**Original article: Functional biogeography of the thermal thresholds for post-dispersal embryo growth in *Conopodium majus***

Cristina Blandino1,2, Brith Natlandsmyr3, Sylvi M. Sandvik4, Hugh W. Pritchard1,5, Eduardo Fernández-Pascual6, \*

1 Royal Botanic Gardens, Kew, Wakehurst, Ardingly, Haywards Heath, West Sussex RH17 6TN, UK

2 Department of Biological, Geological and Environmental Science, University of Catania, Catania, Italy

3 Department of Natural History, University Museum, University of Bergen, Bergen, Norway

4 Department of Natural Sciences, University of Agder, Kristiansand, Norway

5 Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, PR China

6 Biodiversity Research Institute (IMIB), University of Oviedo - CSIC - Principality of Asturias, Mieres, Spain <https://orcid.org/0000-0002-4743-9577>

Running title: Functional biogeography of embryo growth

\* Corresponding author: Eduardo Fernández-Pascual, Instituto Mixto de Investigación en Biodiversidad, Campus de Mieres, Edificio de Investigación, 5ª planta, c/ Gonzalo Gutiérrez Quirós s/n, E-33600 Mieres, Spain. Email: fernandezpeduardo@uniovi.es. Telephone: +34985104781.

**ABSTRACT**

* **Background and Aims** Plant regeneration by seeds is driven by a set of physiological traits, many of which show functional intraspecific variation along biogeographic gradients. In many species, germination phenology depends on a germination delay imposed by the need for post-dispersal embryo growth (a.k.a. morphological dormancy). Such growth occurs as a function of environmental temperatures and shows base, optimum and ceiling temperatures (i.e. cardinal temperatures or thermal thresholds). However, the biogeographical variation in such thresholds has not been tested.
* **Methods** We used a thermal time approach and field experiments to assess intraspecific variation at the continental scale in the embryo growth thermal thresholds of the geophyte *Conopodium majus* (Apiaceae) across its distribution from the Iberian Peninsula to Scandinavia.
* **Key Results** Thermal thresholds for embryo growth varied across the latitudinal gradient, with the estimated optimum temperatures between 2.5 and 5.2 ºC, ceiling temperatures between 12 and 20.5 ºC and base temperatures between -6.6 and -2.7 ºC. Germination in the field peaked in the months of January and February. The limiting factor for embryo growth was the ceiling temperature, which was negatively correlated with latitude and the bioclimatic environment of each population. In contrast, the optimal and base temperature were independent of local climate.
* **Conclusions** These results indicate that thermal thresholds for embryo growth are functional ecophysiological traits that drive seed germination phenology and seed responses to soil climatic environment. Therefore, post-dispersal embryo growth can be a key trait impacting climate change effects on phenology and species distributions.

**KEYWORDS**

Cardinal temperatures for germination, Apiaceae, *Conopodium majus,* embryo:endosperm ratio, morphological dormancy, post-dispersal embryo growth, functional seed traits, plant regeneration, intraspecific variation

**INTRODUCTION**

The three aims of functional biogeography are to describe the distribution of functions along environmental gradients and across spatial scales; to use this information to explain the geographic distribution of organisms; and to predict their responses to environmental changes using trait-based predictive models (Violle et al., 2014). A relevant aspect of plant function that has been underutilized by biogeographical studies is the physiological thermal control of plant reproduction (Bykova et al., 2012), and especially seed germination (Rosbakh and Poschlod, 2015). The temperature to which imbibed seeds are exposed affects their germination rate (Fernández-Pascual et al., 2019). This phenomenon can be numerically described by the “cardinal germination temperatures”, i.e., the optimum temperature (To), at which the germination rate is maximal and the base (Tb) and ceiling (Tc) temperatures that are, respectively, estimated to be the coldest and the warmest temperature at which the rate of germination tends to zero. The measurement of these temperatures for a given species enables prediction of its seed germination rate and germination success under different temperature scenarios (Orrù et al., 2012, Fernández-Pascual et al., 2015). Therefore, the cardinal temperatures can be key parameters to explain the contribution of regeneration thermal niches on species distributions and responses to climatic changes (Parmesan and Hanley, 2015, Baskin and Baskin, 2022, Walck et al., 2011, Cochrane et al., 2015).

In many species, however, seed dormancy prevents germination even in the presence of suitable conditions, so that the exposure of seedlings to unfavourable environments is avoided and the timing of germination is matched to favourable regeneration windows (Lamont and Pausas, 2023, Pausas et al., 2022). A particular case of seed germination delay occurs when the embryo, at the time of dispersal, is not completely developed and needs to grow to a critical size before germination can occur; a trait known as post-dispersal embryo growth or morphological dormancy (Baskin and Baskin, 2004, Vandelook et al., 2009b). Such embryo growth is a distinct ecophysiological process controlled by complex interactions between hormones and the seed tissues (Walker et al., 2021). As happens with germination, temperature is a major environmental driver of post dispersal embryo growth (Baskin et al., 2000, Phartyal et al., 2009, Vandelook et al., 2007, Vandelook et al., 2009a, Blandino et al., 2019, Vandelook and Van Assche, 2008), influencing the rate of development of the embryo, a mechanism that allows a precise timing of germination (Porceddu et al., 2017).

Within-species biogeographical variation in several germination parameters is well documented. For example, seeds from warmer sites have been shown to have lower minimum temperatures for germination at different geographical scales (Daws et al., 2004, Rosbakh and Poschlod, 2015). The requirements for cold stratification can also vary according to the local climate: populations from habitats with longer winters require a longer period of cold stratification compared with populations from milder habitats (Allen and Meyer, 1998, Fenner, 1991). However, much less research has been dedicated to traits related to post-dispersal embryo growth. Mondoni et al. (2008) compared post-dispersal embryo growth between mountain and lowland populations of the temperate woodland forb *Anemone nemorosa* and found that, although embryo size at dispersal was similar in all the populations, embryo growth at cool temperatures was faster in the mountain population. This suggests a capacity of post-dispersal embryo growth to adapt to local conditions, either by local adaptation or phenotypic plasticity, analogous to that shown by other seed traits (Fernández-Pascual et al., 2013). However, Porceddu et al. (2017) found no variation in the thermal thresholds for embryo growth in two closely located populations of *Aquilegia barbaricina*. Further research is warranted, to measure the thermal thresholds for post-dispersal embryo growth across large biogeographical scales and investigate whether they vary in association with environmental gradients.

