

Germination ecology of seeds of endemic species *Degenia velebitica* (Degen) Hayek (Brassicaceae)

DUBRAVKA NAUMOVSKI

Botanical Garden, Faculty of Science, University of Zagreb, Marulićev trg 9a, HR – 10000 Zagreb, Croatia

The phenology of *Degenia velebitica* seeds, including such topics as maturation, dispersal and germination times, is still largely unknown. For the purpose of the present research, the seeds were collected in the Botanical Garden, Faculty of Science, University of Zagreb and in the natural habitat (above Sibinj, Veliki vrh, Velika kapela mountain) at the time of natural dispersal. Freshly matured and collected seeds were placed under natural conditions (16–28 °C), at room temperature (24 °C), 21 °C and in greenhouse conditions (22/31 °C). Some of the seeds were placed at 5 °C for 3, 6, 9, 12 weeks and checked to see if cold stratification was needed. The most interesting part for the study of germination phenology was the exposure of the seeds to temperature conditions similar to those in nature (in the garden) and the definition of maximum and minimum temperatures of germination. Seed dispersal begins soon after maturation and all seeds (100%) are dispersed in one season. After 28 days, low percentages of germination, of 8.77% (at 24 °C), 6.66% (at 21 °C), 14.55% (at 22–31 °C) and 0% (at 16–28 °C) were found, indicating non-deep physiological dormancy (PD) in *Degenia* seeds. Non-deep PD is broken by cold stratification and by seeds being stored dry at room temperature (afterripening). The highest percentage of germination was at 22–31 °C, and the lowest at 21 °C indicating that *Degenia velebitica* germinates in late summer or early autumn. Seeds do not require light for germination, but germination in the light is 54% better at 22–31 °C, 35% at room temperature and 7% better at 16–28 °C than in the dark. The seeds collected in the Botanical Garden germinated 18%, 28% and 30% better at 22–31 °C, 16–28 °C than seeds collected near the place called Bile after 63 days of germination.

Key words: *Degenia velebitica*, seed, germination, dormancy, endemic, Velika kapela, Croatia

Introduction

Degenia velebitica (Degen) Hayek (Brassicaceae) was discovered by the Hungarian botanist Arpad Degen on July 17, 1907. In July, *Degenia* was already in fruit, without any flowers, so he could not put *Degenia* into its proper genus. Next year he came in June, but this was also too late for flowering. Finally on May 9, 1909 his colleague Kummerle found the species in flower and brought a specimen to Degen. At first Degen assigned the species

* Corresponding author: e-mail: dnaumov@botanic.hr

to the wrong genus – *Lesquerella*. An excellent botanist, he knew little about the *Brassicaceae* family, unlike Hayek, who finally put *Degenia* into the right genus and out of respect for Degen named the plant after him.

The genus *Degenia* is a monotypic genus (one of the rarest on Mt Velebit), endemic, endangered and famous. *Degenia velebitica* is in the 45 most endangered species in Europe, and in the 250 most endangered in the world. It has been protected since 1964 in Croatia and in the world since 1978. The small population of *Degenia* grows only on calcareous rocks in a geographically limited area of the middle and southern part of Mt Velebit. A few years ago, mountain climbers found a new habitat on Mt Mala Kapela, near the place called Bile. Since there is a huge risk of the destruction of natural habitats (especially on Velebit) all botanists in Croatia have a major job to do in the protection, cultivation and micro-propagation of *D. velebitica* (PEVALEK-KOZLINA et al. 1999). Therefore, the aim of this study was to learn more about *Degenia* phenology, i.e. flowering, maturation and dispersal, and to experiment with seed germination response to different temperatures.

Materials and methods

The seeds used in this experiment were collected in Velika kapela mountain, near Bile (above Sibinj, Veliki vrh), and in the Botanical Garden, Faculty of Science, University of

