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**Full title:** Hydrothermal thresholds for seed germination in winter annual forbs from old-field Mediterranean landscapes

**Short running title:** Germination response of annual forbs

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### Abstract

- Under Mediterranean climates with dry-hot summers and cool-wet winters, many forbs with potential for habitat restoration are winter annuals, but there is little information about their germination.
- We performed laboratory germination experiments on 13 ruderal dicots native to Andalusia (southern Spain). We measured the germination of recently harvested seeds from natural populations across nine temperature treatments (from 5 °C to 35 °C, constant and alternate); two storage periods; and eight water stress treatments (from 0 MPa to -1.0 MPa). We then calculated the hydrothermal thresholds for seed germination.
- Final germination ranged from 0-100% and results were mixed in response to temperature. Base temperature was below 6 °C, optimal temperature was around 14 °C and the ceiling temperature around 23 °C. For five species, 10 months of storage improved total germination, indicating a dormancy-breaking effect, but the other species did not respond or had their germination reduced. All species were relatively tolerant to water stress, with base water potential ranging from -0.8 MPa to -1.8 MPa.

- Our results suggest that hydrothermal germination thresholds, rather than physiological dormancy, are the main drivers of germination phenology in annual forbs from Mediterranean semi-dry environments. The germination of these forb species differs from winter annual grasses, but their seeds are all suitable for being stored before restoration.

## 1. Introduction

Mediterranean-type ecosystems (MTEs) are characterized by a wet/cool winter and a dry/hot summer, reflecting an extreme version of temperate seasonal climates (Aschmann 1973). The Mediterranean climate favors plant strategies adapted to match the growing season with the cool months when water is available, and dormant stages or water-conserving traits to endure the hot, dry season (Bell *et al.* 1993; Keeley 1995; Connor 2005; David *et al.* 2007; Nardini *et al.* 2014). Herbaceous winter annuals are a significant part of the MTEs' flora in terms of taxa, biomass and range; and interest is growing to study their regeneration (Bell *et al.* 1993; Bretzel *et al.* 2009; Saatkamp *et al.* 2011; Sánchez *et al.* 2014; Benvenuti 2016). However, despite a number of studies addressing the germination timing of winter annuals from temperate climates, much less information exists from MTEs (Köchy & Tielbörger 2007; Sánchez *et al.* 2014).

Winter annuals are defined as plants with a life cycle in which they flower, disperse seeds and senesce by early summer; persisting in the soil seed bank through the warmest and driest months. The strategy of winter annuals is thus a short life cycle, with resources intensively invested toward seed regeneration. Seeds are generally dormant at dispersal, undergo dormancy loss through exposure to warm summer temperatures, and germinate during autumn or winter (Baskin & Baskin 2014). Those which strictly germinate early in the wet season are *obligate* winter annuals, while those that can germinate over a range of dates and into early spring are *facultative* winter annuals (Cici & Van Acker 2009). Physiologically, this phenology is usually achieved through type 1 non-deep physiological

dormancy, meaning that at dispersal seeds are only able to germinate at cool temperatures associated with the winter season (Baskin & Baskin 1983), but their ceiling temperature for germination increases as they lose dormancy (Soltani et al 2017).

In mid-latitudes, winter annuals use this reproductive strategy to match their growth season with autumn and/or winter, when temperatures are cooler and precipitation more reliable (Baskin *et al.* 1993). Nevertheless, studies on germination of winter annuals have been mainly focused on grasses, given that many of them behave as weeds in crop systems (Cheplick 1998; Scherner et al. 2017). Winter annual forbs are potentially important for the regeneration of degraded habitats in semi-dry ecosystems, supporting nutrient cycling, pollination and related ecosystem services (Valladares & Gianoli 2007; Jaunatre et al. 2014). The use of these and other native herbaceous species for ecological restoration is however limited by the lack of proper scientific information about their seed germination (Ladouceur et al. 2017). Understanding the seed germination of understudied species is important to predict their response to environmental conditions, with implications in community assembly, climate change and ecological restoration (Jiménez-Alfaro *et al.* 2016). In MTEs, the germination of winter annuals after short-time storage is a pre-requisite for successful restoration of degraded habitats, where annual forbs may have an important role in the sustainability of ecosystem services (Nunes *et al.* 2016).

