# Wild species intraspecific germination variability in water-limited alpine environments.

Abstract

## 1. Introduction

Mesoscale heterogeneity of alpine landscape; microclimatic conditions; species adaptations (similar to move along intro)

Snowmelt gradients and summer drought effects

Snow depth and snowmelt patterns strongly influence surface-level temperature, moisture and light, three key abiotic factors determining plant growth and reproduction in the corresponding communities (Winkler et al. 2018; Körner 2021).

Regeneration niche and Mediterranean germination syndrome

The majority of the empirical tests of niche theory focus on adults ignoring the important seed regeneration niche (Briceño et al 2015, Larson and Funk 2016).

Temperature and water, two major environmental drivers of the physiological process of seed germination (Bewley et al. 2013).

Drought effects on germination are more commonly studied in water-limited systems (e.g. Cochrane et al., 2014). Other germination in alpine systems often focus on the impacts of warming (Fernández-Pascual 2021, Hoyle 2013, Mondoni 2012) BUT what about alpine + water limited???

In alpine regions, much of the increased precipitation falls as snow. But as increased temperature leads to earlier snowmelt, increased evapotranspiration, and increased run-off, this can paradoxically result in higher risks of early summer soil moisture deficit in the alpine in the future (Hanssen- Baeuer et al., 2017)

Water stress

Plants from drier habitats tend to produce larger seeds in order to provide more resources for handling drought stress during germination (Wellsteins et al., 2013).

Reduced water availability can result in delayed germination (Cochrane et al 2015, Vázquez-Ramírez and Venn 2021)

In alpine lichen heaths, communities occurring at the snow-poor end of the gradient, we expected seeds to be able to germinate at comparatively low soil water contents, due to the low water supply from the melting snowpack and the fast-drying skeletal soils (Onipchenko2004).

Low and spread out germination (Gya2023) could be an indication of bet-hedging, a strategy well known for germination in habitats with high variability in climate (Evans and Dennehy, 2005). Bet hedging has been found to be a strategy that yields advantages during drought events (Evans and Dennehy, 2005, Lampei et al 2017).

Intraspecific germination variability to water stress

Intraspecific variation in germination responses to abiotic gradients has received less attention (Gya et al., 2023).

Populations within species may vary in their germination responses along environmental variables as a consequence of local adaptations (Grassein et al., 2014; Kawecki & Ebert, 2004), for instance, by optimizing germination timing and rate to population-specific environmental conditions (Baskin & Baskin, 2014; Giménez-Benavides et al., 2007; Meineri et al., 2013; Satyanti et al., 2019). The selective pressures operating on such local adaptations may generally be expected to be high in alpine ecosystems due to harsh environmental conditions, topographically complex landscapes, and high microclimate variability (Scherrer & Körner, 2011). This environmental variability within the alpine habitats leads to high but spatiotemporally variable seedling mortality rates (Graae et al., 2018; Scherrer & Körner, 2011), which further suggests selection for locally adapted seed regeneration responses in alpine species (Giménez-Benavides et al., 2007; Kim & Donohue, 2013; Mondoni et al., 2009). While such local adaptions may increase the total range of conditions

under which the species as a whole can survive, each locally adapted population tolerates a narrower range of conditions and can be vulnerable to climate change, especially if dispersal is low (Atkins & Travis, 2010; Valladares et al., 2014). Local adaptations could therefore confer a higher vulnerability to climate change of each population than inferred from the species-wide geographic distribution and climatic range (Atkins & Travis, 2010; Diamond & Martin, 2020; Peterson et al., 2018, 2019).

General goal:

Study intraspecific germination adaptation to water stress in Mediterranean high mountain grasslands.

Novelty:

Germination in water-limited alpine environments is understudied.

Variability in intraspecific responses to water stress in wild species.