In this study, we assess the functional biogeography of post-dispersal embryo growth in the geophyte *Conopodium majus* (Apiaceae) across its latitudinal distribution, from the Iberian Peninsula to Scandinavia. To our knowledge, this is the first study to quantify the thermal thresholds that regulate embryo growth rate across the whole latitudinal distribution of a species. To do this, we develop a model of embryo growth as a function of temperature to describe its cardinal temperatures for embryo growth. It has been shown that Scottish populations of this species require post-dispersal embryo growth, and that embryo growth and germination occur optimally around 5 °C (Blandino et al., 2019). Such narrow thermal control in a species with a relatively wide latitudinal distribution could make the species’ regeneration vulnerable to climate change (Walck et al., 2011), unless the species shows some functional variation in its embryo growth thermal thresholds. Therefore, we hypothesized that (1) the thermal thresholds for embryo growth will show variation across populations sampled over the species’ latitudinal gradient; and (2) the variation of thermal thresholds will be related to bioclimatic features along the latitudinal gradient. Specifically, we predicted that thermal thresholds for embryo growth would be influenced by high maximum temperatures and drought at the lower latitudes, and by low minimum temperatures at the higher latitudes.

**MATERIALS AND METHODS**

***Study species***

*Conopodium majus* is a geophyte with a European Atlantic distribution from Southern Spain to Central Norway (Tutin et al., 1968). *Conopodium majus* is a member of Apiaceae, a family that originated and diversified in the southern hemisphere, but that today is mostly distributed in the northern hemisphere (Calviño et al., 2016). As is common in *Apiaceae* (Walker et al., 2021, Baskin and Baskin, 2014, Vandelook et al., 2012), seeds of *Conopodium majus* possess undeveloped linear embryos (Martin, 1946) and germinate when they extend to the full length of the endosperm (Blandino et al., 2019). For this reason, in this study, the relative embryo size (i.e. embryo length / endosperm length, hereafter referred to as “E:E ratio”) is used to describe embryo development, and germination is defined as the point at which E:E ratio is ≥ 1. In *Conopodium majus*, germination has been observed to occur both at 0 and 5 °C with a similar rate of embryo growth (Blandino et al., 2019). Such low temperature requirements are indicative of germination in winter. For a species adapted to woodlands as well as oligotrophic grasslands, the control of the germination process in this way can allow seedlings to establish before the development of a tree canopy or of competing vegetation.

***Seed collection***

Mericarps of *Conopodium majus* were collected in the summer of 2016 from nine naturally occurring populations sampled across the western European latitudinal range of the species (Table 1). Since the seed cannot be separated from the fruit in this species, the dispersal unit will be hereafter referred to as the “seed”. A population was sampled only if it consisted of at least 200 individual plants. Seeds were sampled from 50 plants within the population to secure a representative sample of the genetic variability of the population. At least 4000 seeds were collected from each population. All seeds were collected between July and August 2016 and the experiments started within three weeks of seed collection. Seeds were kept at below full hydration under ambient condition on a laboratory bench until the beginning of the tests.

***Initial measurements***

Each collection was cleaned from debris and empty seeds were removed using a gravity seed separator machine. From each population, 10 seeds were selected randomly and allowed to rehydrate overnight at 20 ºC and 100% RH. The seeds were then placed on 1% agar-water for 24 hours to become fully imbibed. Thereafter, seeds were prepared for vital staining with 1% aqueous solution of triphenyl tetrazolium chloride (TZ). A slice of seed coat was removed from the dorsal surface of each seed using a scalpel and seeds were incubated in TZ solution at 30 ºC in the dark for 24 hours. Each seed was then cut longitudinally, and the embryo was extracted. Embryos and endosperms were photographed using a camera (Carl Zeiss Axiocam Colour) mounted on a Stemi SV 11 Microscope (Carl Zeiss, Welwin Garden City, Herts, UK) microscope and their lengths measured using the software Axiovision 3.1.2.1 (Carl Zeiss Vision GmbH). The initial relative embryo length was measured only for the seeds that stained red with the TZ, i.e., indicating viability; unstained seeds / embryos were discarded. Relative embryo size was used because it describes the growth of the embryo regardless of the size of each seed. From each population 99 seeds were placed in a controlled humidity room at 15% RH and left to dry. The dry seed weight of 99 seeds for each population was measured using a precision scale.

***Embryo growth in controlled temperature conditions***

From each population and treatment, 16 subsamples of 15 seeds each were randomly taken and sown in separate, 8 cm diameter Petri dishes containing 1% agar-water substrate. Seeds were sown at -2.5 ºC, 0 ºC, 2.5 ºC, 5 ºC, 7.5 ºC and 10 ºC in incubators with a daily light regime of 12 hours. Every 14 days one subsample from each population and treatment was retrieved and the 15 seeds were placed for 24 hours in 1% TZ solution at 30ºC in the dark, after a slice of the seed coat was removed. From this subsample, the embryo and endosperm length of 10 viable seeds was measured following further dissection of the seed. An E:E value of 1 was assigned to all germinated seeds. Seed measurement was stopped when the seeds ceased germinating. The experiment continued for 224 days, until all the 16 subsample assessments were concluded.

***Calculation of a thermal model for embryo growth***

The mean E:E ratio of 10 seeds for each population x temperature x time combination was calculated. All the temperatures for the same population had the same initial E:E ratio at time = 0, while the maximum value was fixed at 1, after which the seed was able to germinate. Since the data followed a sigmoidal growth distribution, except the treatments at -2.5 °C, a logistic model was fitted to each population x temperature combination using the software OriginLab 9.0. The models of each population were bounded between the initial value of E:E for that population and 1. A linear model was fitted to the -2.5°C treatments. From the equation of the logistic and linear models, it was possible to calculate the time expressed in days (tr) at which each temperature x population combination would have reached the following deciles of relative embryo size: 0.3, 0.4, 0.5, 0.6 and 0.7. Deciles < 0.3 could not be calculated because they were less than the initial E:E. Deciles > 0.7 were not calculated to keep the symmetry of the analyses regarding deciles of the population. For each treatment, the embryo growth rate was calculated as 1/tr.