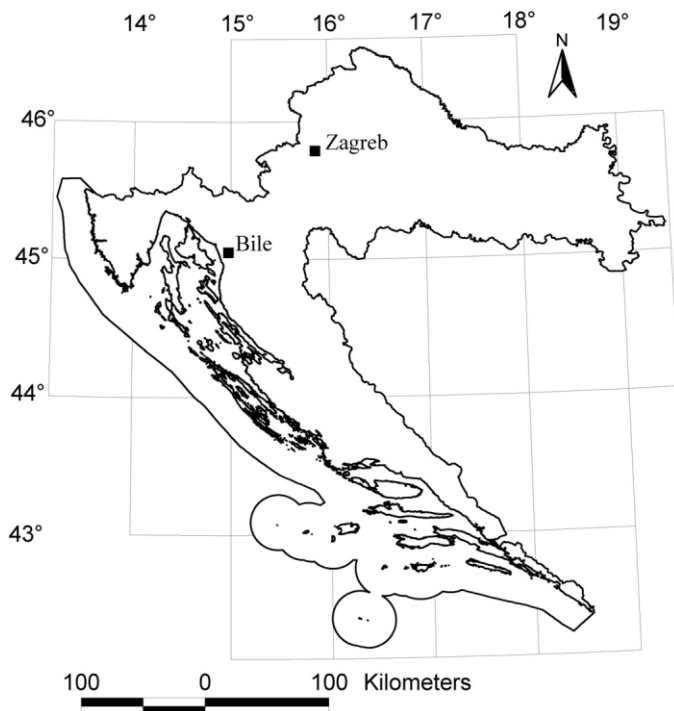


Fig. 1. The locations of of *Degenia velebitica* seed collection: Velika kapela mountain (Bile) and the Croatian capital of Zagreb.

Zagreb (Fig. 1). Mature seeds were placed in a paper bag at room temperature for 10 days and then included in the experiment. This study investigated the details of control of the temperature of germination using: (1) constant temperature, (2) alternating temperatures, (3) varying durations of cold stratification, (4) periods of dry storage at room temperature, (4) differences between germination percentages in the dark and in the light, (5) differences between germination percentages depending on the habitat from which the seeds were collected.

In all these different parts of the research, the seeds were germinated in the same way. Seeds were put on sterile Petri dishes on moist filter paper, 30 seeds per plate, and two plates per different condition.

Room temperature (24 °C) and a temperature of 21 °C were used for the constant temperature, and hot greenhouse (22/31 °C) and outside temperatures (16/28 °C) were used for alternating temperatures. Seeds were put at 5 °C for 3, 6, 9 and 12 weeks for cold stratification and after that plates were held at room temperature (24 °C) and the temperature of the hot greenhouse (22/31 °C). Seeds collected in the Botanical Garden were placed in dry storage at room temperature and tested for germination after 12 weeks. For the research in dark conditions, the seeds were placed as before, but in addition the plates were wrapped in two layers of aluminium foil.

In all the experiments the plates were examined twice a week for at least 28 days, while some of them were examined until the germination was complete (70 days). Seven-day-old seedlings were moved from the Petri dishes and counted.

Seed morphology and morphometry were also studied. For morphology studies, seeds were stained in a 1% solution of 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) for two hours (COTTRELL 1947). For morphometric studies t-test was used to evaluate the differences in means between the two groups. The length of seeds was measured in the Coral program, and 500 seeds were measured at each habitat.

Results and discussion

The first part of the research concerned the flower and seed phenology of *Degenia velebitica*. *Degenia* flowers from the beginning of April until the middle of May (or after the snow melts), which means about 45 days. The first fruit appears 25 days after the first flower and needs 45 days to mature (first dispersal begins). Ninety days after the first flower has appeared, all the seeds have matured. The interval between the appearance of the first flower and the dispersal of the last seed is 100 days. Species from the same family *Brassicaceae* have different dispersal times. *Arabis leavigata* var. *leavigata*, for example, needs more than 2.5 years for all seeds to be dispersed (BLOOM et al. 2002).

According to the studies of MARTIN (1946) there are 12 different types of seeds and embryo positions, sizes and shapes. *Degenia* is characterized by its curved or bent embryo with cotyledons folded back along the hypocotyls/radicle. The seed and embryo are large, while the endosperm is small (Fig. 2).

Light and darkness affected germination differently. Light was not required for germination but seeds germinated much better in light than in darkness (Fig. 3). This light requirement for a higher percentage of germination is not unusual, because many species germinate much better in light than in darkness (GRIME et al. 1981, BASKIN and BASKIN 1988).



Fig. 2. Bent type of seeds and embryo of *Degenia velebitica* stained with 1% solution of 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) Length = 3.75 mm.

The seeds collected in the Botanical Garden, Faculty of Science, University of Zagreb germinated in higher percentages than those collected in the natural habitat near Bile, at all temperatures (Fig. 4). There are at least two possible reasons for this observation. The seeds from Bile natural habitat grow in much harder conditions and in soil with a lower percentage of nutrients and water in the soil than do the seeds collected in the Botanical Garden. The second is that plants in Botanical Garden are mixed, from several habitats, so genetic studies should be done. According to t-test analysis, there are no statistically significant morphometric differences among seeds from these two habitats (Tab. 1).

When cold stratification was longer, the seeds germinated in higher percentages, 13% higher after 6 weeks and 12% after 9 and 12 weeks of cold stratification, after 42 days of germination at 22/31 °C for seeds collected in the Botanical Garden (Fig. 5).