Here, we focus on the germination strategy of ruderal forbs in old-field Mediterranean landscapes of Andalusia, southern Spain. Our study system is characterized by old agricultural landscapes in semi-arid conditions, most of them cultivated with large extensions of olive orchards and vineyards. Our main aim was to assess the germination response of 13 understudied ruderal herbaceous dicot species under varying environmental treatments. We tested whether these species from ruderal and semi-arid habitats with similar ecological requirements had a common germination response to temperature and water stress. We evaluated the germination traits of seeds soon after dispersal and after 10 months of storage, reflecting the scenarios for seeds used in the native seed industry. We expected the

germination of both post-dispersal and stored seeds to be higher under cooler temperatures representative of autumn. We also expected lower germination rates in non-stored seeds, given a requirement for dry after-ripening (physiological dormancy) or softening of the seed coat (physical dormancy). Finally, we expected a relatively high ability of the seeds to germinate under water stress, as an adaptation to germinate with the intermittent precipitation which is characteristic of the beginning of autumn, the natural germination season for Mediterranean winter annuals.

## 2. Materials and methods

### 2.1 Species selection; seed collection, cleaning and storage

From a list of 979 taxa recorded in a plant inventory of cultivated and ruderal habitats in the Córdoba Province (Pujadas Salvá 1986), we identified a subset of 284 native, annual angiosperm taxa observed in habitats related to olive orchards and vineyards. From those, we chose 13 understudied herbaceous dicot species (Table 1), from a range of plant families which are representative of extensive old-field habitats, and whose seeds mature in early summer. Two of the study species, *Anthyllis vulneraria* and *Scabiosa atropurpurea*, can also grow as biennial or perennials, but in our study system they are mostly found as annuals.

In June 2015, we collected seeds by hand from wild populations along ruderal right-of-ways and field margins in the Spanish provinces of Córdoba and Jaén. All the collection sites fell within the “Mediterranean South” environmental zone of Europe (Metzger *et al.* 2005). Sampled populations had a minimum of 500 individuals and seeds were collected from at least 100 haphazardly selected individuals, following the European Native Seed Conservation Network protocol (ENSCONET 2009). The harvested plant material was kept under ambient conditions (~22 °C, ~20 % RH) for an average of 4 weeks before being cleaned (Table 1). Accompanying herbarium vouchers were deposited at the Jardín Botánico Atlántico, Gijón (JBAG).

Small quantities and/or small-seeded species were cleaned by hand, roughing up the seed heads against metal sieves and then sifting to remove the inert material. Large quantities were cleaned using a stationary threshing machine (Wintersteiger LD 350) at 500 rpm with a 3x9 mm metal basket concave followed by separation with a winnower (Seed Processing Holland type 4111.10.00.2). Care was taken to avoid excessive cleaning and separation in order to maintain a more complete range of seed sizes and densities (Basey *et al.* 2015).

As the focus of this study was to address the natural response of native seeds, for the 4 species with supposed physical dormancy, we did not apply any additional scarification or nicking beyond what the seeds received through the mechanical cleaning process described above. Additionally, with *Anthyllis vulneraria*, we included 4 diaspore types to evaluate any differences in dormancy due to scarification: the natural dispersal unit (single seeded legume inside of calyx), partially processed dispersal unit (single seeded legume with calyx removed), fully cleaned seed with scarification (seeds with light scarification from mechanical cleaning with calyx and fruit covering removed), and fully cleaned seed without scarification (cleaned by hand to mimic seed in nature without scarification and with covering structures removed to reduce infection and improve imbibition). The seed lots were stored in opaque breathable packages within a seed warehouse where fluctuations of temperature and relative humidity conditions (Fig. SI 1) were comparable to those in the original collection sites.

## 2.2 Germination tests

Three laboratory germination experiments were done to determine the effect of temperature, storage and water potential. In the first experiment (temperature), we tested the effect of temperature on the germination of recently dispersed (up to 5 weeks since collection) diaspores. A range of four constant and four alternating temperature treatments was chosen to represent field temperatures in autumn, winter, spring and summer (Table 2).