For each population and decile, embryo growth rate was plotted against temperature. Each dataset was visually divided into sub-optimal and supra-optimal ranges, using the point with the highest value of 1/tr as the dividing point. Liner regressions were fitted separately to the sub- and supra-optimal ranges. The intersection with the temperature axis of the sub-optimal and supra-optimal regression are, respectively, the base (Tb) and the ceiling (Tc) temperatures; these estimates are the temperatures below and above which the embryo growth rate is projected to be zero. The optimal temperature (To), defined as the temperature at which the rate of embryo growth is estimated to be fastest, is the x-coordinate of the intersection point between sub-optimal and supra-optimal regressions. Then, for each population, the cardinal temperatures (Tb, Tc and To) were averaged across all the deciles calculated to define a mean value of the population (Ellis et al., 1986). The regression lines of each decile were recalculated and forced to pass through a common origin defined by the mean Tb (for the sub-optimal regressions) or the mean Tc (for the supra-optimal regressions) (Hardegree, 2006). For the three southernmost populations, only the cardinal temperatures calculated for the relative embryo size of 0.4, 0.5 and 0.6 were used, because it was not possible to fit a supra-optimal regression to the 0.3 decile.

The slopes of these new linear regressions were then taken as a reciprocal to estimate the sub-optimal (θb) and supra-optimal (θc) thermal times for embryo growth. θ, expressed in degree days (°Cd), indicates the cumulative thermal time units above Tb (θb) or below Tc (θc) that the seed must accumulate for the embryo to reach successive E:E deciles. For each population, the deciles were plotted against θb and θc, expressed both as their value and as the natural logarithm of the value, and linear regressions were fitted to the data. The regressions fitted to θ and to log(θ) were compared in each case by their R2 (Hardegree, 2006). The regression models with the highest R2 were chosen to represent the rate of embryo growth as a function of thermal time for each population. The R2 of the models obtained fitting embryo growth and log-normal (log °Cd) were slightly higher than the R2 of the model obtained using normal distributed thermal times (°C). The only exception was constituted by the Spanish population of Central del Chorro (CHO), for which the best model fit was obtained using the non-transformed thermal time values, thus describing a linear increase of relative embryo size with accumulated heat.

***Embryo growth in natural conditions***

Embryo growth in the soil was recorded for three population representing the southern (CHO), middle (Wakehurst Place, UK, “WAK”) and northern (Bergen, Norway, “BER”) distribution of the species. The experiment was replicated in two locations where *Conopodium majus* naturally occurs: at Wakehurst Place, England (site of collection of the “WAK” population); and in a meadow on the periphery of Bergen, Norway (close to the site of collection of the “BER” population). Sixteen subsamples of 20 seeds for each population and experimental site were mixed with 20 g of soil collected at the site and passed through a 3 mm sieve. Seeds and soil were placed in mesh net bags and buried at a depth of 5 cm. A datalogger that recorded soil temperature every 30 min was placed in each location (Tinytag View 2, Gemini Dataloggers Ltd., Chichester. UK and EasyLog USB-2, Lascar Electronics, in Norway). The seeds were buried in England on 1st September 2016 and in Norway on 14th September 2016. Every 14 days a bag for each population was retrieved and the soil washed. Seed bags buried in Norway were shipped to England for measurements. All the seeds retrieved were prepared for TZ staining and their embryo and endosperm lengths measured. It was easiest to measure the seeds when most of the seeds were not germinated. With an increasing number of germinated seeds and seedlings, the number of empty seed coats left in the soil bags made it difficult to distinguish between mouldy or germinated seeds. At this point, the experiment was terminated, representing nine measurements in Norway and thirteen in England.

***Validation of the thermal time model with field data***

To compare the embryo growth predicted by the thermal time model with embryo growth in natural conditions, embryo growth in the field sites was plotted against time. A logistic regression was fitted to these curves, and from the equations, the tr to reach every decile of relative embryo growth was calculated. The units of thermal time required by each population to reach every tr during the field experiment were calculated for both field locations using the data recorded by the loggers. To account for every temperature fluctuation during the day, the thermal time was expressed in “°C 30 min” and the heat accumulated by the seed was calculated for every 30 min temperature record. The difference (ΔT) between each temperature record and the population To (averaged between deciles) was summed. When the temperature was higher than the mean Tc or lower than the mean Tb the heat accumulated was considered = 0 and the difference (ΔT) between each temperature record and the To was summed.

The time necessary in the field to accumulate enough heat to reach the thermal time necessary for each tr was compared with the tr estimated from the embryo growth data. The time (in days) needed to sum enough heat to reach the θTb and θTc calculated in the model, for each tr decile (tr model) in each population was compared with the time needed by each population to reach the same decile of relative embryo growth in the field (tr field). These estimates were then graphically compared expressing the different tr in function of E:E.

***Relationship between embryo growth and germination***

Germination was scored for each independent sample before measuring the relative embryo size and expressed as percentage of germinated seeds vs time. For each population, the germination data for the treatments at 2.5 and 5°C were fitted with the Boltzmann equation using the software OriginLab9. The other temperatures were not used because germination was too slow. For each population, from the fitted Boltzmann equation the day to reach 50% germination (tg50) was calculated. The tg50 was then used to calculate the corresponding E:E ratio at the same day using the logistic regression of the E:E data for the same treatment. For each population, the mean E:E ratio corresponding to the tg50 for germination at the two temperatures used was displayed as the mean E:E ratio for 50% germination in that population. The mean between all the populations represented the mean for the species.

***Relationship between environmental data and germination parameters***

The relationship between embryo development and seed germination parameters and geographical and bioclimatic data was explored for each population. A data matrix was built including latitude, altitude, mean annual temperature, precipitation of the driest month, mean maximum temperature of the hottest month and minimum mean temperature of the coldest month, seed dry mass, initial E:E ratio and cardinal temperatures for each population. Climatic data for the seed collection sites was extracted from WorldClim (Fick and Hijmans, 2017). Data was checked for autocorrelation using the Pearson correlation coefficient to exclude the variables with a strong autocorrelation. Finally, a PCA was run on the dataset, scaling the axis.

