

## Phenotypic plasticity in seed germination relates differentially to overwintering and flowering temperatures

Eduardo Fernández-Pascual<sup>1\*</sup>, Borja Jiménez-Alfaro<sup>2</sup>

<sup>1</sup> Jardín Botánico Atlántico, Universidad de Oviedo, Avda. del Jardín Botánico 2230, 33394 Gijón/Xixón, Spain; <sup>2</sup> Department of Botany and Zoology, Masaryk University, Kotlářská 267/2, 611 37 Brno, Czech Republic

\* Corresponding author: Telephone 0034985185134; Fax 0034985130685; Email [eduardofp@indurot.uniovi.es](mailto:eduardofp@indurot.uniovi.es).

This is a pre-copyedited, author-produced PDF of an article accepted for publication in *Seed Science Research*. The version of record (Fernández Pascual, E. & Jiménez-Alfaro, B. (2014) Phenotypic plasticity in seed germination relates differentially to overwintering and flowering temperatures. *Seed Science Research* 24: 273-280) is available online at: <http://dx.doi.org/10.1017/S0960258514000269>.

**Key words:** *Centaurea somedanum*, dormancy variation, Gentianaceae, plant-climate interactions, plant regeneration, seed ecology

### Abstract

Phenotypic plasticity in seed dormancy may allow plant species to cope with fast environmental changes such as climate warming. In controlled experimental settings, plasticity in dormancy has been found to relate to temperature during seed maturation, but this relationship has not been tested in field conditions. Here we analyse for the first time the variation in dormancy during five successive years in wild populations of the study species *Centaurea somedanum*, to determine how this variation is related to average temperatures during specific seasons of plant activity. We performed laboratory germination experiments to measure (1) the degree of dormancy at dispersal, and (2) the sensitivity to dormancy breaking factors. We calculated average temperatures during four seasons of plant activity (overwintering, vegetative growth, flowering and seed maturation) for each year, and tested the relationship between these temperatures and patterns of dormancy variation using Generalized Linear Models. Dormancy varied among sites and years, seeds being more dormant in colder years. For the degree of dormancy at dispersal, we found a positive relationship with flowering temperature and a significant effect of collection site. For the sensitivity to dormancy breaking factors, we found no significant differences among sites, a positive relationship with flowering temperature and a negative relationship with overwintering temperature. Phenotypic plasticity in dormancy in *C. somedanum* is thus especially marked in the sensitivity to dormancy breaking factors. Temperatures during overwintering and flowering have differential effects on this plasticity, allowing the plant to detect the gradient of seasonality, a main ecological feature of its distribution. These results highlight the importance of taking into account more than average temperatures when assessing the response of plant phenotypic plasticity to climate change.

### Introduction

Phenotypic plasticity will be a major player in plant responses to climate change (Nicotra et al., 2010; Reed et al., 2011). Global warming (IPCC, 2013) can potentially disrupt the many interactions between climatic patterns and biological processes, such as the thermal control of seed germination (Probert, 2000). In seasonal regions, the timing of seed germination is closely linked to annual climatic cycles to ensure that seedling establishment occurs in the favourable season (Donohue et al., 2010), but there is a risk that this relationship will be disrupted by climate warming (Walck et al., 2011). Future temperatures may no longer match germination requirements (Cochrane et al., 2011), or be inadequate to fulfil dormancy-breaking requirements (Orrù et al., 2012). Such effects can modify recruitment from the soil seed bank (Hoyle et al., 2013; Ooi, 2012; Ooi et al., 2009) and shift germination timing (Mondoni et al., 2012), ultimately compromising species survival. Nonetheless, predicting germination responses to climate change is a challenge for plant ecologists. In seeds with physiological dormancy, which are the majority in temperate regions (Baskin and Baskin, 1998),

it is necessary to understand the influence of environmental temperature in four layers: germination itself, dormancy loss, phenotypic plasticity in dormancy and selection of dormancy genotypes.

The rate of germination is a linear function of temperature between base and ceiling thermal thresholds beyond which no germination occurs (García-Huidobro et al., 1982; Hardegree, 2006; Pritchard and Manger, 1990). Germination thermal thresholds depend on the level of dormancy; i.e. a seed characteristic, the degree of which defines what conditions should be met to make the seed germinate (Finch-Savage and Leubner-Metzger, 2006; Vleeshouwers et al., 1995). Physiologically, changes in dormancy are translated into changes in germination thermal thresholds, and the rate of this change is again a linear function of temperature below a thermal threshold beyond which dormancy loss stops (Pritchard et al., 1999; Pritchard et al., 1996; Steadman and Pritchard, 2004). Differences among individuals and seed lots in their thermal thresholds are noticed as spatial and temporal variation in seed dormancy, either in dormancy levels (Daws et al., 2004; Orrù et al., 2012) or in the sensitivity to dormancy-breaking factors (Pritchard

et al., 1999). These differences in dormancy can be the result of local differences in environmental temperatures acting in the long and short terms. In the long term, local thermal differences can select genetically-based differences in dormancy through clinal variation and local adaptation (Fernández-Pascual et al., 2013; Wagmann et al., 2012). In the short term, temperature variation during seed maturation can produce phenotypic plasticity in dormancy (Fenner, 1991; Fenner and Thompson, 2005; Gutterman, 2000).