Additionally, an extreme treatment of 35/5 °C was included to test if extreme diurnal temperature fluctuation released dormancy in the physically-dormant species (McKeon & Mott 1982; Santana et al. 2013; Santana et al. 2010; Vázquez-Yanes & Orozco-Segovia 1982). In the second experiment (storage), we tested the effect of 10 months of storage (Fig. SI 1) on the subsequent germination of the same seed lots. Stored seeds were germinated at a single temperature of 20/10 °C, representing spring (Table 2). In the third experiment (water potential), we tested the ability of seeds to germinate under drought stress. For this experiment, we germinated stored seeds at 20/10 °C, as described above. We prepared eight treatments of water stress: 0 MPa (control), -0.1 MPa, -0.2 MPa, -0.3 MPa, -0.4MPa, -0.6MPa, -0.8 MPa, -1.0MPa. These were chosen based on similar studies (Bradford, 1990; Bochet *et al.*, 2007; Cubera and Moreno, 2007; Ahmadian, Shiri and Froozandeh, 2015; Luna and Chamorro, 2016). We used solutions of polyethylene glycol (PEG) 8000 (Panreac AppliChem brand) to achieve the water potential treatments. Since our experiments were carried out under an alternating temperature regime, we used the average PEG concentration that corresponded to the two temperatures (Michel 1983; Money 1989).

For the temperature experiment, the constant temperature treatments were programmed in walk-in rooms (Trident Refrigeration, United Kingdom) and the alternating temperature treatments in upright chambers (LMS Ltd., United Kingdom). The storage and water potential experiments were conducted in an upright chamber (JP Selecta, Spain). For every experimental treatment of each species, four replicates of 25 seeds each were placed inside 9 cm polyethylene petri dishes with 2 layers of filter paper (Whatman Grade #1 85 mm) and moistened with 4 mL distilled water. Throughout the experiments, distilled water was added as needed to maintain availability of free water. The water potential experiment ended before any additional PEG solution had to be added. Light conditions in the chambers cycled through 12 hours of 30-35W cool white fluorescent light and 12 hours of darkness. Dark periods coincided with the cooler temperature in the alternating temperature regimes. Germination was defined as visible radicle emergence. The tests were ended once the

germination rate had slowed to 0 (4-10 weeks depending upon the species). Ungerminated seeds were cut and examined to determine viability. The germination proportion was calculated on the basis of the total number of viable seeds.

### 2.3 Data analysis

We used R (version 3.2.3 (2015-12-10)) (R Core Team 2015) to analyze the results of the experiments. To assess the effect of the experimental treatments on the final germination proportions, we fitted Generalized Linear Models (binomial error, logit link) to each experiment and species. We started by fitting fully factorial models, and then removed non-informative interactions and model parameters, until achieving the minimal adequate model for each experiment and species (Crawley 2013).

We also calculated the hydrothermal-time thresholds of the different seed lots (Garcia-Huidobro *et al.* 1986; Hardegee 2006; Orrù *et al.* 2012). We only performed this part of the analysis with datasets that met certain quality criteria: final germination above 45 % and time to 50 % germination of at least 2 days. As a first step, we fitted cumulative germination curves for each experimental treatment, and used the curves to estimate the time needed to reach successive deciles of final germination (10-90 %). Then we calculated the germination rates for each decile as the inverse of the times. We plotted the germination rates against the values of the experimental treatments (temperature or water potential) to compute the hydrothermal-time thresholds.

For the thermal thresholds, we plotted the germination rates against temperature, and then divided the plots in suboptimal and supraoptimal temperature ranges determined by visual inspection. We fitted a linear regression to each range; and calculated the base temperature ( $T_b$ ) as the x-intercept of the suboptimal regression, the ceiling temperature ( $T_c$ ) as the x-intercept of the supraoptimal regression; and the optimal temperature ( $T_o$ ) as the intercept of the two regression lines. For the hydro thresholds, we plotted the germination rates against PEG concentration and calculated the base water potential ( $\psi_b$ ) as the x-



intercept of a fitted linear regression (Gummerson 1986; Bradford 2002). We repeated these calculations for each available germination decile and averaged the results, thus obtaining the final hydrothermal thresholds for the germination of each species.

### 3. Results

#### 3.1 Effect of temperature

The final germination of 6 unscarified seed lots with physical dormancy (*Helianthemum ledifolium*, *Tuberaria guttata*, *Medicago orbicularis*, and three diaspore types of *Anthyllis vulneraria*) was very low (< 5%), and we did not include these lots in further analyses. In the extreme alternating temperature treatment of 35/5 °C, all seeds were ungerminated and infected at the end of the experiment, and those results are not presented either.

