afra*	*Ziziphora hispanica*
Seasonal scenario	4	**106.3*****	**38.8*****	**132.6*****	**274.1*****
Irrigation	1	**12.5*****	**10.4****	**34.7*****	**106.6*****
S. scenario × Irrigation	4	**88.1*****	**30.3*****	**253.0*****	**426.6*****
D^2		0.77	0.40	0.83	0.80

with cool white fluorescent light with an irradiance of $40\,\mu\mathrm{mol\,m^{-2}\,s^{-1}}$.

Data analysis

In order to test for the effect of seasonal scenarios, water availability and their interactions on total germination percentage we performed for each species quasi-binomial Generalized Linear Models (GLMs) using the logit link function. Quasi-binomial GLMs allowed the correction of overdispersion in our data.

Seasonal scenarios (cool and warm autumn, warm winter, and cool and warm spring) and water availability (single irrigation versus continuous irrigation) treatments were set as categorical fixed factors. The interaction between seasonal scenarios and water availability treatments was also evaluated. Since we also aimed to detect the effect of winter temperature on spring seed germination percentage, we performed a quasi-binomial GLM with the spring scenarios considering germination temperature, water availability and storage conditions (cold dry temperature versus room temperature) as fixed factors. D-squared (D^2) was calculated for each GLM as: (null deviance − residual deviance)/null deviance. Statistical analyses were performed in the R environment (R Core Team, 2013). Germination probability was also estimated by means of a non-parametric Kaplan–Meier approach, considering right-censored data in continuous and single irrigation in each scenario. Germination curves in spring scenarios with cold dry and room-stored seeds were also compared. Pairwise differences in germination curves were tested by non-parametric log-rank tests. Both water availability and season (each photoperiod × temperature combination) were considered as factors. SPSS v. 17 was used to perform these analyses (SPSS Inc., Chicago, Illinois, USA).

Results

Seasonal scenarios and water availability significantly affected final germination percentage of the four studied species (Table 3). D^2 values indicate that the predictors included in the models are highly explicative. Every species showed higher germination in autumn and winter scenarios regardless of water availability. In the absence of water restrictions, final germination was gradually reduced along the growing season, from autumn to spring scenarios (Fig. 1). Nevertheless, in the simulated cool spring
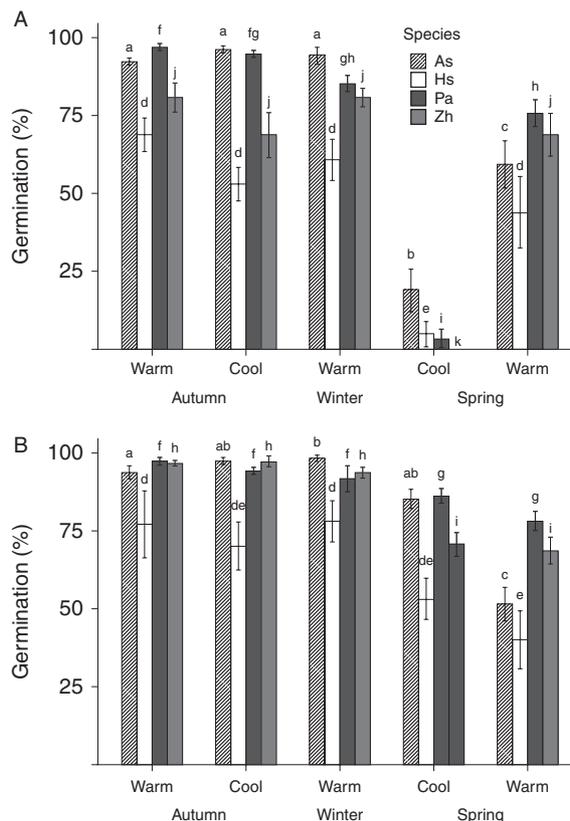

Figure 1. Final germination percentage along the simulated season under two different water availability treatments: (A) initial single irrigation and (B) continuous irrigation. Different letters indicate significant differences among simulated seasons for each species (Tukey test, $P < 0.05$). As, *Alyssum simplex*; Hs, *Helianthemum salicifolium*; Pa, *Plantago afra*; Zh, *Ziziphora hispanica*.

scenario, germination was very sensitive to low water availability conditions and it dramatically decreased for all species, as indicated by a significant interaction between scenario and water availability (Table 3 and Fig. 1).

