Data for Vandvik et al. 2016 Botany:**Seedling recruitment in subalpine grassland forbs: Predicting field regeneration behaviour from lab germination responses**

SITE/STUDY INFO:

We studied eleven species(*Campanula rotundifolia* L., *Gentianella amarella* L. Börner, *Gentiana nivalis* L.,  *Geranium sylvaticum* L., *Knautia arvensis* Coult., *Potentilla crantzii* Crantz. Fritsch, *Primula scandinavica* Brunn, *Ranunculus platanifolius* L., *Trollius europaeus* L., *Veronica alpina* L.,and *Viola biflora* L.)that co-occur within perennial subalpine grasslands in the study area (nomenclature follows the International Plant Names Index ([www.ipni.org/index.html](http://www.ipni.org/index.html)). The species were selected to represent a range in seed mass, life histories (annual/biennial to long-lived clonal perennial), seed dormancy classes (physical, physiological, morphophysiological), established plant size, distributional patterns (alpine, lowland, or ubiquitous), regional abundances (scattered or common), habitat preferences (strictly grassland or generalist), bedrock requirements (basic vs. indifferent), and soil fertility requirements (low or high) (based on data from Lid and Lid, 2005; see Table 1 in Vandvik *et al.* 2016).

Seeds (the whole single-seeded fruits of *Knautia arvensis* and *Ranunculus platanifolius* are denoted as seeds hereafter) were collected during the summer of 1998 from perennial grasslands, all within 150 km of the field experimental site (see below) and at 700-1000 metres above sea level. Only ripe and undamaged seeds were used, and these were air-dried at room temperature, and, to obtain enough seeds per sample for both the field and laboratory experiments, combined in bulk samples, each containing seeds from 1-4 different localities, and from at least 50 mother plants. This implies that our results should be interpreted as species-level responses, with no information about population or individual-level variation. For the field germination experiment, 20 batches of 100 seeds were prepared and sown within two weeks from collection (see below). The remaining seeds were stored dry in paper bags at 4ºC until batches of 50 apparently ripe and undamaged seeds were placed on moist filter paper in a 100 mm seal-tight Petri dish for the lab experiments.

1. *Lab germination experiments*

The germination data (Vandvik\_Germination.csv) reports on a laboratory experiment to assess seed germination requirements. Seeds were tested in four growth-chamber experiments, investigating the effect of (A) light and temperature, and (B) fluctuating temperatures, (C) a moist chilling pre-treatment (cold-stratification *sensu* Baskin and Baskin 2014), and (D) dormancy breaking by means of gibberellic acid, GA3.

Light treatments were full light, using standard artificial greenhouse light for a photoperiod of 16 hours per day to match summer light conditions at our latitude, or darkness. Petri dishes receiving dark treatment were wrapped individually in two layers of aluminium foil, and these were opened and counted under a safe green light (< 0.05 μmol/m2s).

In experiments (A) and (B), four constant temperature regimes (10ºC, 15ºC, 20ºC and 25ºC) and a diurnal cycle (16 hours at 25ºC and 8 hours at 10ºC) were compared in light and darkness. The 24-hour temperature sums of the 20ºC and 25/10ºC treatments are identical; by testing germination responses to fluctuating temperature against germination at 20ºC we investigate the effect of the diurnal variation *per se.*

Seeds were subjected to moist chilling in darkness at 4ºC in a moist environment (achieved by keeping the filter paper moist and sealing the petri dishes, ensuring access to free water) for two months prior to experiments (A) and (B), and germinability in seed batches not subjected to the moist chilling pre-treatment (C) was included to quantify dormancy levels in fresh seeds (Grime *et al.,* 1981; Baskin and Baskin, 2014). In experiment (C) fresh seeds (these were kept in dry storage during the moist chilling period, see above) were set to germinate at 20ºC in light and darkness, using the germination of moist chilled seeds at 20ºC in light and darkness as controls.