In a changing climate this phenotypic plasticity becomes of the utmost importance, as it may be the key for quick plant responses to new conditions (Matesanz et al., 2010; Walck et al., 2011). Controlled seed maturation in experimental gardens has shown that warmer maturation environments produce less dormant seeds (Chiang et al., 2011; Figueroa et al., 2010; Luzuriaga et al., 2006; Qaderi et al., 2006), but there is little information on how this relationship holds in field conditions. A possible approach to address this question might be to study dormancy levels of wild populations in successive years, so the genetic background remains relatively constant and any differences can be attributed to phenotypic plasticity in response to varying weather conditions. Analysing these temporal changes in relation to climate is a key step towards understanding the effects of climate change on seed ecology (Walck et al., 2011). It is for instance necessary to establish the specific influence of temperature during different seasons of plant growth, as climate change is expected to be focalized in certain parts of the year (IPCC, 2013). However, to our knowledge no field studies have evaluated the influence of climate on seed dormancy during consecutive years.

In this work, we investigate how dormancy variation is related to climatic trends along five successive years in the study species *Centaurium somedanum* M. Laínz (Jiménez-Alfaro et al., 2013). At dispersal, seeds of *C. somedanum* have non-deep simple morphophysiological dormancy (Fernández-Pascual et al., 2012). The physiological component of this dormancy varies following an altitudinal cline that is related to long-term climatic differences among sites and also to neutral genetic variation (Fernández-Pascual et al., 2013). Moreover, in a previous common garden experiment we demonstrated phenotypic plasticity in seed dormancy in response to the seed maturation environment (Fernández-Pascual et al., 2013). These preceding works point to this species as a good biological system to test the influence of short-term climatic changes. To this end, we tested the relationship between environmental temperatures experienced by wild populations and two seed traits: (1) level of dormancy at dispersal and (2) sensitivity to dormancy breaking factors. To assess the potential role of thermal variation within each year, we tested the effect of temperatures during different seasons of plant growth.

## Materials and methods

### *Study species*

*Centaurium somedanum* M. Laínz (Gentianaceae) is a perennial herb that grows in small-sedge and brown moss communities associated to tufa-forming springs (Jiménez-Alfaro et al., 2013). Its distribution is confined to a 210 km<sup>2</sup> area in the Cantabrian Mountains of north-western Spain where discrete populations can be found from 600 to 1700 m above sea level (Jiménez-Alfaro et al., 2010). Regional climate is transitional between Oceanic and Continental-Mediterranean, with local differences depending on altitude and exposure. The species is a facultative outcrosser and shows a high reproductive output: average individuals produce two-four fruits with c. 140 seeds each, although the number of fruits can reach up to 15 (Jiménez-Alfaro et al., 2005); and the proportion of viable seeds is generally over 95 % (Fernández-Pascual et al., 2012). Flowering peaks in July, seeds mature during August and September, dispersal lasts until October and seedlings emerge next summer (Jiménez-Alfaro et al., 2010).

### *Seed collection and germination*

We collected seeds of *C. somedanum* during five consecutive years (2008 - 2012). To assess within-species variation in seed dormancy, we replicated the study in the two largest populations, La Malva (43° 07' 05'' N, 06° 15' 05'' W, 600 m asl, c. 230 reproductive individuals) and El Valle (43° 04' 19'' N, 06° 11' 49'' W, 1280 m asl, c. 280 reproductive individuals). These two sites are 7.5 km apart, in the northern side of the Cantabrian Mountains and have a strong Oceanic influence. Each year we made one collection per site at the time of seed dispersal, sampling 20 medium-sized individuals. Marking individuals was unfeasible because of the small and frail plants; each year we selected individuals randomly from the same areas. From each individual we took two capsules (dry and brownish). Capsules spent 3 weeks in our laboratory (c. 22 °C, 50 % relative humidity) before being cleaned and used in germination experiments.

