Carasso V., Gallino B., Gosmar V. & Mucciarelli M., 2015. Optimal temperature range for seed germination of the rare Delphinium fissum *Waldst. & Kit. (Ranunculaceae). In: Mariotti M. & Magrini S. (Eds.), Conservation of threatened species: activities and collaborations within the network. RIBES Series 1: 11-14.*

Optimal temperature range for seed germination of the rare *Delphinium fissum* Waldst. & Kit. (Ranunculaceae)

Valentina Carasso[1], Bruno Gallino[1], Valeria Gosmar[1] & Marco Mucciarelli[2]

[1]Ente di gestione del Parco Naturale del Marguareis, Chiusa di Pesio (Cuneo), Italy. [2]Department of Life Sciences and Systems Biology, Viale P.A. Mattioli 25, 10125 Torino (Italy).
E-mail: *marco.mucciarelli@unito.it*

Introduction

Conserving rare and endangered species is a main focus of the Centro per la Biodiversità Vegetale (CBV) of the Marguareis Natural Park and its Seed Bank. In order to achieve this goal species-specific viability tests are designed and routinely performed on each accessions before and after banking.

FIGURE 1. Delphinium fissum *in full bloom*

Delphinium fissum Waldst. & Kit. (Ranunculaceae) is a rare species in the South West Alps. Plants sampled for this study (Fig. 1) originated from a single wild *D. fissum* population growing next to the Roccasparvera village (CN). This population is presently protected within a Site of Community Importance, SCI/SPA IT1160036 'Stura di Demonte', according to the Natura 2000 network (Directive 92/43/EEC).

This mountain area is characterized by rocky calcareous cliffs, with south-west orientation and a xeric climate conditions. In this site no more than 20 adult plants of *D. fissum* were censused in 2013.

Species belonging to the Ranunculaceae family of plants have morphologically and morpho-physiologically dormant (MPD) seeds (Baskin & Baskin, 2001). As a consequence, a suitable incubation protocol must be designed to meet the needs for germination in these species. In this type of seeds, in fact, embryos must grow before germinating, and this process is often controlled by temperature.

In particular, seeds of *D. fissum* subsp. *sordidum* (Cuatrec.) Amich, E. Rico & J. Sánchez have been found to possess intermediate complex MPD and need a three-month incubation at 5°C, in darkness, to promote embryo growth and germinate (Herranz et al., 2010).

The aim of the present study was to explore if *D. fissum* seeds presented temperature requirements for germination similar to those reported in literature for the genus, in order to develop a suitable protocol to test seed viability and longevity during long-term storage at the CBV seed bank.

Material and methods

Source of germplasm

Seeds used for this study were collected in summer at the time of dispersal at SCI/SPA IT1160036 'Stura di Demonte' (Valle Stura di Demonte, CN). Seeds were immediately moved to the seed bank and kept for a few days at laboratory conditions (20°C and 60% RH). Thereafter, seeds were cleaned and analysed as addressed in the following sections.

Viability and germination analysis

Original seed viability was tested by *Tetrazolium* staining (TTC); the same test was applied to non-germinating seeds at the end of each experiment. Germination tests were performed with a range of temperatures as follows: five constant temperature regimes, in darkness (5°C, 10°C, 15°C, 20°C and 25°C); one alternating temperature regime at 20/10°C thermoperiod, 12 h/12 h light/darkness. For each experiment, three repetitions of 25 seeds were employed. Seeds were sown on sterile 1% w/v water/agar medium in 9 cm diameter Petri dishes. Seed germinations were checked once every fortnight. Number of germinating seeds were expressed as both cumulative germination percentages and as the percentage of seeds germinating at a given time. Midpoints of the germination period (MGP) were also calculated for each temperature regime.