Treatment with gibberellins (GA3 acids) has proven highly effective in dormancy-breaking in a number of alpine species (e.g., Hoyle *et al.,* 2013; and references therein) and for different kinds of dormancy (Baskin and Baskin, 2004), and experiment (D) was therefore included to indicate whether any important environmental cues that may break dormancy and initiate germination had been missed. In experiment(D), the Petri dishes were watered with 800 mg L−1 GA3, (selected to be within the range reported in Hoyle et al., 2013) and set to germinate in light at 20ºC, using the germination of seeds exposed to a moist chilling pre-treatment at 20ºC in light as controls.

For each factorial combination of treatments x species four replicate Petri dishes containing 50 seeds were used, for a total of 572 Petri dishes and 28 600 seeds. Germination was recorded and seedlings removed after 2, 4, 6, 10, 16, 24, and 32 days. At each count, the positions of the Petri dishes were shuffled and water was added to maintain equal light and moisture levels and prevent drying at warmer incubation temperatures. The experiments were carried out at the Centre for Plant Research in Controlled Climate at the Norwegian University of Life Sciences ([www.nmbu.no/tjenester/sentre/skp](http://www.nmbu.no/tjenester/sentre/skp)). Six growth chambers were available, and in order to obtain replication for temperatures, experiments (A) and (B) were run twice with different chamber x temperature combinations in the two replicate runs. There was no measurable chamber-to-chamber difference in light intensity and spectral quality, or any differential effect of the light treatments on temperatures within Petri dishes (monitored by the technical staff at the Centre). For further details, see Vandvik *et al.* (2016).

**List of variables:**

Species 2-letter abbreviation (first letter for species and genus name)

PETRI ID Unique ID for each petri dish

CHAMBER ID Unique ID for each growth chamber (1-6)

Light 1 = germination in light, 0 = germination in darkness

Temperature Growth chamber temperature (10°C – 25°C)

Diurnal fluctuation Diurnal temperature fluctuation (1= 16 hrs of 25°C and 10 hrs of 10°C; 0 = 24 hrs of 20°C)

Stratification 1 = seeds exposed to cold-stratification, 0 = unstratified seeds

Giberillic acid 1 = giberillic acid added 0 = water

Day 2 – day 32 Number of seeds germinating at each census day

Total germination Total number of seeds germinated

1. *Seed regeneration in perennial grassland*

The seedling recruitment data (Vandvik\_Recruitment.csv) reports on a field experiment to investigate the seedling emergence and mortality of the 11 species in contrasting grassland microsites. The experiment was set up at a semi-natural grassland at Såttåhaugen (10º49’50’’E, 62º37’80’’N) at 880 m.a.s.l..

Four experimental treatments that reflect different environmental and competitive settings for seeds and seedlings were compared; bare-ground gaps with both above- and below-ground plant parts removed, vegetation cut at ground level (0cm), vegetation cut at 5cm above ground, and nontreated plots with a field layer of ca. 20cm in height.

In October 1998, a fully factorial randomized block design with five replicate blocks, each containing a grid of 44 25cm x 25cm plots, was set up. Each combination of species x treatment was represented once within each block, and batches of 100 recently collected seeds (see above) were sown into the central 20cm x 20cm of each plot, for a total of 22 000 seeds and 220 plots.

In 1999 and 2000, seedling emergence and mortality were recorded as the grassland sward closed at the end of spring (late June), and at the end of the growing season (late August). No precautions were taken against seed predation or seedling herbivory, and the observed emergence and survival hence reflect those realized by naturally-dispersed seeds and seedlings at the site. For further details, see Vandvik *et al.* (2016).

**List of variables:**

PLOT ID unique Id per plot

plot number plot number per block

block id block ID (1-5)

SPECIES species id (abbreviation, see above)

TREATMENT 1 - 4 (see above)

count autumn 1999 total # seedlings at census

count spring 2000 total # seedlings at census

count autumn 2000 total # seedlings at census

germination spring 2000 # new seedlings sprin 2000

germination autumn 2000 # new seedlings sprin 2001

mortality spring 2000 # of old seedlings lost / dead at census

mortality autumn 2000 # of old seedlings lost / dead at census

germination total sum of unique seedlings germinated across censuses

mortality total sum of unique seedlings dead across censuses

Other notes:

Data

**Literature cited:**

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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. Glob. Change Biol. **19**: 1549–1561. DOI: 10.1111/gcb.12135

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