For the experiments we sowed seeds on 1 % distilled water agar held in 6-cm-diameter Petri dishes (eight dishes with 25 seeds each per collection), sealed with Parafilm to prevent desiccation. Half of the dishes (fresh seeds) went immediately into germination incubation to check dormancy at dispersal; the other half (stratified seeds) were exposed to wet-cold stratification (12 weeks, 1 % agar, 3 °C, darkness) before being placed in germination incubation, to assess their receptiveness to dormancy-breaking factors. Germination incubation was conducted in a growth chamber (Grow-S 360, Ing. Climas, Barcelona, Spain) programmed with a 12h-light/12h-darkness photoperiod (c. 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during the light phase provided by six Philips TLD30W/54-765 cool fluorescent tubes) coupled with a 22/12 °C thermal regime. These conditions promote *C. somedanum* germination once dormancy is broken (Fernández-Pascual et al., 2012). Although this procedure does not allow for interpreting the effect of dormancy on the base and ceiling thermal thresholds for germination (Steadman and Pritchard, 2004), tests

performed under a single temperature treatment offer valuable information on differences in dormancy among seed collections (Andersson and Milberg, 1998; Fernández-Pascual et al., 2013; Wagmann et al., 2012). During incubation, we counted and discarded germinated seeds three times a week. Radicle protrusion was the criterion for germination. We terminated experiments after four weeks and opened non-germinated seeds with a scalpel, classifying them as full, empty or fungus infected. We excluded empty ( $3.1\% \pm 0.5$ ) and infected ( $1.9\% \pm 0.4$ ) seeds from statistical analyses and germination percentages.

#### Temperature data

To describe temperature during the study period we used climatic data recorded by the Spanish Meteorological Agency. Data for the entire study period were only available from Xinestosu meteorological station ( $43^{\circ} 03' 53''$  N,  $06^{\circ} 23' 30''$  W, 1170 m asl). The station is located 15 km away from the study area, in a valley under the same climate. Daily records show a very high correlation ( $R^2 > 97\%$ ) to those obtained in the stations which are closest to the study sites, La Pola and La Peral. Therefore, although absolute values from Xinestosu are not suitable to establish a predictive model, they allow assessing differences among years at the regional level as is our goal.

We divided the year into four seasons of plant activity: (1) overwintering (November - February), which is the unfavourable season in this area with cold temperatures, relatively frequent snow and null plant activity; (2) vegetative period (March - May) with rising temperatures and vegetative plant growth; (3) flowering (June - July), including flower development and pollination; and (4) seed maturation (August - October) up to dispersal. Then we calculated average temperature for each season and year (Table 1).

#### Statistical analyses

To analyse variation in germination data, we fitted a fully factorial Generalized Linear Mixed Model (GLMM, binomial distribution, logit link function) with site, year and stratification as fixed factors and Petri dish as random factor. Afterwards, to test the relationship between germination and temperature we used a variable selection procedure (forward / backwards, Akaike's Information Criterion) to fit a model to germination data (separately for fresh and stratified seeds) with seasonal temperatures and collection site as predictors. We performed statistics with the R-Commander package (v1.5-3) for R (v2.10.1, The R Foundation for Statistical Computing). We also plotted the best predictors for each model using SigmaPlot 12.5 (Systat Software Inc., San José, USA).

#### Results

Dormancy varied among years and sites (Table 1). Germination was significantly lower in fresh ( $6.7\% \pm 1.2$ ) versus stratified ( $79.2\% \pm 2.2$ ) seeds ( $\chi^2 = 298.247$ ,  $p < 0.001$ ). Average germination was significantly

different among years, being higher in 2008 and 2011 ( $63.3\% \pm 4.9$ ), intermediate in 2009 and 2012 ( $31.5\% \pm 4.9$ ) and lower in 2010 ( $6.0\% \pm 2.0$ ) ( $\chi^2 = 28.067$ ,  $p < 0.001$ ). Conversely, collection site had not a significant main effect on germination ( $\chi^2 = 1.801$ ,  $p = 0.185$ ). Stratification had a significant interaction with both year ( $\chi^2 = 17.004$ ,  $p < 0.001$ ) and site ( $\chi^2 = 6.640$ ,  $p = 0.012$ ); as dormancy release was stronger in certain years (e.g. 2012); and La Malva seeds were less dormant when fresh but more dormant after stratification than El Valle seeds. Interaction was also significant between year and site ( $\chi^2 = 7.113$ ,  $P < 0.001$ ); especially as a consequence of year 2008, whose fresh germination was the highest of all years at La Malva but one of the lowest at El Valle. The 3-way interaction was not significant ( $\chi^2 = 0.492$ ,  $p = 0.742$ ). Overall, sizeable fresh germination occurred in 2008, 2009 and 2011; whereas a high alleviation of dormancy by cold stratification occurred in 2008, 2011 and 2012 (Fig. 1). Highest germination thus took place in 2008 and 2011, years with warmer winters (Table 1).