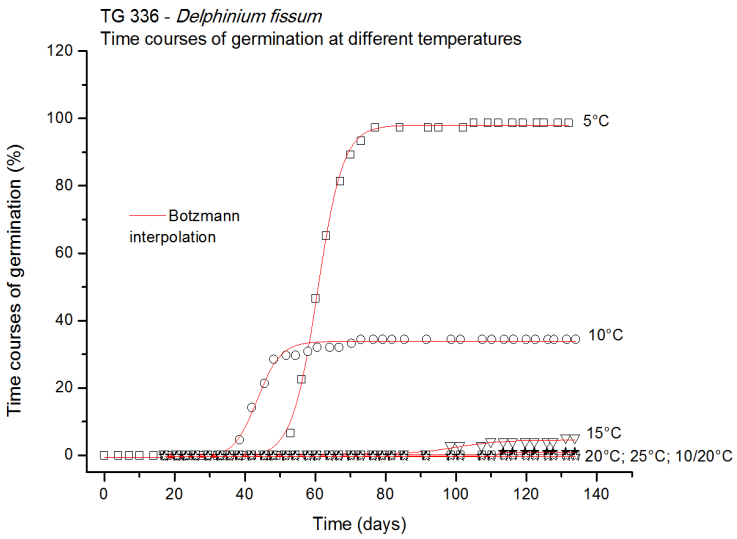
Statistical analysis

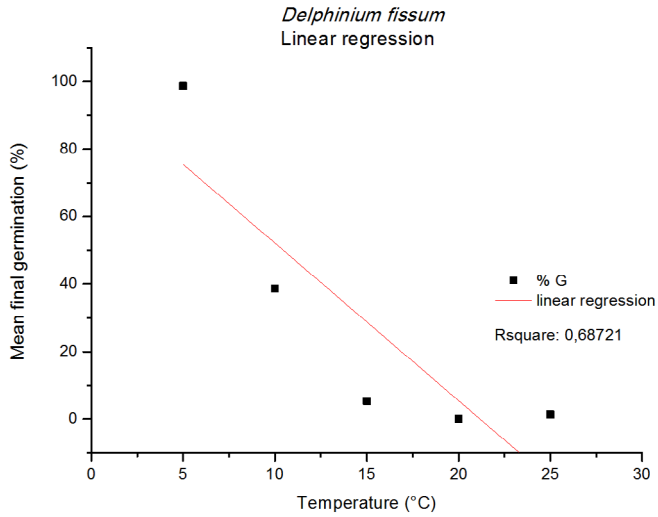
Germination percentages at different temperature regimes (six levels) were statistically compared by ANOVA one-way at 0.05 level, using the Tukey's test for pairwise comparison among means. Cumulative germination percentages were also analysed by linear regression analysis and interpolated using the Boltzmann model in Origin 6.1 (OriginLab Corporation, Massachusetts, USA).

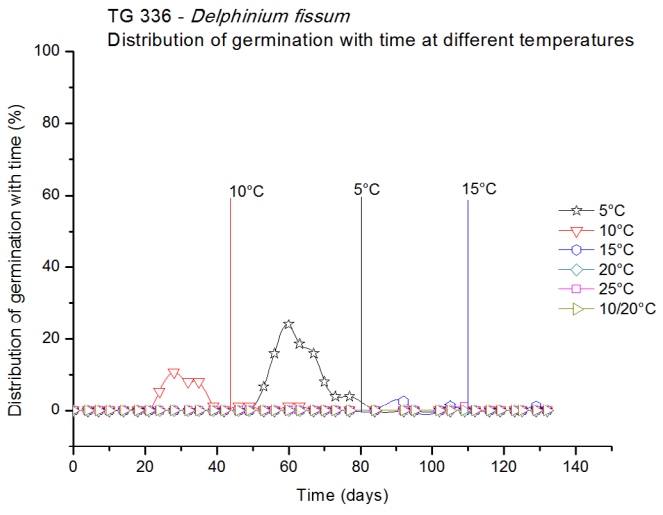
Results and discussion

Germination test

First germinations in *D. fissum* seeds occurred after 24 days at 10°C, after 53 days at 5°C and after 92 days at 15°C. The longest germination delay (time between the first and the last germination) was observed at 25°C and the shortest at 10°C (Fig. 2).







Captions from top to bottom:

FIGURE 2. Cumulative germination curves of D. fissum *at the six different temperature regimes. Sigmoidal positive curves were interpolated by Boltzmann function*

FIGURE 3. The correlation between incubation temperatures and final germination of D. fissum *was analysed by linear regression*

FIGURE 4. The distribution of germination of D. fissum *with time. Germination picks are displayed as well as midpoint of the germination period (MGP) (vertical lines)*

The highest final cumulative germination percentage was recorded at 5°C, where 98.67% of the seeds germinated. Cumulative germination percentages proportionally decreased with the other temperature regimes (Fig. 3) as follows: 38.67% at constant 10°C, 5.33% at constant 15°C, 1.33% at constant 25°C. No seed germinated at 20°C and at the alternating temperature regime. Results of ANOVA showed that germination percentages at 5°C and 10°C were significantly different between them and differed from all the other temperature regimes ($p \leq 0.05$). By contrast, germination percentages at 15°C, 20°C and 25°C were not statistically different. Progress of germination with time is shown in Fig. 4. Although the low final germination percentage, seeds of *D. fissum* incubated at 10°C germinated faster than at 5°C (Fig. 2), and their MGPs were 43 days and 80 days, respectively (Fig. 4). Results at the end of the experiments showed that all the seeds incubated at 5°C had germinated; by contrast the TTC analysis displayed that non-germinating seeds incubated at the other temperature regimes were still viable after 132 days. This implies that seeds incubated at temperatures greater than 5°C were still dormant. Even if we didn't measure embryo length inside seeds of *D. fissum*, on the bases of the long germination delay (greater than 30 days), we can assume that seeds of *D. fissum* are morphologically dormant. This speculation is supported by literature data available for other species in the same genus (Williams & Cronin, 1968) and for the subsp. *sordidum* from Herranz et al. (2010).

Our results seem to address that a cold stratification is necessary in this species for the germination of fresh seeds. Further tests will be conducted in order to asses: 1) if ultra-drying before seed banking can alter seed responses to incubation temperatures; 2) whether the faster germination observed in some seeds incubated at 10°C can be ascribed to a small population of seeds having greater embryo size at dispersal.

Acknowledgments *- This study was funded by the European Structural Funds (Piano di Sviluppo Rurale 2007-2013, Asse 3 - Misura 323 - Azione 1 - tipologia 1b) under the Project named 'Il Centro per la Biodiversità Vegetale' managed by the Ente di gestione del Parco Naturale del Marguareis. We thank also the staff of the Parco Fluviale of Cuneo for granting us permission to collect seeds at SIC/ZPS IT1160036 'Stura di Demonte'*

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