# 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

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

Intraspecific germination variability to water stress

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).

General goal:

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

Novelty:

Germination in water-limited alpine environments is understudied.

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

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 sampling

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. On the 7th and 8th of August 2023, we collected the mature seeds directly from the mother plants, following standard protocols for sampling seeds of wild populations to maximize intraspecific genetic diversity (ENSCONET 2009). Sampling took place within a 2m radius of the datalogger location, we collected seeds from at least 20 individuals chosen at random. The goal was to collect 600 seeds from each subpopulation. After collection, seeds were manually clean and air-dried the following 10 days. We were able to collect enough seeds from 18 plots/subpopulations. For 6 of them more than 1200 seeds were collected.

### 2.4. Germination trials

In total, we sampled 18 plots/subpopulations of *D. langeanus*, and from all of them, we measured seed mass by weighting 5 replicates of 50 seeds. 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.

We decided to divide the plots/subpopulations into 3 groups: one group of 6 subpopulations sowed immediately after collection (10 days after collection), another group of 6 subpopulations sowed after 1 - 1.5 months in room conditions (22ºC and 35% RH approx., i.e., 30 - 45 days after collections) and a final group of 6 subpopulations sowed both immediately and after 1.5 months in room conditions.

We subjected all subpopulations to 7 water potential treatments (h2O = 0, -0.2, -0.4, -0.6, -0.8, -1 and -1.2 MPa). For each WP treatment we used 4 Petri dishes 90mm in diameter, as replicates, with 25 seeds for each WP treatment (100 seeds x treatment except in -1 and -1.2 Mpa treatments with only 2 replicates of 25 seeds each). We sowed the seeds in two layers of germination paper (Filtros Anoia S.A. paper for germination assays, Ref.518G085) moistened with 5ml of either distilled water or a Polyethylene glycol (PEG) 6000 solution. We prepared PEG solutions according to Michel and Kaufmann (1973). We added 5 ml of PEG solution in each Petri dish and sealed Petri dishes with parafilm to avoid evaporation of the solution, mantening filter paper moist and thus maintaining relatively constant the water potential throughout the experiment.

All seeds were exposed to constant 20ºC temperature treatment with 12-12h photoperiod 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). The germination trial ran for 28 days (4 weeks) with daily germination scores until day 21 and afterwards every 2-3 days until the end of the experiment. We removed germinated seeds from the Petri dishes once radicle >1.5mm. At the end of the experiment, we visually checked non-germinated seeds via cut-test. Seeds with firm and white embryos were considered potentially germinable (Baskin and Baskin 2014); empty, broken or infected seeds were removed from further analysis.

### 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)

## 3. Results (preliminary)

### 3.1. Populations realized niche

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

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

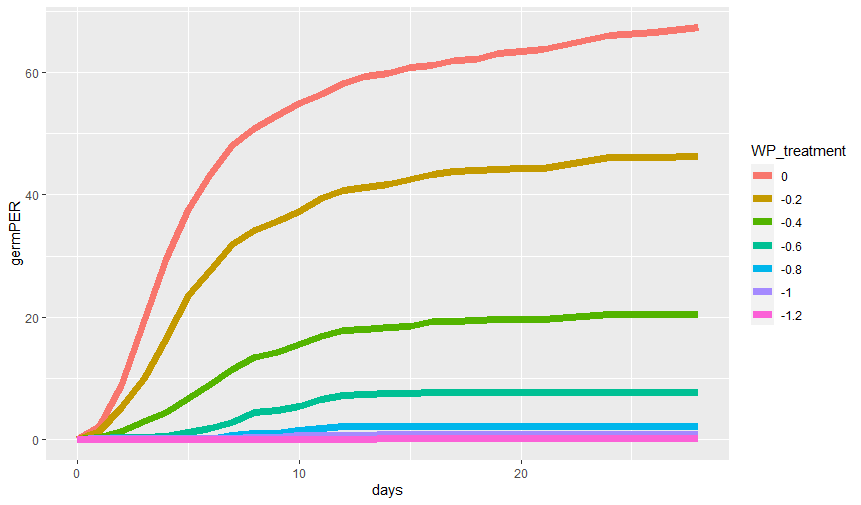
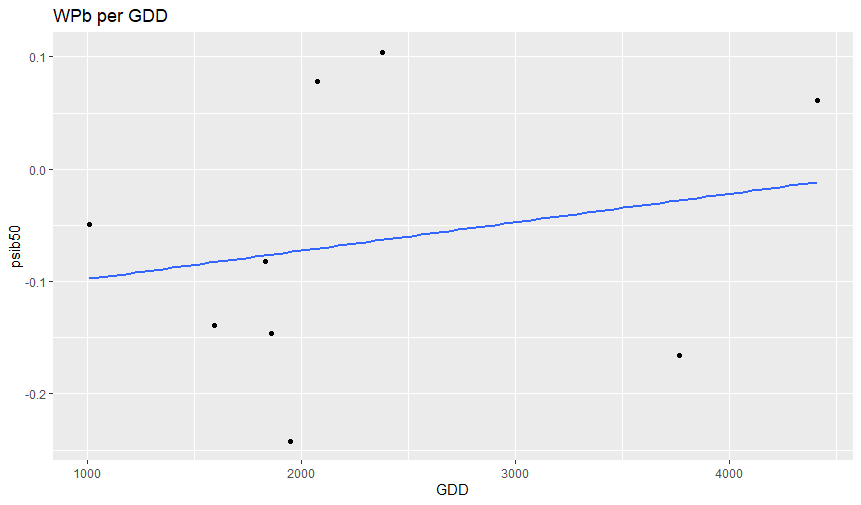
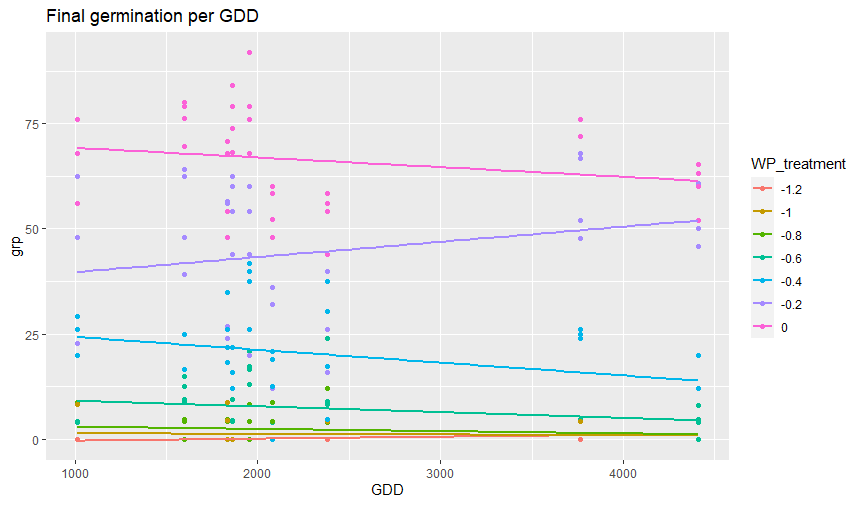


Table with full base water potential summary “immediate\_bwpsummary.csv” for all plots



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

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).

## 5. References

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