For fresh seeds, the variable selection procedure fitted a model with flowering temperature and collection site as explanatory variables (Table 2a). For stratified seeds, the model dropped collection site and included temperatures of overwintering and flowering (Table 2b). The explained deviance of the stratified model (36%) was higher than that of the fresh model (6%) suggesting a stronger effect of the selected predictors. On one hand, flowering temperature was positively related to dormancy levels of fresh (Fig. 2) and stratified seeds (Fig. 3), producing lower germination with increasing mean temperatures from June and July. However, in fresh seeds this effect was not significant in one of the populations (El Valle,  $\chi^2 = 3.154$ ,  $p = 0.076$ ). On the other hand, overwintering temperature was negatively related to the dormancy levels of stratified seeds (Fig. 3), resulting in higher germination rates with increasing winter year temperatures.

#### Discussion

Our study suggests that wild populations of *C. somedanum* show phenotypic plasticity in seed dormancy along successive years. While similar plasticity has been found in other species (Andersson and Milberg, 1998; Herranz et al., 2010; Schütz and Rave, 2003), to our knowledge this is the first time that inter-annual germination plasticity has been related to inter-annual differences in temperature. Influences of local temperatures on dormancy have usually been tested *in space*, i.e. in different populations at a given year (Fernández-Pascual et al., 2013; Moyer and Lang, 1976; Wagmann et al., 2012); but it seems that the same relationship holds in the field *in time*, i.e. in different years in a given population. We also found that plasticity is stronger for the sensitivity to dormancy-breaking factors (in stratified seeds), while dormancy at dispersal (in fresh seeds) is more heritable. This agrees with our previous experiments with *C. somedanum* seeds grown in a common garden, where an altitudinal dormancy cline detected in the field was strongly maintained at dispersal,

while the sensitivity to dormancy-breaking factors showed more plasticity in response to the seed maturation environment (Fernández-Pascual et al., 2013).

It must be taken into account that the variation we have detected in this study cannot be attributed exclusively to phenotypic plasticity. Sampled individuals were selected at random each year, and thus the slightly different genetic makeup of each seed collection may be driving some of the differences identified. However, differences do not follow a random pattern as would be expected if they were influenced by sampling, but are related to annual temperatures. As the study species is not annual but perennial, and does not flower until its second year, a selection of dormancy genotypes by each year's winter is not likely. Thus we may assume, with the mentioned limitations, that the variation we have detected is mainly caused by phenotypic plasticity.

Our analyses at different phenological stages provide interesting outputs about the potential effect of environmental temperatures within the same year. In years with warmer winters, *C. somedanum* seeds are more sensitive to dormancy breaking treatments and their dormancy release is quicker. This follows the traditional rule of dormancy variation: warmer temperatures, be they in the garden or field, equal less dormant seeds (Chiang et al., 2011; Hoyle et al., 2008; Luzuriaga et al., 2006; Qaderi et al., 2006; Wagmann et al., 2012). Surprisingly, we found the opposing effect for the temperature during flowering, as warmer temperatures during that season determine higher dormancy levels. This contrasts with the mentioned rule, especially with those experiments of controlled seed maturation where warmer maturation environments produced less dormant seeds (Fenner, 1991; Fenner and Thompson, 2005). It must be taken into account that, in the five studied years, overwintering and flowering temperatures were negatively related. Thus, the negative influence of flowering temperatures on dormancy could actually be an indirect detection of overwintering temperatures. The critical point then is to evaluate which one of the two temperature regimes (or both) has a causal effect on dormancy variation. In our previous experiments in a common garden (Fernández-Pascual et al., 2013) we detected a negative effect of flowering temperatures in seeds produced in an environment that was warmer all year through: while the sensitivity to dormancy breaking factors was higher in the common garden than in the field, dormancy at dispersal was also higher, showing the response to a warmer flowering season that our model predicts with seeds collected in the field.

We note that models left a percentage of unexplained variation, showing that temperature is only one of the factors determining phenotypic plasticity in dormancy. Water availability was related to subsequent dormancy levels in controlled garden experiments (Allen and Meyer, 2002; Hoyle et al., 2008; Luzuriaga et al., 2006; Wright et al., 1999), while long-term differences in rainfall influenced dormancy in our study species

(Fernández-Pascual et al., 2013). Similarly, day length during seed maturation has been found to have an effect on dormancy (Fenner, 1991; Fenner and Thompson, 2005). This could explain why La Malva population, placed at the bottom of a shadowy gorge, consistently showed deeper dormancy than sunnier El Valle despite being at a lower altitude. This suggests that potential seed responses to global warming through phenotypic plasticity might be mediated by more factors than the change in temperature.