Germination curves were significantly different among all pairwise comparisons between simulated climate scenarios for all the species, except for a few curves under prolonged water availability conditions for *H. salicifolium* and *Z. hispanica* (Table 4). Moreover, the water availability effect differed greatly among simulated climate scenarios and species. In the warm-spring scenarios and in the warm-winter scenarios water availability did not affect germination probability of any species (except for *Z. hispanica* in winter). Germination curves of *A. simplex* and *P. afra* were not affected by water availability treatments except for the cool-spring scenario, where the single irrigation treatment greatly reduced germination of every species (Fig. 2). Finally, germination of *Z. hispanica* and *H. salicifolium* was generally reduced by the single irrigation treatment, except in the warm spring scenario.

Cold dry storage of seeds significantly reduced final germination and germination probability of all species in spring scenarios (Table 5, Fig. 3), especially in cold spring and under the single irrigation treatment.

Discussion

Germination was significantly higher in autumn and winter scenarios, when almost all of the seeds of the four studied species germinated. However, during spring scenarios, germination slowed down and the process was highly dependent on temperature and water availability. Low water availability together with simulated low spring temperature jointly emphasized the reduction in germination observed in cold dry stored, relative to room stored, spring seeds. This is consistent with most studies on Mediterranean annual species, which revealed a clear prevalence of autumn–winter germination (Espigares and Peco, 1993; Ortega *et al.*, 1997).

Germination was favoured by prolonged water availability along the study seasons. This germination behaviour is generally found irrespective of plant habitat, and has been documented for other species living in arid and semi-arid habitats (Freas and Kemp, 1983; Tang *et al.*, 2009). However, our results showed that, even under prolonged high water availability conditions, final germination was gradually reduced as the growing season progressed from autumn to spring scenarios. This means that the studied species are able to discriminate seasons. In autumn, seed germination was mainly restricted by water

Table 4. χ^2 and *P* values from the comparison of Kaplan–Meier germination curves between experimental scenarios, in single (white) and continuous irrigation (shaded) treatments. W-A, warm autumn; C-A, cool autumn; W-W, warm winter; C-S, cool spring; W-S, warm spring. Spring scenarios were performed with cold dry stored seeds. Significance levels: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