For most species, we found that in the cooler treatments of 10°C and 15°C, there was higher final germination percentages in the constant treatments, compared to the corresponding diurnally alternating treatments of 15/5°C and 20/10°C (Fig. 1). The opposite was true for the warmer temperature treatments of 20°C and 25°C. In this case, final germination was higher in the diurnally alternating treatments (25/10°C and 30/20°C) and lower in the constant treatments (Fig. 1). Some exceptions to this pattern were *T. barbata* and *A. vulneraria* which germinated at high proportions across most treatments; while for *A. cotula*, germination was low and there was no effect of temperature on germination (Fig. 1). *Scabiosa atropurpurea* had the highest final germination at higher temperatures, while *Stachys arvensis* had the highest final germination in alternating regimes, except for the coolest treatment of 10°C (Fig. 1). We were able to calculate the cardinal germination temperatures with the germination rates of *A. vulneraria*, *C. lusitanica*, *S. atropurpurea*, and *T. barbata* (Table 3a).  $T_b$  ranged between 3.6°C and 6°C,  $T_o$  were around 14°C, and  $T_c$  were around 23°C.

### 3.2 Effect of storage

Ten months of storage increased the final germination of five species: *M. moricandioides*, *S. atropurpurea*, *C. lusitanica*, *A. cotula* and *T. maximum* (Fig. 2). Two species, *T. barbata* and *A. vulneraria*, which reached high final germination regardless of temperature treatment, likewise germinated at high final germination regardless of storage treatment (Fig. 2). Three species, *S. arvensis*, *N. damascena*, *E. plantagineum* had higher final germination when fresh than following storage (Fig. 2).

### 3.3 Effect of water potential

Five species (*S. atropurpurea*, *T. maximum*, *C. lusitanica*, *T. barbata* and *A. vulneraria*) had high final germination percentages in the control and a decrease in germination with increased water stress (Fig. 3). *Echium plantagineum*, *M. moricandioides* and *S. arvensis* had overall low final germination percentages even in the control, and additional water stress lowered germination further (Fig. 3). Of note, increased water stress did not affect the final germination percentages of *N. damascena* and *A. cotula*, even under the highest treatment of water stress (-1.0 MPa) (Fig 3). We were able to calculate  $\psi_b$  using the germination rates of *A. vulneraria*, *C. lusitanica*, *N. damascena*, *S. atropurpurea*, *T. barbata* and *T. maximum* (Table 3b). All of them had a relatively low base water potential, from -0.8 MPa to -1.8 MPa, thus indicating their ability to germinate under low moisture conditions.

## 4. Discussion

### 4.1 Thermal germination niche

In general, the studied species had a broad thermal germination niche, as they germinated across a wide range of temperatures with at least 50% final germination in all species except *A. cotula*. These results indicate that the studied species have the potential

to function as facultative winter annuals, since they germinated within the range of 6°C to 22°C. This strategy agrees with a recent survey of field germination of Mediterranean herbs, which found that most of them are facultative annuals with similar germination whether sown in early winter (November) or in late winter (February) (Benvenuti & Pardossi 2016). Despite a range of responses across temperatures, the values for the cardinal temperatures were similar across species:  $T_b$  were between 3°C to 6°C,  $T_o$  around 14°C, and  $T_c$  around 23°C. These values indicate that the species would not germinate when field temperatures remain above 23 °C, i.e. from June to September; whereas the fastest germination rates would be reached in November. Given the general lack of frost or temperatures close to 0 °C in the studied sites, field temperatures are expected to remain above  $T_b$  during almost all the year. Thus  $T_b$ , which incidentally showed more variation across species, does not seem to have the functional importance of  $T_o$  or  $T_c$  in these habitats.

Another noticeable pattern was the contrasting effect of alternating temperatures, which improved germination under the higher temperature treatments (20°C and 25°C), but reduced it at the lower temperatures (10°C and 15°C). This could be due to the inhibiting effect of the cooler alternating temperature on germination while in warmer treatments, the cooler of the alternating temperatures was not cool enough to have an inhibiting effect on germination. The lower final germination under some temperature treatments could be explained by the higher degree of dormancy expected in these relatively fresh, post-dispersal seed lots. In a study of *A. cotula* as a non-native weed, achenes which had been stored for about 6 months and were germinated in darkness reached final germination of 20% to 45% under constant temperatures of 10°C, 15°C, 20°C and 25°C but germination was 12% or less in the extreme temperatures of 5°C , 30°C and 35°C (Gealy *et al.* 1985). In our study, there was no effect of temperature or diurnal oscillation, and final germination was low (less than 3%) for *A. cotula*. A partial explanation for the low final germination could be the effect of the pericarp on lowering germination when compared to seeds (Gealy *et al.* 1985).