Nonetheless, the combined effect of overwintering and flowering temperatures has a direct ecological relevance in our study species. *C. somedanum* occurs from low valleys with oceanic influence to high altitude and continental sites in the southern side of the Cantabrian Mountains (Fernández-Pascual et al., 2013; Jiménez-Alfaro et al., 2013). Shallower dormancy would be beneficial in milder winters of the northern valleys, as it could favour earlier germination without the associated risks of winter mortality (Donohue et al., 2010). Colder summers and warmer winters (i.e., low seasonality), which we have found to correlate with shallower dormancy, are characteristic of oceanic low valleys. This plasticity could allow individuals dispersing between continental and oceanic areas to quickly adjust their dormancy phenotype to the more favourable one. Additionally, it might help to cope with some of the expected effects of climate change (Nicotra et al., 2010; Reed et al., 2011; Walck et al., 2011) acclimatizing dormancy loss to new thermal scenarios (Orrù et al., 2012) and avoiding changes in emergence timing (Mondoni et al., 2012).

Despite uncertainties about the causal effect of overwintering and flowering temperatures on the observed patterns, our results highlight that the rule of thumb 'the colder the weather, the deeper seed dormancy' should consider intra-annual variations in temperature. Accurate predictions of climate change effects on plant regeneration by seed will therefore require quantifying how temperatures during plant development are translated into subsequent dormancy levels. Moreover, differential effects of temperature depending on the plant activity season highlight the importance of incorporating more information than average temperatures to assess trait plasticity under climate change.

#### Acknowledgements

C. Álvarez, R. Álvarez, Á. Bueno, V. García, A.I. García, E. Moratón and J.I. Sanzo contributed to seed collection, which was authorized by the Government of Asturias. Two anonymous reviewers improved the manuscript with their comments. The Spanish National Meteorology Agency sold temperature data. Work was supported by the Spanish Ministry of the Environment (*Fundación Biodiversidad* project 2008-bis-ASTERCENT). E.F.P. was supported by the Government of Asturias (Grant BP09-107, Programa de Ayudas Predoctorales 'Severo Ochoa', Plan de Ciencia, Tecnología e Innovación del

Principado de Asturias) and the European Commission (LIFE11/NAT/ES/707-TREMEDAL); B.J.A. by the project 'Employment of Best Young Scientists for International Cooperation Empowerment' (CZ.1.07/2.3.00/30.0037) co-financed by the European Social Fund and the state budget of the Czech Republic.