| Sp.	Season	W-A		C-A		W-W		C-S		W-S	
Alyssum simplex	W-A	–		74.7	***	185.8	***	206.3	***	284	***
	C-A	105.2	***	–		232	***	252.9	***	313.5	***
	W-W	188.7	***	247.2	***	–		29.4	***	122	***
	C-S (S)	317.5	***	341.8	***	266.6	***	–		70.4	***
	W-S (S)	236.9	***	288.1	***	83	***	49.3	***	–	
Helianthemum salicifolium	W-A	–		1.7		19.2	***	61.7	***	66	***
	C-A	0.8		–		4.1	*	29.9	***	48.8	***
	W-W	2.5		0		–		13.6	***	21.9	***
	C-S (S)	163.2	***	106.8	***	129	***	–		84.4	***
	W-S (S)	15.6	***	5.7	*	5.3	*	3.1		–	
Plantago afra	W-A	–		71.4	***	271.5	***	286.9	***	327.4	***
	C-A	117.2	***	–		199.8	***	218.4	***	181.1	***
	W-W	273.9	***	207.2	***	–		5.1	*	15.7	***
	C-S (S)	383.3	***	395	***	302.7	***	–		227.2	***
	W-S (S)	309.4	***	203.7	***	14.2	***	22.3	***	–	
Ziziphora hispanica	W-A	–		2		193.2	***	248.5	***	80.8	***
	C-A	3.2		–		235.5	***	280.9	***	54.1	***
	W-W	41.8	***	7.3	**	–		44.9	***	0.8	
	C-S (S)	290.6	***	218.5	***	291.1	***	–		219.9	***
	W-S (S)	0.9		0.6		8.6	**	15	***	–	
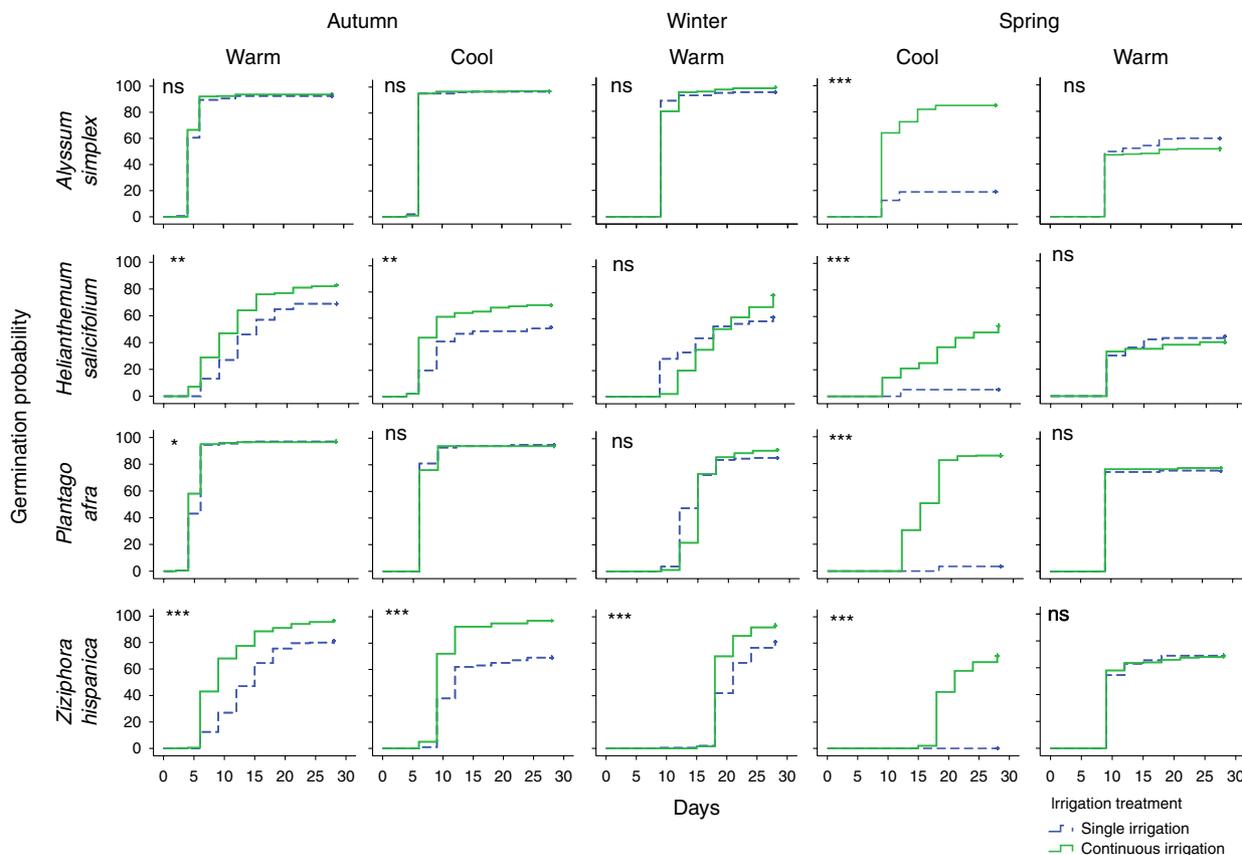
Figure 2. (colour online) Germination curves by means of the Kaplan–Meier method for every scenario. Continuous irrigation: solid line; single irrigation: dotted line. Significance levels: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

availability. In winter, germination was slowed down by low temperatures, but final germination still remained close to autumn percentages. Nevertheless, the effect of winter conditions on germination may have been underestimated in our study, because cool-winter conditions could not be set up in the germination chambers. In spring scenarios, even under prolonged high water availability conditions, higher temperatures decreased germination, contrary to the situation in autumn scenarios, and thus germination was lower in warm spring than in cool

spring. Furthermore, spring germination probability decreased under low water availability conditions, single irrigation treatment, combined with cool temperatures (Fig. 2). This strong interaction could be related to the fact that low temperatures slowed down water absorption (Murphy and Noland, 1982) and other temperature-dependent biochemical processes, so probably a single downpour event was not enough to imbibe seeds and trigger germination.

Our results show clearly that seeds germinated less during spring than during the previous seasons, even

Table 5. Environmental effects on final spring germination tested by means of quasi-binomial Generalized Lineal Models (GLMs) using logit link function. LR chi-squares are indicated together with their significance levels: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. D-squared (D^2), amount of deviance accounted for each model, is also provided.