**RESULTS**

***Initial embryo length and seed mass***

The initial relative embryo size ranged from a mean value of 0.10 (±0.03 SD) for the population BER to a mean value of 0.19 (±0.03 SD) for the population CHO (Table 2). Mean seed dry mass ranged just under two-fold from 1.21 mg (±0.38 SD) in TRE to 2.03 mg (±0.53 SD) in FLE (Table 2).

***Embryo growth in controlled temperature conditions***

The rate of embryo growth was strictly dependent on the temperature and the increase in embryo size could be appreciated already after 14 days of sowing for germination. For all the populations, the temperature treatments with the highest rate of embryo growth were 2.5 and 5 °C. Clearly 0 °C was sub-optimal for embryo growth rate, and 7.5 and 10 °C were supra-optimal (Fig.1). The seeds survived cooling to -2.5 ºC but the embryo did not grow at this temperature.

***Relationship between embryo growth and germination***

The first germination was scored after 84 days of imbibition in the four Spanish populations at temperatures of 0, 2.5 and 5 °C. The populations from WAK and BER first germinated after 112 days of imbibition. The last population to begin germinating was SCO, after 126 days of imbibition. Germination occurred when the embryo reached the same length of the endosperm (E:E = 1) and a mean E:E = 1 corresponded to 100% germination in the sample. The treatments that had the highest mean germination across all the populations after 32 weeks of imbibition were 2.5 °C, and 5 °C with, respectively, 97.7 and 98.4 % of seeds germinated at the last sampling. The lowest germination was observed at -2.5 and 10 °C. The population that reached, across all the treatments, the highest mean germination at week 32 (the end of the experiment), was TRE (80% ± 32 SD) while the lowest was achieved by SCO (59%, ± 42 SD). The time to reach 50 % germination (Tg50), interpolated with the Boltzmann equation ranged between 111 (BAS) and 147 days (FLE) at 2.5 °C and between 116 (LEO) and 150 days (SCO) at 5 °C. The values of E:E corresponding to the estimated T50 in these two treatments were averaged between population and temperatures to describe a value of 0.89 (± 0.02 SD) for the species.

***Cardinal temperatures for embryo growth***

Between populations, Tb estimates varied between -2.63 (SCO) and -6.65 °C (BER). In addition, To varied from 2.54 (LEO) and 5.23 °C (CHO). Finally, Tc was between 12.08 (BER) and 20.54 °C (TRE) (Table 2).

***Embryo growth in natural conditions***

The minimum temperature recorded in Norway in winter was -2 °C in mid-November while the highest (18.5 °C) was recorded at the beginning of the experiment, on 15th September 2016. In England the minimum temperature recorded was 1.6 °C at the end of January and the maximum 17.0 °C, recorded on the same day as the Norwegian site, during an autumn heat wave. Embryo growth in natural condition was faster, for all the population tested, in the northern most location of Bergen (BER) where daily mean temperatures were lower than at Wakehurst (WAK), UK. However, in both sites the rate of embryo growth started to increase when the temperatures fell below 10 °C (Fig. 2). Even if the southern population (CHO) had the greater initial E:E ratio, its growth rate was not different from the other populations tested. Eventually, the three growth curves tended to converge when a mean E:E ratio approached 0.8 (Fig. 2). Germination in nature tended to peak in the months of January and February. Fitting a logistic regression to the curves permitted an estimation of the time, in days, to reach different deciles of relative embryo size.

***Comparison of the model with field data***

The comparison of the thermal models against estimates of embryo growth in the field gave different results between the three populations but was consistent between experimental sites (Fig. 3). Estimates of time to reach successive deciles of E:E ratio were similar if calculated using the ƟTb and ƟTc of the WAK population for both sites but higher than the Trg estimated from the logistic regression of embryo growth in the field. The BER population shown a rate of embryo growth that could be better predicted by the ƟTc rather than by ƟTb while both models diverged from the observed pattern of embryo growth in the southern population CHO.

***Environmental correlates of embryo growth traits***

A PCA (Fig. 4) ordered the populations according to their seed and germination parameters and to the climate of the collection site. The first axis, that explained 50% of the variability in the data, separated the southern populations from the northern ones. The axis was described mostly by geographic and bioclimatic variables and the only seed traits that had a significant correlation with it were seed dry mass and the ceiling temperature for embryo growth (Tc). In particular, there was a strong negative correlation between precipitation of the driest month and Tc. Mountain populations of *Conopodium majus* were in the southern portion of the distribution range of the species and were characterized by higher maximum temperatures and more severe drought stress. The seeds from these populations had a lower dry mass but a greater initial relative embryo size than the northern, lowland populations. The second axis explained 18.8 % of the variability in the data and had a significant correlation only with Tb and To. The two cardinal temperatures showed opposite trends, such that a higher optimum corresponded to a lower Tb. The two southern most populations, CHO and TRE, remained separated from the others: they came from the highest altitude and are exposed to the strongest heat and drought stress. The remaining populations can be separated in three groups: SCO, BAS and LEO had the highest Tb, HER and FLE the biggest seeds and BER and WAK had the higher To.

**DISCUSSION**

*Conopodium majus* shows considerable intraspecific variability in the thermal thresholds for post-dispersal embryo growth along its European distribution. Additionally, variability in post-dispersal embryo growth thresholds appears related to the climate of the collection sites, with warmer and drier sites correlating with a capacity for embryos to grow at warmer temperatures. These results indicate that there is functional variation in the embryo growth thermal thresholds, and therefore that these thresholds might be functional ecophysiological traits.To our knowledge, this is the first work showing biogeographical variation in the thermal thresholds for embryo growth. A previous study had found no variation in embryo thresholds between closely located populations of the Ranunculaceae species *Aquilegia barbaricina*, even if the same populations showed variation in the thermal thresholds for germination (Porceddu et al., 2017).