## References

- Allen, P.S. and Meyer, S.E. (2002) Ecology and ecological genetics of seed dormancy in downy brome. *Weed Science*. 50, 241-247.
- Andersson, L. and Milberg, P. (1998) Variation in seed dormancy among mother plants, populations and years of seed collection. *Seed Science Research*. 8, 29-38.
- Baskin, C.C. and Baskin, J.M. (1998) Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination. San Diego, Academic Press.
- Cochrane, A., Daws, M.I. and Hay, F. (2011) Seed-based approach for identifying flora at risk from climate warming. *Austral Ecology*. 36, 923-935.
- Chiang, G.C., Bartsch, M., Barua, D., Nakabayashi, K., Debieu, M., Kronholm, I., Koornneef, M., Soppe, W.J., Donohue, K. and De Meaux, J. (2011) DOG1 expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in *Arabidopsis thaliana*. *Molecular Ecology*. 20, 3336-3349.
- Daws, M.I., Lydall, E., Chmielorz, P., Leprince, O., Matthews, S., Thanos, C.A. and Pritchard, H.W. (2004) Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe. *New Phytologist*. 162, 157-166.
- Donohue, K., de Casas, R.R., Burghardt, L., Kovach, K. and Willis, C.G. (2010) Germination, postgermination adaptation, and species ecological ranges. pp 293-319 in Futuyma, D.J., Shafer, H.B. and Simberloff, D. (Eds) *Annual Review of Ecology, Evolution, and Systematics* 41. Palo Alto, Annual Reviews.
- Fenner, M. (1991) The effects of the parent environment on seed germinability. *Seed Science Research*. 1, 75-84.
- Fenner, M. and Thompson, K. (2005) *The Ecology of Seeds*. Cambridge, Cambridge University Press.
- Fernández-Pascual, E., Jiménez-Alfaro, B., Caujapé-Castells, J., Jaén-Molina, R. and Díaz, T.E. (2013) A local dormancy cline is related to the seed maturation environment, population genetic composition and climate. *Annals of Botany*. 112, 937-945.
- Fernández-Pascual, E., Jiménez-Alfaro, B., García-Torrico, A.I., Pérez-García, F. and Díaz, T.E. (2012) Germination ecology of the perennial *Centaurium somedanum*, a specialist species of mountain springs. *Seed Science Research*. 22, 199-205.
- Figuerola, R., Herms, D.A., Cardina, J. and Doohan, D. (2010) Maternal environment effects on common groundsel (*Senecio vulgaris*) seed dormancy. *Weed Science*. 58, 160-166.
- Finch-Savage, W.E. and Leubner-Metzger, G. (2006) Seed dormancy and the control of germination. *New Phytologist*. 171, 501-523.
- García-Huidobro, J., Monteith, J.L. and Squire, G.R. (1982) Time, temperature and germination of pearl millet (*Pennisetum typhoides* S. & H.): I. Constant temperature. *Journal of Experimental Botany*. 33, 288-296.
- Gutterman, Y. (2000) Maternal effects on seeds during development. pp 59-84 in Fenner, M. (Ed) *Seeds: The Ecology of Regeneration in Plant Communities*. Wallingford, CABI.
- Hardegee, S.P. (2006) Predicting germination response to temperature. I. Cardinal-temperature models and subpopulation-specific regression. *Annals of Botany*. 97, 1115-1125.
- Herranz, J.M., Copete, M.A., Ferrandis, P. and Copete, E. (2010) Intermediate complex morphophysiological dormancy in the endemic Iberian *Aconitum napellus* ssp. *castellanum* (Ranunculaceae). *Seed Science Research*. 20, 109-121.
- Hoyle, G.L., Steadman, K.J., Daws, M.I. and Adkins, S.I. (2008) Pre- and post-harvest influences on seed dormancy status of an Australian Goodeniaceae species, *Goodenia fascicularis*. *Annals of Botany*. 102, 93-101.
- Hoyle, G.L., Venn, S.E., Steadman, K.J., Good, R.B., McAuliffe, E.J., Williams, E.R. and Nicotra, A.B. (2013) Soil warming increases plant species richness but decreases germination from the alpine soil seed bank. *Global Change Biology*. 19, 1549-1561.
- IPCC (2013) *Climate Change 2013. The Physical Science Basis. Summary for Policymakers*. Geneva, Intergovernmental Panel on Climate Change.
- Jiménez-Alfaro, B., Bueno Sánchez, Á. and Fernández Prieto, J.A. (2005) Ecología y conservación de *Centaurium somedanum* M. Laínz (Gentianaceae), planta endémica de la Cordillera Cantábrica (España). *Pirineos*. 160, 45-67.
- Jiménez-Alfaro, B., Fernández-Pascual, E. and García-Torrico, A.I. (2010) *Centaurium somedanum* M. Laínz. pp 100-101 in Bañares, Á., Blanca, G., Güemes, J., Moreno, J.C. and Ortiz, S. (Eds) *Atlas y libro rojo de la flora vascular amenazada de España. Adenda 2010*. Madrid, Dirección General de Conservación de la Naturaleza.
- Jiménez-Alfaro, B., Fernández Menéndez, S., Bueno Sánchez, Á. and Fernández Prieto, J.A. (2013) Vegetation and hydrogeology along the distribution range of *Centaurium somedanum*, an endemic plant of mountain calcareous springs. *Alpine Botany*. 123, 31-39.
- Luzuriaga, A.L., Escudero, A. and Pérez-García, F. (2006) Environmental effects on seed morphology and germination in *Sinapis arvensis* (Cruciferae). *Weed Research*. 46, 163-174.
- Matesanz, S., Gianoli, E. and Valladares, F. (2010) Global change and the evolution of phenotypic plasticity in plants. *Annals of the New York Academy of Sciences*. 1206, 35-55.
- Mondoni, A., Rossi, G., Orsenigo, S. and Probert, R.J. (2012) Climate warming could shift the timing of seed germination in alpine plants. *Annals of Botany*. 110, 155-164.
- Moyer, J.L. and Lang, R.L. (1976) Variable germination response to temperature for different sources of winterfat seed. *Journal of Range Management*. 29, 320-321.
- Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E., Mathesius, U., Poot, P., Purugganan, M.D., Richards, C. and Valladares, F. (2010) Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*. 15, 684-692.
- Ooi, M.K.J. (2012) Seed bank persistence and climate change. *Seed Science Research*. 22, 53-60.
- Ooi, M.K.J., Auld, T.D. and Denham, A.J. (2009) Climate change and bet-hedging: interactions between increased soil temperatures and seed bank persistence. *Global Change Biology*. 15, 2375-2386.
- Orrù, M., Mattana, E., Pritchard, H.W. and Bacchetta, G. (2012) Thermal thresholds as predictors of seed dormancy release and germination timing: altitude-related risks from climate warming for the wild grapevine *Vitis vinifera* subsp. *sylvestris*. *Annals of Botany*. 110, 1651-1660.
- Pritchard, H.W. and Manger, K.R. (1990) Quantal response of fruit and seed germination rate in *Quercus robur* L. and *Castanea sativa* Mill, to constant temperatures and photon dose. *Journal of Experimental Botany*. 41, 1549-1557.
- Pritchard, H.W., Steadman, K.J., Nash, J.V. and Jones, C. (1999) Kinetics of dormancy release and the high temperature germination response in *Aesculus hippocastanum* seeds. *Journal of Experimental Botany*. 50, 1507-1514.
- Pritchard, H.W., Tompsett, P.B. and Manger, K.R. (1996) Development of a thermal time model for the quantification of dormancy loss in *Aesculus hippocastanum* seeds. *Seed Science Research*. 6, 127-135.
- Probert, R.J. (2000) The role of temperature in the regulation of seed dormancy and germination. pp 261-292 in Fenner, M. (Ed) *Seeds: The Ecology of Regeneration in Plant Communities*. Wallingford, CABI.
- Qaderi, M.M., Cavers, P.B., Hamill, A.S., Downs, M.P. and Bernards, M.A. (2006) Maturation temperature regulates germinability and chemical constituents of Scotch thistle (*Onopordum acanthium*) cypselas. *Canadian Journal of Botany*. 84, 28-38.
- Reed, T.E., Schindler, D.E. and Waples, R.S. (2011) Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conservation Biology*. 25, 56-63.
- Schütz, W. and Rave, G. (2003) Variation in seed dormancy of the wetland sedge, *Carex elongata*, between populations and individuals in two consecutive years. *Seed Science Research*. 13, 315-322.
- Steadman, K.J. and Pritchard, H.W. (2004) Germination of *Aesculus hippocastanum* seeds following cold-induced dormancy loss can be described in relation to a temperature-dependent reduction in base temperature (T<sub>b</sub>) and thermal time. *New Phytologist*. 161, 415-425.