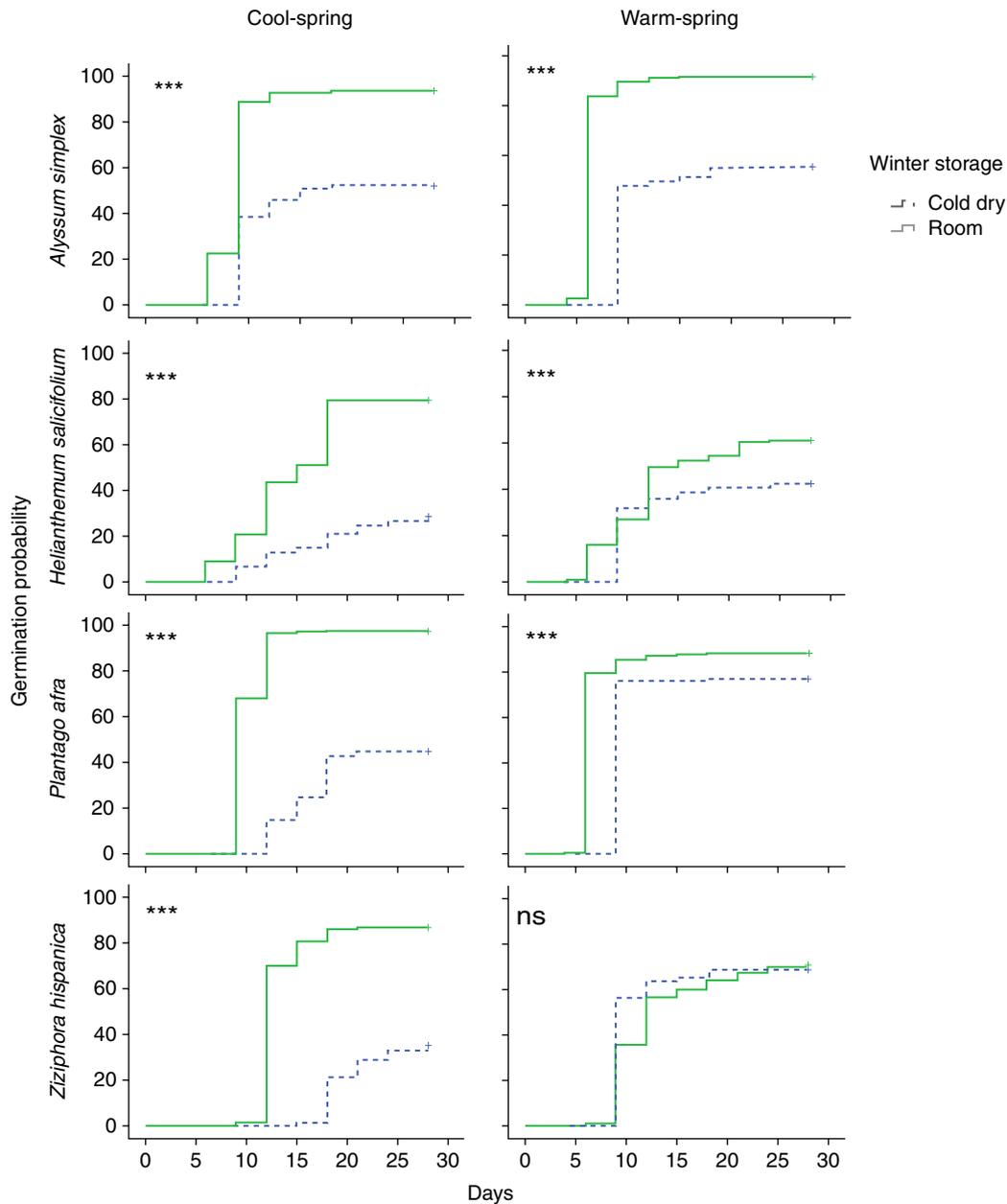|	df	*Alyssum simplex*	*Helianthemum salicifolium*	*Plantago afra*	*Ziziphora hispanica*
Temperature	1	0.15	0.6	**33.6***	**4.0*
Irrigation	1	**34.4***	2.3	**77.9***	**28.2***
Winter storage (WS)	1	**157.4***	**33.4***	**196.2***	**37.4***
Temperature × Irrigation	1	**45.9***	2.2	**62.7***	**11.6***
Temperature × WS	1	3.6	**10.9***	31.3***	**37.5***
Irrigation × WS	1	0.04	**6.0*	1.25	2.6
Temp × Irrigation × WS	1	**7.9**	**15.8***	**13.2***	**34.1***
D^2		0.74	0.47	0.85	0.67