## 4.2 Effect of storage

Winter annuals are typically dormant at the time of dispersal (Hilhorst & Toorop 1997; Thompson 2001; Baskin & Baskin 2014). Contrary to the expectation that germination would be lower in the post-dispersal (dormant) seed lots than in the stored lots, instead there were three types of response. Two species, *A. vulneraria* and *T. barbata*, had the same final germination post-dispersal and after storage. For three species (*S. arvensis*, *N. damascena* and *E. plantagineum*), 10 months of storage resulted in higher final germination of the post-dispersal treatments compared to the stored treatments. Five species (*T. maximum*, *A. cotula*, *M. moricandioides*, *C. lusitanica* and *S. atropurpurea*) responded as expected, with higher final germination following several months of storage. Similarly, storing of more than one year increased germination in *A. cotula* from the cold Himalayan deserts (Rashid *et al.* 2007), suggesting a species-level trait of losing dormancy during long periods of storage. Other studies found that seeds of *M. moricandioides* collected from wild Spanish populations and stored at 5°C for 4-8 months germinated to nearly 90% under the alternating temperatures of 20/7°C (Herranz *et al.* 2006), although that experiment did not assess baseline germination of fresh seeds.

In general, we found less dormancy than expected in the recently dispersed seeds, and reduced germination in stored seeds that can be explained by dormancy. Warmer maternal environments can lower the primary dormancy of fresh seeds (Gutterman 2000; Donohue 2005) and May 2015 was unseasonably warm which may have affected the studied seed lots (Dwyer & Erickson 2016), which were ripening on the mother plants in that period. Another possible explanation for the levels of dormancy that we observed is that the post-dispersal and storage/after-ripening treatments that we chose were not adequate to elicit a response in our study species. The post-dispersal seeds had been stored 2 to 7 weeks when the experiments began, and could have undergone some after-ripening during that period, thus being less dormant than would be expected with a shorter storage period. Nevertheless, field temperatures during summer are well above the  $T_c$  of germination, thus

seed dormancy may not be needed to prevent germination until autumn. Our forb species also showed varied dormancy responses, in contrast with the results of six ruderal annual grasses sampled in the same study system, which were all non-dormant both when fresh and after storage (Jiménez-Alfaro *et al.* 2018).

Although we were not explicitly testing scarification treatments on the physically dormant species, we can conclude that the passive scarification received via the mechanical cleaning process was not sufficient to alleviate dormancy and allow for imbibition in these experiments. In our separate field studies of the same species (Frischie *et al.*, unpublished data), the two Fabaceae (*M. orbicularis* and *A. vulneraria*) germinated and established well, despite low germination response in the lab tests. Additional abrasion from soil particles or wider contrasts in temperature fluctuation at the soil surface may explain this difference between lab and field results (Baskin *et al.* 2000; Santana *et al.* 2010). In contrast, the two Cistaceae (*H. ledifolium* and *T. guttata*) did not imbibe and germinate in the lab nor did they emerge and establish in the field trials. This could be due to field planting depth which was too much for the small-seeded species. It is also possible that different conditions such as heat treatments akin to fire exposure (Keeley 1995; Luna & Chamorro 2016) or additional scarification beyond the mechanical cleaning we used may be required to alleviate dormancy in these Cistaceae species.

#### 4.3 Water potential

As expected, there was a general tolerance to water stress in the seed lots we studied with a decrease in germination as water potential decreased (Bradford 1990). All ten species germinated well under moderate levels of water stress and the base water potential ranged from -0.8 MPa to -1.8 MPa. This exceeds the soil water potential which has been measured in ruderal Mediterranean habitats (Bochet *et al.* 2007; Ben-Gal *et al.* 2009; Gómez-del-Campo 2013). Two species (*A. cotula* and *N. damascena*) were not limited by the lowest water potential (-1.0 MPa) in this experiment, indicating they would germinate well

under the dry conditions between rainfall events in Mediterranean climates. The base water potential for *N. damascena* was very low, at -1.8 MPa. Interestingly, *N. damascena* also had its germination strongly inhibited by the warmer temperature treatments, so it may rely only on cold temperatures as a germination cue and attempt to germinate even in the driest conditions. Water potential from -0.4 to -1.0 MPa reduced germination in *A. cotula* achenes (Gealy *et al.* 1985) from Oregon (USA), where the plants were observed as weeds and limited to moister parts of fields. The other eight species responded as expected with a decrease in final germination as water potential decreased. These results are similar to those for 22 ruderal species which colonize road cuts in Spain. In that study, there was a notable reduction in germination when water potential decreased from -0.05 MPa to -0.35 MPa and no species germinated at the lowest water potential of -1.5 MPa (Bochet *et al.* 2007). Ability to germinate under water stress was correlated with colonizing ability in these disturbed habitats.