The ceiling temperature for embryo growth varied between 12 and 21 °C and had a strong negative correlation with latitude and precipitation. Populations from northern and wetter locations had lower ceilings; while the two southernmost populations (CHO and TRE) had a ceiling above 20 °C. Interestingly, the two southernmost populations (CHO and TRE) belong to the subspecies *Conopodium majus* subsp. *marizianum*, while the others belong to the typical subspecies *Conopodium majus* subsp. *majus* (the LEO population grows in the contact area between the two subspecies). *Conopodium majus* subsp. *marizianum* and all the other species in the genus are restricted to the Iberian Peninsula and northern Africa, with the only exception of *Conopodium majus* subsp. *majus,* whichis the only taxon to be widely distributed in western and northern Europe (Mateo and López Udias, 2003). This may suggest that the lower ceiling temperature for embryo growth in *Conopodium majus* subsp. *majus* played a key role in allowing the subsp. to colonize colder regions, a hypothesis that should be explored with broader comparative studies in the genus. From a functional point of view, the correlation between the ceiling temperature and precipitation reflects the general role of drought and frost as the major drivers of regeneration strategies (Jurado and Flores, 2005). A higher ceiling temperature in *Conopodium majus* subsp. *marizianum* would allow embryo growth during late summer and autumn, and therefore seedling emergence during winter, the season of lowest water stress in the Mediterranean mountains where the subspecies lives. Conversely, the lower ceiling temperature in *Conopodium majus* subsp. *majus* would retard embryo growth until winter, and lead to seedling emergence in early spring, a more favourable regeneration environment in cold-limited regions. Moreover, embryo growth (and the potential to germinate) under cold temperatures (close to 0°C) would enable the start of growth during winter and emergence around the time of snowmelt, a behaviour that has been described in sub-alpine meadows where *Conopodium majus* can be found (Fernández-Pascual et al., 2017, Shimono and Kudo, 2005). This could give the seedling an early start that can result in a competitive edge, making full use of a short growing season. In another frequent habitat of *Conopodium majus*, forest understoreys, early seedling emergence is a common trait in herbaceous species because it allows the young plant to start photosynthesis before the tree canopy closes.

The optimum temperature for embryo growth ranged between 2.5 and 5 °C (Table 2). This is a very low optimal temperature when compared to the optimal temperatures for germination found in other species: in the review by Dürr et al. (2015), the mean optimal temperature for germination across species was 27 ºC, with the lowest value being 7 ºC in the tree *Acer saccharum* (McCarragher et al., 2011). In the case of the Apiaceae crops *Apium graveolens* and *Daucus carota*, optimal embryo growth or germination occurred at 20-25 ºC (Finch-Savage et al., 1998, Rowse and Finch-Savage, 2003, Walker et al., 2021), but it has been shown that crops tend to have higher thermal threshold for germination than wild species (Dürr et al., 2015). In the future, it would be interesting to explore whether *Conopodium majus* is an outlier, or embryo growth temperatures tend to be lower than the temperatures for seed germination across species. It is also worth noting that, in our case, To had a negative correlation with Tb, a phenomenon already observed for germination temperatures by Dürr et al. (2015). In other words, the populations with the higher Tb (BAS, LEO and SCO) also have the lower To, and therefore they have a narrower window of suboptimal conditions for embryo growth. In practical terms, these populations are at greater risk from climate change, which could produce a mismatch between their narrow germination niche and soil temperatures (Walck et al., 2011, Orrù et al., 2012, Maleki et al., 2024).

All the populations considered are estimated to have a negative base temperature for embryo growth, ranging from -6.7 °C in BER to -2.7 in BAS. Although it cannot be discarded that such low values are an artefact of the thermal time modelling approach, values of Tb lower than zero have been reported for some temperate trees, crops (mainly legumes) and wild plants but are not common (Dürr et al., 2015). However, to our knowledge, no values as low as -6.7 °C have been reported previously, the lowest being a Tb of -3.9 °C for *Cryptantha minima* (Boraginaceae) (Wei et al., 2009) and -4.5 °C for *Krascheninnikovia lanata* (Amaranthaceae) (Wang et al., 2006). The germination of *Cryptantha minima* at negative temperatures was explained as an adaptation to take advantage of the water of the snowmelt in early spring and develop its annual cycle before the summer drought (Wang et al., 2006). In the case of *Conopodium majus*, that is a perennial, this strategy could, however, offer some advantage at the southern range of its distribution, where summer drought can be a recurrent issue, as already observed for Mediterranean subalpine species (Fernández-Pascual et al., 2017). *Krascheninnikovia lanata* seeds show a positive effect of seed size on the ability to germinate at sub-zero temperatures (Wang et al., 2006). The authors demonstrate that bigger seeds had a higher concentration of sugars (glucose, raffinose and sucrose) that probably lower the freezing point of the seed tissues. There are no reports on sub-zero germination in Apiaceae but an optimal temperature for embryo growth of 2 °C has been described for *Heracleum sphondylium* (Stokes, 1953) and it is not unlikely that this species, or others from the same family, could present equally low Tb for embryo growth. However, in practical terms, it is unlikely that such low temperatures do have a functional ecological role in embryo growth in the field: our field collected data and averaged climatic data from 1970-2000 for the collection sites show that such low mean temperatures are rare in the natural environment of *Conopodium majus.* Therefore, in the field it is likely that embryo growth is possible throughout the winter season and is limited only by the higher temperatures (and the ceiling threshold) in autumn. In fact, results from the ordination analysis, showed that Tb is independent from climatic and geographic factors and is not even correlated to seed size or initial E:E ratio. Therefore, we can conclude that the limiting factor for embryo growth in *Conopodium majus* is the ceiling temperature and its interaction with warmer temperatures during the annual cycle.

Our thermal model outputs are supported by the results of the field sowings, although these should be taken with caution since we were not able to perform field sowing at the southernmost distribution of our experiment, in central Spain. The responses of different populations to the warmer, drier sites in central Spain could provide useful data for predicting possible effects of a warming climate on seed regeneration in *Conopodium majus*. A most interesting follow up study would involve reciprocal transplants of populations across the latitudinal gradient of the species, to establish how much of the variation measured and modelled is genetic and how much is due to phenotypic plasticity (Franks et al., 2014). This would inform on the role for adaptation in any species' response to environmental change through seed regeneration.