Research questions:

1. Will seeds from warmer/drier subpopulations germinate better under higher water stress levels? Intraspecific adaptation to realized niche from adult plants, transgenerational effects into the regeneration niche?
2. Will the ripening stage (fresh vs. after-ripening) modify their response to water stress? Physiological responses to explore bet-hedging strategies?

Hypothesis:

1. Seeds from warmer/drier subpopulations will germinate better and faster at higher levels of water stress.
2. Fresh seeds will have higher variability of germination responses and will germinate worse.

Approach/General methods

We conducted a growth chamber experiment to investigate subpopulation-level variation of germination to water availability.

Seed collection of 1 specialist/strict alpine species, *Dianthus langeanus*, from Mediterranean high mountain grasslands. Seed collection the 7th-8th of August 2023, from 16 different collection sites (considered as subpopulations). Collections sites where previously iButtons were buried for 11 months (from 12/7/2021 to 29/05/2022) and we have temperatures registered every 4 hours. Additionally, we have hourly temperature and water potential data from 7 plots (A00, B00, B07, C00, C18, D00, D12).

Fresh seeds germination trial: (A00, B00, B03, B07, B17, B19, C00, C06, C19, D00, D19, D12). Seeds from 12 subpopulations were subjected to 7 water stress treatments (h2O, -0.2, -0.4, -0.6, -0.8, -1, -1.2 MPa, following standard typical with PEG 6000). 4 Petri dish 90mm diameter with 25 seeds for each WP treatment (100 seeds x treatment). Seeds sowed in two layers of germination paper. Added 5ml of PEG solutions and sealed Petri dishes with parafilm to avoid evaporation of the solution.

After-ripening germination trial: (A00, A02, A11, B03, B19, C00, C18, C19, C20, D00, D11, D19) Seeds from 12 subpopulations were subjected to 7 water stress treatments (h2O, -0.2, -0.4, -0.6, -0.8, -1, -1.2 MPa, following standard typical with PEG 6000). 4 Petri dish 90mm diameter with 25 seeds for each WP treatment (100 seeds x treatment, number were adapted in subpopulations with lower seeds). Seeds sowed in two layers of germination paper. Added 5ml of PEG solutions and sealed Petri dishes with parafilm to avoid evaporation of the solution.

6 populations were subjected to both fresh and after-ripening sowing (A00, B03, C00, C19, D00, D19)

## 2. Methods

### 2.1. Study area

This study was conducted in the alpine grasslands above 2000 m a.s.l from the southern slope of the Cantabrian Mountains, a mountain range running E-W in northern Spain, and within the Valles de Omaña and Luna Biosphere Reserve (coordinates?). The climate in the study system is typically Mediterranean, characterized by a 2-month dry period in summer (average annual precipitation of 1050 mm, mostly accumulated in spring and autumn). The growing season stretches from March to October with a mean annual soil temperature of 8ºC ADD days with water stress in the growing season and mean of days with snow (high variations according to orientation of slope). Grazing impact is also restricted to wild populations of Cantabrian chamois. The sampling sites are located above very acidic bedrock (pH 3.8 – 4.8, own non-published data). Local community richness ranges from 20 to 30 species dominated mostly by *Poaceae* and *Cyperaceae*, but they are also rich in Hemicryptophytes and Chamaephytes.

We established a systematic sampling across 4 summits with a central representative plot where we buried, at 5 cm deep, a Microlog SP3 datalogger, which hourly records temperature and water potential values (Microlog SP3 ref). The recording period for the Microlog SP3 went from XXXX to XXXX (xxx days). We also established 20 additional plots, 5 in each cardinal direction separated by 10 m (cruces) where we buried, also at 5 cm deep, iButtons dataloggers (Thermochron, iButton, Newbury, UK; accuracy: +/- 0.5 ºC from -10 ºC to +65 ºC, resolution: 0.5 ºC, records every 4 hours). The recording period for the iButtons went from 12/7/2021 to 29/05/2022 (xx days). Each plot with *D. langeanus* is considered a different subpopulation of Dianthus langeanus.