## 5 Conclusions

The hydrothermal thresholds for germination among our species seem to be in accord with the few studies that addressed similar traits in Mediterranean annuals. Our results are also comparable to those of the perennial grass from semi-arid Mediterranean grasslands, *Stipa tenacissima*, (Krichen *et al.* 2014) which germinated most between 10-20°C and was limited by water potentials lower than -0.8 MPa. However, even though our species are all native winter annuals from ruderal habitats, this study suggests that there was no single, general response of winter annual forbs to environmental cues. This contrasts with the homogeneous responses detected across Mediterranean winter annual grasses (Jiménez-Alfaro *et al.* 2018), which might imply different phenological strategies in the populations restored with native seeds.

The variation in germination responses can be understood in the context of the high diversity of ruderal and semi-arid habitats due to both anthropogenic and natural disturbances (Fernández-Alés *et al.* 1993; Rey Benayas & Scheiner 2002; Bonet 2004). In our study area, a mosaic of micro-habitats is formed by the interplay of disturbances, stresses, topography, aspect, soil type and precipitation (Gallego Fernández *et al.* 2004). Other studies have discussed the disturbances and stresses of Mediterranean habitats, mainly heat and drought, which lead to diverse floras and often local adaptations (McIntyre *et al.* 1999; Pausas 1999; Millington *et al.* 2009; McIntyre & Grigulis 2013; Matesanz & Valladares 2014; Nardini *et al.* 2014). For example, among four annuals from gypsum soils, germination response fell within the winter annual strategy, yet plasticity allowed for bet hedging and micro-adaptation to the mosaic of Mediterranean habitats (Sánchez *et al.* 2014).

Our study provides new information about germination traits of species with potential for restoration, which is urgently needed at regional and continental scale (Ladouceur *et al.* 2017). In our system, hydrothermal germination thresholds, rather than physiological seed dormancy, seem to be the main driver of germination phenology. This agrees with a parallel study focused on annual grasses of the same old-field Mediterranean landscapes (Jiménez-Alfaro *et al.* 2018) and it is also consistent with the seasonal variability expected in Mediterranean climates. Sowing seeds in October or November (i.e., when field temperatures fall below 23 °C) should ensure a rapid and successful establishment in habitats subject to ecological restoration, while species from *Fabaceae* and *Cistaceae* will need mechanical external factors to break physical dormancy. Although the populations established by native seeds of the studied species will probably differ in their germination rates, many of them are suitable to be stored for several months before restoration.

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## Conflicts of Interest

None.

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**Table 1.** Study species, main habitat requirements and number of weeks between seed collection and germination experiments. Taxonomy follows theplantlist.org; soil and habitat are from Castroviejo (1986-2012)). \* classification for the genus. † classification for the family. In the cases where plants and/or fruits were entirely senescent and brittle, no herbarium voucher was made.

Scientific Name	Family	Fruit type	Dormancy class (Baskin & Baskin 2014)	Embryo type (Martin 1946)	Voucher collection number (JBAG)	Soil	Habitat	Collection date	Days between collection and onset of experiments for post-dispersal seed lots
<i>Anthemis cotula</i> L.	Asteraceae	Achene (2 forms)	PD	spatulate	SF - 0304	--	fields and disturbed areas	23 June 2015	36
<i>Anthyllis vulneraria</i> L.	Fabaceae	Legume (single seeded, dispersal unit retains corolla)	PY	bent	--	indifferent	seaside sand and cliffs, rocky clefts and plains, pastures, openings, matorral	18 July 2015	27
<i>Cleonia lusitanica</i> (L.) L.	Lamiaceae	Nutlet	PD†	spatulate†	SF - 0320	limestone, clay, gypsum, sandy or gravelly and generally poor soils	dry pastures and matorral scrublands, openings in oak woodlands (encinar, quejigar) and juniper woodlands (sabinar)	18 July 2015	11