Figure 3. (colour online) Spring germination curves by means of the Kaplan–Meier method. Curves compare germination of cold dry stored seeds versus seeds stored at room conditions, under both cool and warm spring scenarios. Significance levels: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

though simulated spring temperatures were in the same range as autumn ones. We interpret this seasonal germination response as an adaptive strategy that allows seedlings to counteract the variable risk to fail during each season. Early germination (autumn) increases the time span for plants to grow and to produce seeds. At the same time, autumn germination confers a competitive advantage in the use of space and resources during the growing season (Ross and Harper, 1972; Keddy and Shipley, 1989). In addition, plants need to counteract the detrimental experience of suffering freezing conditions during winter at earlier

life stages. Since most annuals have a relatively low risk of suffering damage by freezing conditions, the net balance seems to be favourable for this autumn response. Spring germination is a riskier option as the time plants have to complete their life cycle is shorter, and the probability of early plant death and soil seed bank depletion is higher. In this environmental context, it makes sense to reserve some seeds as a way of spreading the risk of failure. This bet-hedging strategy has been documented in desert annuals (Clauss and Venable, 2000; Venable, 2007), with rates of dormancy always being higher in those situations

with larger uncertainty for plant reproductive success: species with more variability in their reproductive success (Venable, 2007), populations in drier locations (Clauss and Venable, 2000; Volis, 2012) or during drier years (Tielborger and Valleriani, 2005). In line with the previously mentioned studies, we report that seeds germinate more readily during the secure autumn than in the riskier spring.

Nevertheless, under the unpredictable semi-arid Mediterranean conditions, dry periods during autumn and winter are not unlikely, and then spring rains would be an extra opportunity for germination. Thus, the facultative winter annual strategy may allow these species to germinate and take advantage of a rainy spring (see reports of massive field emergence in these ecosystems in Escudero *et al.*, 1999, 2000). Our study also highlights the role of water availability as a trigger for spring germination. The relevance of spring germination in terms of population dynamics is broadly unknown. To our knowledge, there are no studies recording field germination all year round in semi-arid annual plant communities to assess when seeds germinate and if there is a differential rate of successful reproduction related to germination timing. However, it is well known that winter annual plants in desert and semi-arid communities are able to germinate, grow and set seeds in a very short period of time (Petrů *et al.*, 2006), and thus spring germination may possibly lead to successful establishment and reproduction.

Although the four studied species showed the typical facultative winter annual pattern of germination, they also presented some slight differences in their germination responses. Seeds of *Plantago* and *Alyssum* germinated faster and in larger proportions, while *Ziziphora* and *Helianthemum* seemed to need a higher threshold of water requirements to germinate. As previously mentioned, faster species may have a competitive advantage in terms of site occupancy, but at the cost of a higher risk of seedling mortality when a single downpour event is followed by persistent drought conditions (Baskin and Baskin, 2004). Moreover, this species-specific germination pattern could be the basis of a storage effect process, favouring coexistence of species in this species-rich community. Our results support the first factor necessary for this mechanism to operate, a species-specific response caused by environmental variability, and the other two factors have been widely recognized: higher germination rates lead to a higher level of competition; and the seed bank is a persistent life stage that buffers populations during unfavourable periods (Facelli *et al.*, 2005).

In conclusion, our study demonstrates that environmental conditions strongly modulated the germination response of annual plants in semi-arid Mediterranean gypsum environments, which resulted in season-dependent germination, following the described germination pattern of facultative winter annuals.

Therefore, it could be said that germination in the studied species is under strict environmental control, but in a more complex way than that described for plants under more predictable and mesic environments. Under highly stressful and stochastic environments, without a clear environmental cue leading to a high probability of reproductive success, the evolution of a highly plastic germination response has been favoured. This implies that species are able to take advantage of favourable environmental conditions from autumn to spring. This environmental cuing is combined with an increasing rate of seed dormancy that is inversely related to the time to the next summer season.

Other studies have documented how germination is influenced by the predispersal environment (Donohue, 2005; Luzuriaga *et al.*, 2006), and even how the rate of dormant seeds produced by a species in a given site also varies depending on population density, plant size or plant crop (Tielborger and Valleriani, 2005). As a consequence, each year the process of community assemblage will start from a different amount of seeds per species, with seeds of each species varying in the level of dormancy, and with the germinable fraction of seeds responding in a different way to each year's and season's particular environmental conditions (Adondakis and Venable, 2004; Facelli *et al.*, 2005). The great environmental variability under which community regeneration occurs, together with a large variety of germination niches, undoubtedly will be one of the major processes behind the surprising species richness of these communities and their large inter-annual variability (Luzuriaga *et al.*, 2012). A thorough understanding of species germination niches gained from experimental studies simulating field conditions will lead to a better knowledge of germination itself and its ultimate importance as a determining process (Donohue *et al.*, 2010) in Mediterranean annual community assemblage and dynamics.

Conflicts of interest

None.

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