Figura 1. Mapa zona de estudio con las cruces de los plots recolectados, imagen de Dianthus langeanus y de los sensores enterrados.

### 2.2. Soil Bioclimatic Indices (from Picos paper)

We used the microclimatic soil data of our dataloggers to calculate soil bioclimatic indices. For comparison, we homogenized the data between the two types of dataloggers: (1) Microlog SP3 (Temperature + water potential hourly data) in 6 plots and iButtons temperature data at four-hour intervals for the 12 resting plots, keeping the same XXX calendar days (but from different years if we include the extremes WP loggers buried one year later). In total, we obtained XX data points. We calculated bioclimatic indices based on WorldClim standard variables (Fick & Hijmans 2017), together with other relevant variables on alpine microtopography. Following the paper by (Picos Paper) the selected variables were: (1) bio1 = annual mean temperature; (2) bio2 = mean diurnal range, i.e. the mean of the monthly differences between maximum and minimum temperatures; (3) bio7 = temperature annual range; i.e. the difference between the maximum temperature of the warmest month and the minimum temperature of the coldest month; (4) snow = the number of days of snow cover, when the soil temperature is around 0 ºC, calculated for the period in which the maximum temperature was < 0.5 ºC and the minimum temperature was > -0.5 ºC; (5) FDD = freezing degree days, i.e. the sum of daily mean temperatures for days in which the mean temperature was below 0 ºC (Choler 2018); and (6) GDD = growing degree days, i.e. the sum of daily mean temperatures for days in which the soil mean temperature at five cm deep was above 5 ºC (Körner 2021). For FDD, we transformed the values from negative to positive, so higher values represent more freezing. To identify the main gradients of microclimatic variability, we conducted a principal component analysis (PCA) includign all bioclimatic indices.

### 2.3. Seed collection

The study focuses on *Dianthus langeanus*, a wild species endemic to grasslands in high Mediterranean mountains, very abundant in our study area and with high seed production. We collected mature seeds directly from the mother plants from at least 20 randomly selected individuals following standard protocols for sampling seeds of wild populations (ENSCONET, 2009) within a 2m radius from the datalogger location, at the time of natural dispersal (August 7-8th, 2023). To enable equal conditions among seeds, we manually clean and air-dried at room conditions (22ºC and 35%RH) before germination experiments. In total, we sampled 18 plots (subpopulations), and we measured fresh seed mass by weighting 5 replicates of 50 seeds from each subpopulation. In some cases, with less than 50 seeds left after sowing, we annotated the number of seeds and the weight to have a proxy of mass per individual seed.

### To our knowledge there are no drought germination studies on our study species, therefore by testing across such a large gradient of water potentials, we aimed to identify the species minimum water potential for germination (from Gya 2023). 2.4. Germination experiments

To test the seed germination responses to water stress we performed two four-level full factorial experiments (12 subpopulations x 7 WP treatments x 4 replicates x 25 seeds), using fresh seeds (10 days after collection) and after ripened seeds (45 days after collection).

We sowed four replicates of 25 seeds from each subpopulation in a Petri dish (90 mm Ø) with two layers of filter paper (Filtros Anoia S.A. paper for germination assays, Ref.518G085) moistened with 5 ml of either distilled water or a polyethylene glycol 6000 solution (PEG) prepared according to Michel & Kaufmann (1973) to reach osmotic potentials of −0.1, −0.2, −0.4, −0.6, −0.8, −1 and −1.2 MPa (100 seeds per treatment except in -1 and -1.2 MPa treatments with only 2 replicates of 25 seeds each). Previous pilot study showed 0 germination at -1.4 and -1.6 MPa, thus we excluded those concentrations from the final experiment. We sealed Petri dishes with parafilm to avoid evaporation of the solution, keeping the filter paper moist and thus maintaining relatively constant water potential throughout the experiment.