**CONCLUSION**

Species responses to climate determine how plants cope with ongoing global change. Parmesan and Hanley (2015) identified three key issues for climate change research on plants: changing phenology, changing distributions and the role of plasticity and adaptation. Our study suggests that the thermal thresholds for post-dispersal embryo growth may have functional relevance for these three issues. The dependence of embryo growth on a relatively low ceiling temperature means that warmer winter temperatures could slow down post dispersal embryo growth and potentially shift the emergence phenology from one season to another, as has been described in alpine systems in relation to another seed process, physiological dormancy release (Mondoni et al., 2012). Regarding distribution, we have found that two vicariant subspecies show diverging thermal thresholds for embryo growth. This supports the concept that seed ecophysiology can be a major driver of the distribution of taxa (Bykova et al., 2012), and of species migrations as a response to climate change (Walck et al., 2011, Baskin and Baskin, 2022). At the same time, our study also shows intraspecific variation in the embryo thermal thresholds, and therefore the potential for plasticity and/or adaptation to changes in the climatic environment (Cochrane et al., 2015, Nicotra et al., 2010, Franks et al., 2014).

To date, most studies on the responses of seeds to climate have focused on germination. To complete the picture, our study highlights the importance of post-dispersal embryo growth, showing intraspecific variation in this key ecophysiological trait across the latitudinal distribution of a species. Thus, embryo growth seems to be one of the many ways by which plants interact with changing soil temperatures (Amstutz et al., 2024). We make the case for more ecological, evolutionary and comparative studies on post-dispersal embryo growth, fully incorporating this trait into the research agenda on plant regeneration (Saatkamp et al., 2019) and plant responses to climate change (Parmesan and Hanley, 2015).

**AKNOWLEDGEMENTS**

Álvaro Bueno Sánchez, Joseba Garmendia, Luis Carlón, Giles Laverack and Maria Marin helped with seed collection.

**FUNDING**

This research received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n°607785.

**CONFLICT OF INTEREST STATEMENT**

The authors declare no conflict of interest.

**AUTHOR CONTRIBUTIONS**

**Cristina Blandino**: Conceptualization; Methodology; Investigation; Data Curation; Formal Analysis; Visualization; Writing – Original Draft Preparation; Writing – Review & Editing. **Brith Natlandsmyr**: Investigation; Writing – Review & Editing. **Sylvi M. Sandvik**: Investigation; Writing – Review & Editing. **Hugh W. Pritchard**: Funding acquisition; Conceptualization; Methodology; Writing – Review & Editing. **Eduardo Fernández-Pascual**: Conceptualization; Methodology; Investigation; Writing – Review & Editing.

**DATA AVAILABILITY STATEMENT**

Upon acceptance, all data will be deposited into Zenodo.

**LITERATURE CITED**

**Allen PS, Meyer SE.** **1998**. Ecological aspects of seed dormancy loss. *Seed Science Research,* **8**: 183-191.

**Amstutz A, Firth LB, Spicer JI, De Frenne P, Gómez-Aparicio L, Graae BJ, Kuś S, Lindmo S, Orczewska A, Rodríguez-Sánchez F, Vangansbeke P, Vanneste T, Hanley ME.** **2024**. Taking sides? Aspect has limited influence on soil environment or litter decomposition in pan-European study of roadside verges. *Pedobiologia,* **102**: 150927.

**Baskin CC, Baskin JM.** **2014**. *Seeds. Ecology, Biogeography and Evolution of Dormancy and Germination. Second Edition.* San Diego: Academic Press.

**Baskin CC, Baskin JM.** **2022**. *Plant regeneration from seeds: A global warming perspective.* San Diego: Academic Press.

**Baskin CC, Milberg P, Andersson L, Baskin JM.** **2000**. Deep complex morphophysiological dormancy in seeds of *Anthriscus sylvestris* (Apiaceae). *Flora,* **195**: 245-251.

**Baskin JM, Baskin CC.** **2004**. A classification system for seed dormancy. *Seed Science Research,* **14**: 1-16.

**Blandino C, Fernández-Pascual E, Marin M, Vernet A, Pritchard HW.** **2019**. Seed ecology of the geophyte *Conopodium majus* (Apiaceae), indicator species of ancient woodland understories and oligotrophic meadows. *Plant Biology,* **21**: 487-497.

**Bykova O, Chuine I, Morin X, Higgins SI.** **2012**. Temperature dependence of the reproduction niche and its relevance for plant species distributions. *Journal of Biogeography,* **39**: 2191-2200.

**Calviño CI, Teruel FE, Downie SR.** **2016**. The role of the Southern Hemisphere in the evolutionary history of Apiaceae, a mostly north temperate plant family. *Journal of Biogeography,* **43**: 398-409.

**Cochrane A, Yates CJ, Hoyle GL, Nicotra AB.** **2015**. Will among-population variation in seed traits improve the chance of species persistence under climate change? *Global Ecology and Biogeography,* **24**: 12-24.

**Daws MI, Lydall E, Chmielarz P, Leprince O, Matthews S, Thanos CA, Pritchard HW.** **2004**. Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe. *New Phytologist,* **162**: 157-166.

**Dürr C, Dickie JB, Yang XY, Pritchard HW.** **2015**. Ranges of critical temperature and water potential values for the germination of species worldwide: Contribution to a seed trait database. *Agricultural and Forest Meteorology,* **200**: 222-232.

**Ellis RH, Covell S, Roberts EH, Summerfield RJ.** **1986**. The influence of temperature on seed germination rate in grain legumes: II. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. *Journal of Experimental Botany,* **37**: 1503-1515.

**Fenner M.** **1991**. The effects of the parent environment on seed germinability. *Seed Science Research,* **1**: 75-84.

**Fernández-Pascual E, Jiménez-Alfaro B, Bueno A.** **2017**. Comparative seed germination traits in alpine and subalpine grasslands: Higher elevations are associated with warmer germination temperatures. *Plant Biology,* **19**: 32-40.

**Fernández-Pascual E, Jiménez-Alfaro B, Caujapé-Castells J, Jaén-Molina R, Díaz TE.** **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, Mattana E, Pritchard HW.** **2019**. Seeds of future past: Climate change and the thermal memory of plant reproductive traits. *Biological Reviews,* **94**: 439-456.