<i>Echium plantagineum</i> L.	Boraginaceae	Nutlet	PD†	spatulate	SF - 0278	basic or acidic	fields, disturbed sites, right of ways	3 June 2015	48
<i>Moricandia moricandioides</i> (Boiss.) Heywood	Brassicaceae	Non-dehiscent silique	PD*	bent†	SF - 0307	limestone	Marl slopes, clay or sandy hills, rock clefts	24 June 2015	34
<i>Nigella damascena</i> L.	Ranunculaceae	Dehiscent capsule	MPD*	rudimentary - linear †	SF - 0305	--	crop fields, untilled areas, rocky or sandy pastures	24 June 2015	36
<i>Scabiosa atropurpurea</i> L.	Caprifoliaceae	Achene (2 forms)	PD*	spatulate*	--	indifferent, nitrophile	pastures, fallow areas, right of ways, slopes	11 July 2015	19
<i>Stachys arvensis</i> (L.) L.	Lamiaceae	Nutlet	PD*	spatulate*	SF - 0287	silica, sand, clay or rarely basic	annual grasslands, openings in woodlands and matorral, fallow and cultivated fields	10 June 2015	41
<i>Tolpis barbata</i> (L.) Gaertn.	Asteraceae	Achene (2 forms)	PD*	spatulate†	SF - 0318	sand	understory of woodlands and shaded fields	11 July 2015	48
<i>Tordylium maximum</i> L.	Apiaceae	Schizocarp	PD†	linear†	SF - 0310	--	right of ways, crop fields and fallow areas	25 June 2015	34

**Table 2.** Average daily maximum and minimum air temperature for the region of Córdoba for January, April, July and October and corresponding temperature treatments (Instituto de Investigación y Formación Agraria y Pesquera).

	Average day/night T in Córdoba	Constant Treatment	Alternating Treatment
Autumn (October)	25/13°C	20°C	25/15°C
Winter (January)	15/4°C	10°C	15/5°C
Spring (April)	23/10°C	15°C	20/10°C
Summer (July)	37/19°C	25°C	30/20°C
Experimental extremes	n/a	5°C	35/5°C

**Table 3.** Cardinal temperatures (a) and base water potential (b) for germination. Missing values are due to lack of three or more responding temperatures in the corresponding suboptimal or supraoptimal ranges.

a)						
	$T_b$	SD	$T_o$	SD	$T_c$	SD
<i>A. vulneraria</i> T alternating	--	--	--	--	23.9	± 0.4
<i>A. vulneraria</i> T constant	6.1	± 0.8	13.4	± 3.4	23.7	± 0.6
<i>C. lusitanica</i> T constant	3.6	± 2.0	14.0	± 1.4	23.7	± 0.3
<i>S. atropurpurea</i> T constant	6.5	± 3.4	--	--	--	--
<i>T. barbata</i> T alternating	--	--	14.6	± 0.1	24.2	± 0.3
<i>T. barbata</i> T constant	4.1	± 3.8	--	--	--	--
b)						
	$\psi_b$	SD				
<i>A. vulneraria</i>	-0.84	± 0.04				
<i>C. lusitanica</i>	-1.03	± 0.06				
<i>N. damascena</i>	-1.82	± 0.13				
<i>S. atropurpurea</i>	-0.81	± 0.20				
<i>T. barbata</i>	-0.86	± 0.20				
<i>T. maximum</i>	-0.85	± 0.07				

**Figure 1.** Final germination proportions of post-dispersal seeds across all temperature treatments. Species are generally ordered from higher to lower final germination. Darker bars are constant temperatures and lighter bars are alternating temperatures; e.g. 10°C constant is displayed with 15°C/5°C alternating. Error bars are 95% confidence interval. Letters indicate no significant difference ( $p < 0.001$ ) between treatments. See Table SI 1 for the parameters of the GLM tests for differences in final germination proportions.

**Figure 2.** Effect of storage (10 months) on final germination proportion. Final germination was higher for fresh seeds of species to the left of center and lower for species to the right of center. Error bars are 95% confidence interval. Letters indicate no significant difference ( $p < 0.001$ ) between treatments. See Table SI 2 for the parameters of the GLM tests for differences in final germination proportions.

**Figure 3.** Effect of water stress on final germination proportion. Error bars are 95% confidence interval. Letters indicate no significant difference ( $p < 0.001$ ) between treatments. See Table SI 3 for the parameters of the GLM tests for differences in final germination proportions.