Seeds were incubated at constant 20°C with a daily photoperiod (12-12h) in Aralab incubators (Aralab climatic chamber Fitoclima S600 PL, equipped with 4 led modules 11W 350mA, using Fitolog 9000 software version 9308, Aralab Pharmaceutical Stability software). Conditions simulate late summer conditions in the field, when germination has been previously showed to happen (paper move-along??)

### We monitored germination (radicle emergence > 1.5mm) daily for 28 days until the cumulative germination curve flattened (day 21) and we checked germination every two or three days until the end of the experiment. We removed germinated during the scorings and when the experiments were finished, we cut non-germinated seeds (Ooi et al., 2004; FAO, 2014), under the binocular loupe and classified them as viable, dead or empty. Seeds with firm and white embryos were considered viable i.e., potentially germinable (Baskin and Baskin 2014). The subsequent analysis took into account germinated and viable seeds. 2.5. Data Analysis

### All analyses were done in R software (R core Team 2022), using seedR package (Fernández-Pascual and González-Rodríguez, 2020) and GerminaR package (Lozano-Isla et al., 2019). visualization was done with ggplot2 package (Wickham, 2016)

Germination (final/t50/rate/synchrony) ~ maturity\_level\*WP\_treatment\*Bioclimatic\_indices\*seed mass (several?).

## 3. Results (preliminary)

### 3.1. Populations realized niche

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site | ID | bio1 | bio2 | bio7 | Snw | FDD | GDD | Mean seed mass |
| Rabinalto | A00 | 6.67 | 6.35 | 32.87 | 108 | 184.94 | 4410.08 | 1.098 |
| Rabinalto | A02 | 7.88 | 6.14 | 28.23 | 19 | 0.00 | 2190.85 | 1.144 |
| Rabinalto | A11 | 7.73 | 7.89 | 33.18 | 28 | 4.50 | 2190.29 | 0.949 |
| Cañada | B00 | 18.77 | 16.34 | 18.18 | 0 | 0.00 | 1013.40 | 0.949 |
| Cañada | B03 | 8.54 | 8.68 | 29.85 | 0 | 11.08 | 2380.34 | 1.025 |
| Cañada | B07 | 7.38 | 10.17 | 40.32 | 23 | 12.75 | 2078.20 | 1.2 |
| Cañada | B17 | 6.50 | 4.52 | 26.19 | 41 | 8.50 | 1833.33 | 1.2 |
| Cañada | B19 | 6.51 | 6.27 | 28.31 | 14 | 1.58 | 1775.83 | 0.776 |
| Cañada | B20 | 6.75 | 7.00 | 28.37 | 33 | 0.00 | 1860.21 | 1.603 |
| Solana | C00 | 10.22 | 10.71 | 34.49 | 30 | 30.86 | 3766.36 | 1.119 |
| Solana | C06 | 6.84 | 5.27 | 23.90 | 15 | 3.75 | 1951.94 | 1.908 |
| Solana | C18 | 6.39 | 6.68 | 28.95 | 20 | 7.00 | 1896.90 | 0.81 |
| Solana | C19 | 5.51 | 4.51 | 22.69 | 8 | 8.42 | 1595.75 | 0.995 |
| Solana | C20 | 6.47 | 7.51 | 27.95 | 16 | 6.25 | 1869.46 | 0.765 |
| Penauta | D00 | 8.92 | 8.11 | 30.38 | 51 | 58.26 | 3883.65 | 1.112 |
| Penouta | D11 | 6.60 | 5.31 | 25.56 | 31 | 12.17 | 1975.60 | 0.853 |
| Penouta | D12 | 7.98 | 7.56 | 31.26 | 6 | 4.17 | 2341.53 | 1.036 |
| Penouta | D19 | 4.83 | 2.69 | 16.90 | 56 | 5.00 | 1375.27 | 1.074 |