**Fernández-Pascual E, Seal CE, Pritchard HW.** **2015**. Simulating the germination response to diurnally alternating temperatures under climate change scenarios: Comparative studies on *Carex diandra* seeds. *Annals of Botany,* **115**: 201-209.

**Fick SE, Hijmans RJ.** **2017**. WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology,* **37**: 4302-4315.

**Finch-Savage WE, Steckel JRA, Phelps K.** **1998**. Germination and post-germination growth to carrot seedling emergence: predictive threshold models and sources of variation between sowing occasions. *New Phytologist,* **139**: 505-516.

**Franks SJ, Weber JJ, Aitken SN.** **2014**. Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications,* **7**: 123-139.

**Hardegree SP.** **2006**. Predicting germination response to temperature. I. Cardinal-temperature models and subpopulation-specific regression. *Annals of Botany,* **97**: 1115-1125.

**Jurado E, Flores J.** **2005**. Is seed dormancy under environmental control or bound to plant traits? *Journal of Vegetation Science,* **16**: 559-564.

**Lamont BB, Pausas JG.** **2023**. Seed dormancy revisited: Dormancy-release pathways and environmental interactions. *Functional Ecology,* **37**: 1106-1125.

**Maleki K, Soltani E, Seal CE, Colville L, Pritchard HW, Lamichhane JR.** **2024**. The seed germination spectrum of 486 plant species: A global meta-regression and phylogenetic pattern in relation to temperature and water potential. *Agricultural and Forest Meteorology,* **346**: 109865.

**Martin AC.** **1946**. The comparative internal morphology of seeds. *The American Midland Naturalist,* **36**: 513-660.

**Mateo G, López Udias S.** **2003.** *Conopodium* W.D.J. Koch. In: Nieto Feliner G, Jury SL, Herrero Nieto A, eds. *Flora iberica Vol. X. Araliaceae-Umbelliferae*: 168-181. Madrid: Real Jardín Botánico, CSIC.

**McCarragher SR, Goldblum D, Rigg LS.** **2011**. Geographic variation of germination, growth, and mortality in sugar maple (*Acer saccharum*): Common garden and reciprocal dispersal experiments. *Physical Geography,* **32**: 1-21.

**Mondoni A, Probert R, Rossi G, Hay F, Bonomi C.** **2008**. Habitat-correlated seed germination behaviour in populations of wood anemone (*Anemone nemorosa* L.) from northern Italy. *Seed Science Research,* **18**: 213-222.

**Mondoni A, Rossi G, Orsenigo S, Probert RJ.** **2012**. Climate warming could shift the timing of seed germination in alpine plants. *Annals of Botany,* **110**: 155-164.

**Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F.** **2010**. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science,* **15**: 684-692.

**Orrù M, Mattana E, Pritchard HW, 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.

**Parmesan C, Hanley ME.** **2015**. Plants and climate change: complexities and surprises. *Annals of Botany,* **116**: 849-864.

**Pausas JG, Lamont BB, Keeley JE, Bond WJ.** **2022**. Bet-hedging and best-bet strategies shape seed dormancy. *New Phytologist,* **236**: 1232-1236.

**Phartyal SS, Kondo T, Baskin JM, Baskin CC.** **2009**. Temperature requirements differ for the two stages of seed dormancy break in *Aegopodium podagraria* (Apiaceae), a species with deep complex morphophysiological dormancy. *American Journal of Botany,* **96**: 1086-95.

**Porceddu M, Mattana E, Pritchard HW, Bacchetta G.** **2017**. Dissecting seed dormancy and germination in *Aquilegia barbaricina*, through thermal kinetics of embryo growth. *Plant Biology,* **19**: 983-993.

**Rosbakh S, Poschlod P.** **2015**. Initial temperature of seed germination as related to species occurrence along a temperature gradient. *Functional Ecology,* **29**: 5-14.

**Rowse HR, Finch-Savage WE.** **2003**. Hydrothermal threshold models can describe the germination response of carrot (*Daucus carota*) and onion (*Allium cepa*) seed populations across both sub- and supra-optimal temperatures. *New Phytologist,* **158**: 101-108.

**Saatkamp A, Cochrane A, Commander L, Guja LK, Jimenez-Alfaro B, Larson J, Nicotra A, Poschlod P, Silveira FAO, Cross AT, Dalziell EL, Dickie J, Erickson TE, Fidelis A, Fuchs A, Golos PJ, Hope M, Lewandrowski W, Merritt DJ, Miller BP, Miller RG, Offord CA, Ooi MKJ, Satyanti A, Sommerville KD, Tangney R, Tomlinson S, Turner S, Walck JL.** **2019**. A research agenda for seed-trait functional ecology. *New Phytologist,* **221**: 1764-1775.

**Shimono Y, Kudo G.** **2005**. Comparisons of germination traits of alpine plants between fellfield and snowbed habitats. *Ecological Research,* **20**: 189-197.

**Stokes P.** **1953**. A physiological study of embryo development in *Heracleum sphondylium* L.: III. The effect of temperature on metabolism. *Annals of Botany,* **17**: 157-174.

**Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA.** **1968**. *Flora Europaea. Volume 2. Rosaceae to Umbelliferae.* Cambridge: Cambridge University Press.

**Vandelook F, Bolle N, Van Assche JA.** **2007**. Seed dormancy and germination of the european *Chaerophyllum temulum* (Apiaceae), a member of a trans-atlantic genus. *Annals of Botany,* **100**: 233-239.

**Vandelook F, Bolle N, Van Assche JA.** **2009a**. Morphological and physiological dormancy in seeds of *Aegopodium podagraria* (Apiaceae) broken successively during cold stratification. *Seed Science Research,* **19**: 115-123.

**Vandelook F, Janssens SB, Probert RJ.** **2012**. Relative embryo length as an adaptation to habitat and life cycle in Apiaceae. *New Phytologist,* **195**: 479-487.

**Vandelook F, Lenaerts J, Van Assche Jozef A.** **2009b**. The role of temperature in post-dispersal embryo growth and dormancy break in seeds of *Aconitum lycoctonum* L. *Flora - Morphology, Distribution, Functional Ecology of Plants,* **204**: 536-542.