Vleeshouwers, L.M., Bouwmeester, H.J. and Karssen, C.M. (1995) Redefining seed dormancy: an attempt to integrate physiology and ecology. *Journal of Ecology*. 83, 1031-1037.

Wagmann, K., Hautekèete, N.-C., Piquot, Y., Meunier, C., Schmitt, S.E. and Van Dijk, H. (2012) Seed dormancy distribution: explanatory ecological factors. *Annals of Botany*. 110, 1205-1219.

Walck, J.L., Hidayati, S.N., Dixon, K.W., Thompson, K. and Poschold, P. (2011) Climate change and plant regeneration from seed. *Global Change Biology*. 17, 2145-2161.

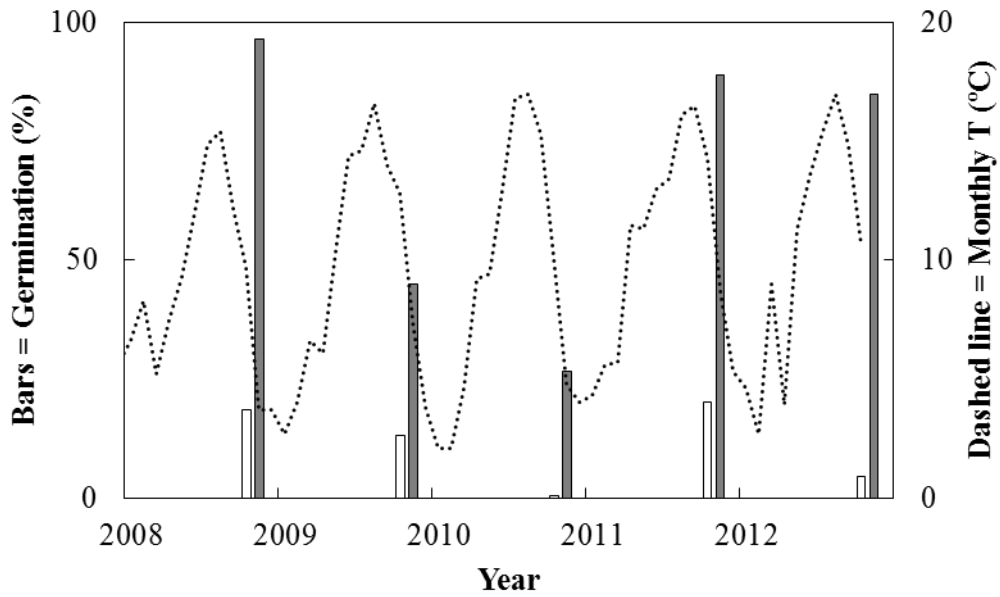
Wright, K.J., Seavers, G.P., Peters, N.C.B. and Marshall, M.A. (1999) Influence of soil moisture on the competitive ability and seed dormancy of *Sinapis arvensis* in spring wheat. *Weed Research*. 39, 309-317.