Diagrama

Descripción generada automáticamente

Escala de tiempo

Descripción generada automáticamente

### 3.2. Immediate sowing

Table with full germination summary “immediate\_germsummary.csv” for all plots

Individual plots for each dianthus subpopulation in results/Dianthus ID graph/immediate

Combined cumulation germination plot

Germination percentage dropped sharply below -0.2MPa water potential. Low water availability also led to delayed germination (confirming results by Gya 2023)

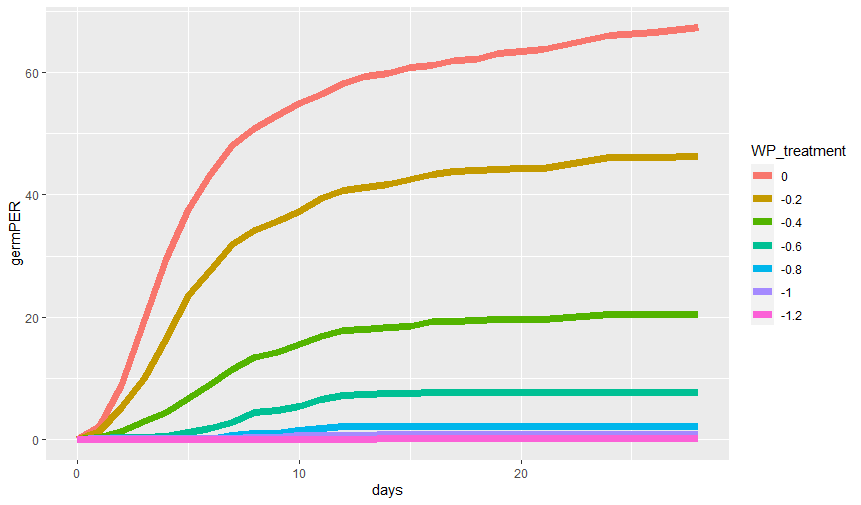
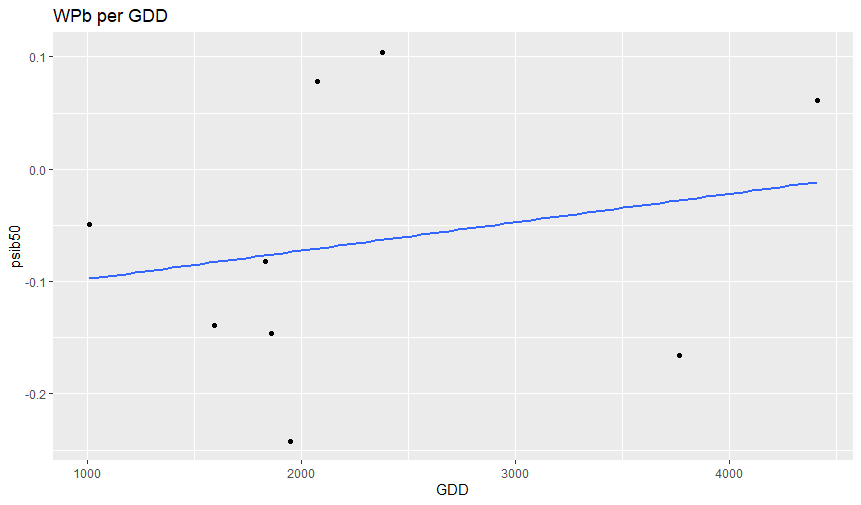
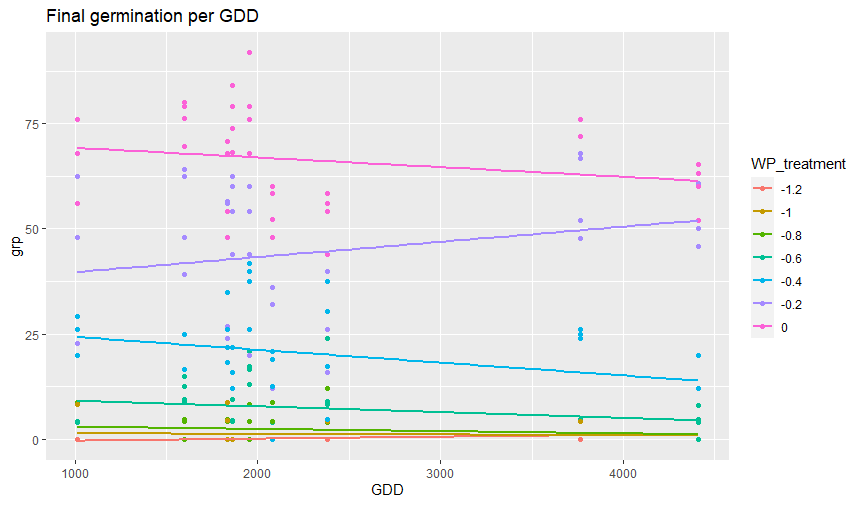