**Vandelook F, Van Assche JA.** **2008**. Deep complex morphophysiological dormancy in *Sanicula europaea* (Apiaceae) fits a recurring pattern of dormancy types in genera with an Arcto-Tertiary distribution. *Botany,* **86**: 1370-1377.

**Violle C, Reich PB, Pacala SW, Enquist BJ, Kattge J.** **2014**. The emergence and promise of functional biogeography. *Proceedings of the National Academy of Sciences,* **111**: 13690-13696.

**Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P.** **2011**. Climate change and plant regeneration from seed. *Global Change Biology,* **17**: 2145-2161.

**Walker M, Pérez M, Steinbrecher T, Gawthrop F, Pavlović I, Novák O, Tarkowská D, Strnad M, Marone F, Nakabayashi K, Leubner-Metzger G.** **2021**. Molecular mechanisms and hormonal regulation underpinning morphological dormancy: a case study using *Apium graveolens* (Apiaceae). *The Plant Journal,* **108**: 1020-1036.

**Wang R, Bai Y, Low NH, Tanino K.** **2006**. Seed size variation in cold and freezing tolerance during seed germination of winterfat (*Krascheninnikovia lanata*) (Chenopodiaceae). *Canadian Journal of Botany,* **84**: 49-59.

**Wei Y, Bai Y, Henderson DC.** **2009**. Critical conditions for successful regeneration of an endangered annual plant, *Cryptantha minima*: A modeling approach. *Journal of Arid Environments,* **73**: 872-875.

**TABLES**

**Table 1:** Provenance of seeds used in the experiments.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Population | Country | Location | Latitude | Longitude | Elevation (m a.s.l.) |
| HER | Norway | Herdla | 60º 34'29.784'' N | 4º 56' 53.627'' E | 37 |
| BER | Norway | Bergen | 60º 20' 7.35 N | 5º 22' 17.79'' E | 97 |
| FLE | Norway | Flekkeroya | 58º 4'5.34'' N | 7º 59' 53.56'' E | 19 |
| SCO | UK | Dalreoch Farm | 56º 44' 47.36'' N | 3º 32' 25.03'' W | 252 |
| WAK | UK | Wakehurst Place | 51º 04' 12.79'' N | 0º 05' 28.28'' W | 114 |
| BAS | Spain | Ondarre | 43º 01' 42.8'' N | 2º 03' 55.7'' W | 809 |
| LEO | Spain | El Tendero | 42º 54' 26,62'' N | 5º 49' 25,87'' W | 1426 |
| CHO | Spain | Central del Chorro | 40º 18' 26.17'' N | 5º 40' 09.39'' W | 1398 |
| TRE | Spain | Tremedal | 40º 22' 00.5'' N | 5º 37' 57.20'' W | 1555 |

**Table 2:**Initial E:E , seed dry mass and cardinal temperatures averaged between deciles (all as mean ± SD) in seeds of all populations of *Conopodium majus* studied. To have a symmetric result around the middle value, when the lower deciles were excluded for being too close to the initial embryo size, the higher ones were excluded too.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Population | Initial E:E | Seed dry weight (mg) | Tb (°C) | To (°C) | Tc (°C) | Deciles used |
| HER | 0.12 ±0.03 | 1.94 ±0.58 | -4.01 ± 0.57 | 4.26 ± 0.80 | 12.90 ± 1.86 | 0.3 - 0.7 |
| BER | 0.10 ±0.03 | 1.80 ±0.55 | -6.65 ± 0.62 | 4.58 ± 0.02 | 12.08 ± 1.32 | 0.3 - 0.7 |
| FLE | 0.17 ±0.04 | 2.03 ±0.53 | -3.90 ± 0.14 | 4.50 ± 0.07 | 13.70 ± 0.71 | 0.3 - 0.7 |
| SCO | 0.13 ±0.02 | 1.63 ±0.54 | -2.63 ± 0.38 | 2.80 ± 0.25 | 14.42 ± 2.47 | 0.3 - 0.7 |
| WAK | 0.10 ±0.02 | 1.75 ±0.42 | -6.20 ± 0.89 | 4.59 ± 0.11 | 14.44 ± 1.72 | 0.3 - 0.7 |
| BAS | 0.15 ±0.02 | 1.43 ±0.59 | -2.75 ± 0.10 | 2.69 ± 0.10 | 13.07 ± 0.93 | 0.3 - 0.7 |
| LEO | 0.12 ±0.03 | 1.66 ±0.52 | -3.17 ± 0.06 | 2.54 ± 0.03 | 14.64 ± 2.23 | 0.4 - 0.6 |
| CHO | 0.19 ±0.03 | 1.24 ±0.38 | -4.09 ± 0.59 | 5.23 ± 1.05 | 20.48 ± 9.09 | 0.4 - 0.6 |
| TRE | 0.11 ±0.02 | 1.21 ±0.38 | -6.47 ± 0.41 | 4.86 ± 0.04 | 20.54 ± 7.25 | 0.4 - 0.6 |

**FIGURES**

**Fig. 1:** Patterns of embryo growth (E:E ratio) for all the seed populations of *Conopodium majus* and all temperatures tested. Each data point represents the mean of ten replicates (± SE).

**Fig. 2:** Embryo growth in the field for buried seeds of *Conopodium majus*. Each data point represents the mean E:E ratio for 20 seeds of three representative populations: CHO for the southern edge of the distribution range, WAK for the middle and BER for the northern; soil temperature is also shown. The burial experiment was performed in England (on the collection site of the WAK population) and in Norway (on the collection site of BER population).The experiment started on 1st September 2016 in England and on 15th September 2016 in Norway . For each site, the experiment finished when all population reached 100% radicle emergence (corresponding to E:E =1).

**Fig. 3:**Time (in days) required by each population of *Conopodium majus* seeds in each field location to reach different deciles of E:E ratio according to: 1) interpolation from the logistic regression of embryo growth in the field (grey line); 2) ƟTb (blue line); and 3) ƟTc (orange line) obtained from the model.

**Fig. 4:** Principal component analysis of seed traits (in red) and geographic and bioclimatic variables (in blue) across the latitudinal distribution of *Conopodium majus*.