**Table 1** Temperature and germination in five studied years. Temperatures are the average of the plant activity seasons of overwintering (O), vegetative growth (V), flowering (F) and seed maturation (S); annual average (A) is also given. Germination of fresh and stratified seeds is given for the two populations. Germination data are the mean  $\pm$  SE of four dishes

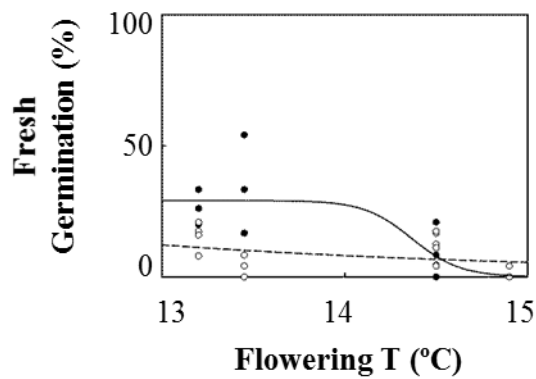
Year	Temperature (°C)					Germination (%)			
						La Malva		El Valle	
	O	V	F	S	A	Fresh	Stratified	Fresh	Stratified
2008	6.8	7.3	13.5	12.4	9.4	34 $\pm$ 8	98 $\pm$ 1	3 $\pm$ 2	95 $\pm$ 1
2009	3.5	7.7	14.5	14.5	9.1	15 $\pm$ 3	37 $\pm$ 2	11 $\pm$ 3	53 $\pm$ 8
2010	3.9	7.7	14.9	14.1	9.2	0	20 $\pm$ 3	1 $\pm$ 1	33 $\pm$ 5
2011	4.7	9.5	13.2	15.6	10.0	24 $\pm$ 4	83 $\pm$ 3	16 $\pm$ 3	95 $\pm$ 1
2012	5.3	8.1	14.5	14.1	9.7	0	72 $\pm$ 3	9 $\pm$ 3	98 $\pm$ 1

**Table 2** Generalized Linear Models (GLM, binomial distribution, logit link) fitted to germination data. Effects appear on the order they were included in the models by the variable selection procedure (forwards/backwards, Akaike's Information Criterion). Flowering T = average temperature during flowering season (June - July); Overwintering T = average temperature during overwintering season (November - February); %V = percentage of variance explained by the model after inclusion of the effect

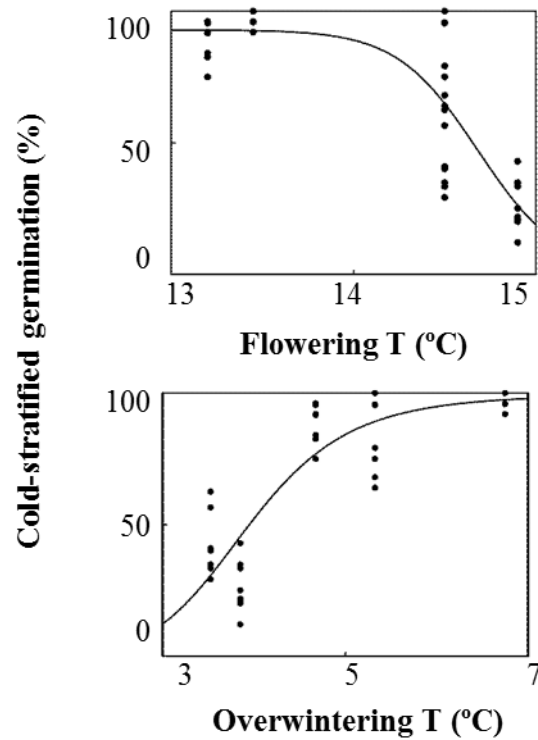
Model	Effect	Coefficient	S.E.	$\chi^2$	p	%V
(a) Fresh AIC=622.4	Intercept	13.441	2.332	33.224	<0.000	
	Flowering T	-1.091	0.169	41.667	<0.000	4.84
	Site [El Valle]	-0.711	0.218	10.602	<0.000	6.00
(b) Stratified AIC=838.5	Intercept	13.888	2.822	24.216	<0.000	
	Overwintering T	0.956	0.116	68.525	<0.000	29.35
	Flowering T	-1.221	0.178	46.923	<0.000	35.52



**Fig. 1** Temperature and germination during the study years. Temperature (dashed line) is the monthly average. Germination of fresh (white bars) and stratified (grey bars) is the average of the two sites. Ticks in the x axis indicate the beginning of each year in January.



**Fig. 2** Effect of flowering temperature on germination of fresh seeds, separated per site (black circles and solid line = La Malva; open circles and dashed line = El Valle). Each circle represents germination % of a Petri dish after 4 weeks of incubation at 22/12 °C. Lines are the logistic functions fitted to data



**Fig. 3** Effect of flowering and overwintering temperatures on germination of cold-stratified seeds, averaging germination data for the two populations (with non-significant differences, see Table 2). Each circle represents germination % of a Petri dish after 4 weeks of incubation at 22/12 °C. Lines are the logistic functions fitted to data.