Table with full base water potential summary “immediate\_bwpsummary.csv” for all plots



Combine total germination, mean germination rate and synchrony (calculated with GerminaR) to check possible correlation with the response according to bioclimatic indices. No patterns visually detected but bioclimatic values need to be updated and homogenised.



### 4. Discussion

Rosback et al., 2022 found that:

“Germination temperature and water potential had significant and positive main effects: overall, germination was higher at higher temperatures and at comparatively higher water potentials. The different communities (lichen heath, grassland, meadow, snowbed) did not differ in their response to water potential. This finding suggests that snowbed species might not depend entirely on habitat specific ample meltwater supply during germination and can tolerate short-term summer droughts common in years with low precipitation. In its turn, the overall positive effects of low osmotic potentials on the germination of the focal species confirms the evidence that seedling establishment in terrestrial habitats with seasonal climates, including arctic and alpine environments (Bell and Bliss 1980; Oberbauer and Miller 1982; Tudela-Isanta et al. 2018b), is water-limited (Orsenigo et al. 2015; Walder and Erschbamer 2015). From the ecological point of view, it implies that alpine seed germination is triggered by comparatively high soil moisture levels (e.g. after a snowmelt or summer rainfalls), a key adaptation to avoid seedling emergence on commonly desiccated surfaces of summer alpine soils (Körner 2021) known to be one of the main reasons for seedling mortality in alpine regions (Welling and Laine 2000; Forbis 2003). High soil moisture is necessary for germination and seedling establishment because small seeds do not contain sufficient carbohydrate reserves for rapid production of deep roots in a drying environment (Oberbauer and Miller 1982).

Gya et al., 2023 (test for local adaptations to drought, 10 different water potentials, in germination and seedling in two alpine forbs with contrasting habitats (generalist vs snowbed). Main findings: drought led to lower germination percentage and slower germination. Seeds from the driest populations had higher germination percentage and shorter time to germination than seeds from the wettest populations, suggesting local adaptation to drought. We found evidence that germination and seedling traits may show adaptation to drought even in populations from wet habitats. Our results also indicate that alpine generalists might be more adapted to drought and show more local adaptations in drought responses than snowbed specialists. In one species, seed mass was also important because populations with lighter seeds reached higher germination percentages but had slower germination rates compared to heavier-seeded populations. While in the other species seeds from populations with higher seed mass germinated at higher percentages and faster rates. (mixed evidence)

Low moisture availability leads to partial or complete inhibition of physiological processes which may slow down the germination processes or hinder seeds from germinating (Baskin and Baskin 2014, Sumner and Venn 2021)

A sharp decrease in germination percentage between -0.57 and -0.7 Mpa. -0.7Mpa is described by Evans and Etherington (1990) as continuous water stress, representative of an intermediate dry environment before rainfall, or according to Young and Nobel (1986), corresponding to approximately 600 mm of rainfall.

As plants respond predominantly to soil water availability rather than precipitation events themselves, drought responses may be driven by different factors (Gya 2023).

## 5. References

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