After 12 weeks of being kept at a dry room temperature (24 °C) seeds germinated in the highest percentage, 41.4%, which is 6% higher than after 12 weeks of cold stratification

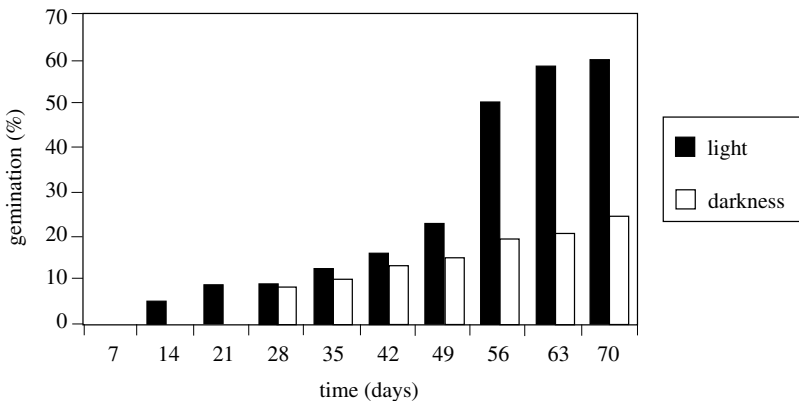


Fig. 3. Germination of *Degenia velebitica* seeds at room temperature (24 °C) incubated on moist filter paper over a 14-hr daily photoperiod and in continuous darkness for 70 days.

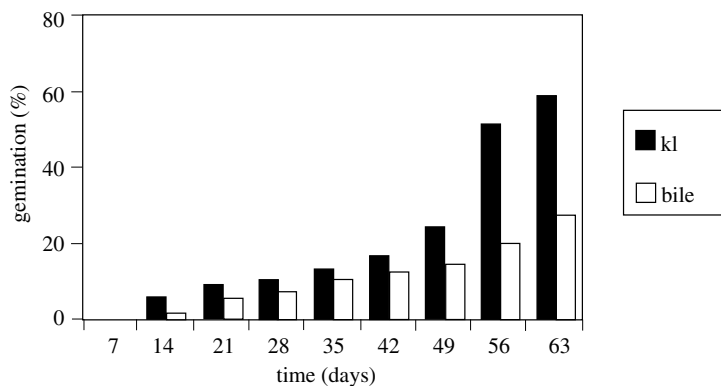


Fig. 4. Germination of *Degenia velebitica* seeds collected in the Botanical Garden, Faculty of Science, University of Zagreb and the Bile natural habitat, at room temperature (24 °C) and incubated on moist filter paper over a 14-hr photoperiod for 70 days.

Tab. 1. Differences in means between seeds of *Degenia velebitica* (from Bile and Botanical Garden in Zagreb) analysed with t-test. The probability of error involved in accepting our research hypothesis about the existence of differences is 0.05. (x_1 and s_1 – arithmetic mean and standard deviation of *Degenia* seeds collected at Botanical Garden; x_2 and s_2 – arithmetic mean and standard deviation of *Degenia* seeds collected in the Bile natural habitat; df – degree of freedom, t – t-test measured; t-tab – standard t value).

x_1	x_2	s_1	s_2	df	t	t-tab
3,774	3,737	0,165	0,155	499	0,164	1,96

(Fig. 6). This behaviour of seeds of *Degenia velebitica* is unusual. The time required for seeds to afterripen is usually much longer than that required for dormancy loss during cold stratification (TOOLE and TOOLE 1941).

When maximum germination percentages at various temperatures were compared it was seen that seeds germinated in the highest percentage at alternating temperatures of 22/31 °C in all different regimes (length of stratification, lightness, habitat, storage at dry room temperature). Minimum germination percentage was at a constant temperature of 21 °C, which leads us to the conclusion that seeds of *Degenia velebitica* do not germinate in late autumn or spring. Most probably, the seeds germinate in the late summer and early autumn of the same year in which they mature (Fig. 7).

Freshly collected seeds did not germinate in a higher percentage after 28 days on moist filter paper: 8.77% at room temperature (24 °C), 6.66% at 21 °C, 14.55% at 22/31 °C and 0% at 16/28 °C, but germination increased after 50 days (Fig. 7). From this it can be concluded that *Degenia velebitica* seeds have nondeep physiological dormancy that can be broken by cold stratification or by keeping seeds in dry place at room temperature (afterripening).

Studies about *Degenia velebitica* seed germination should be continued. The rarity of *Degenia* rareness and the small amount of seeds that can be collected every year have restricted this research, for the primary consideration should be to learn more while not endangering *Degenia* in its natural habitat.

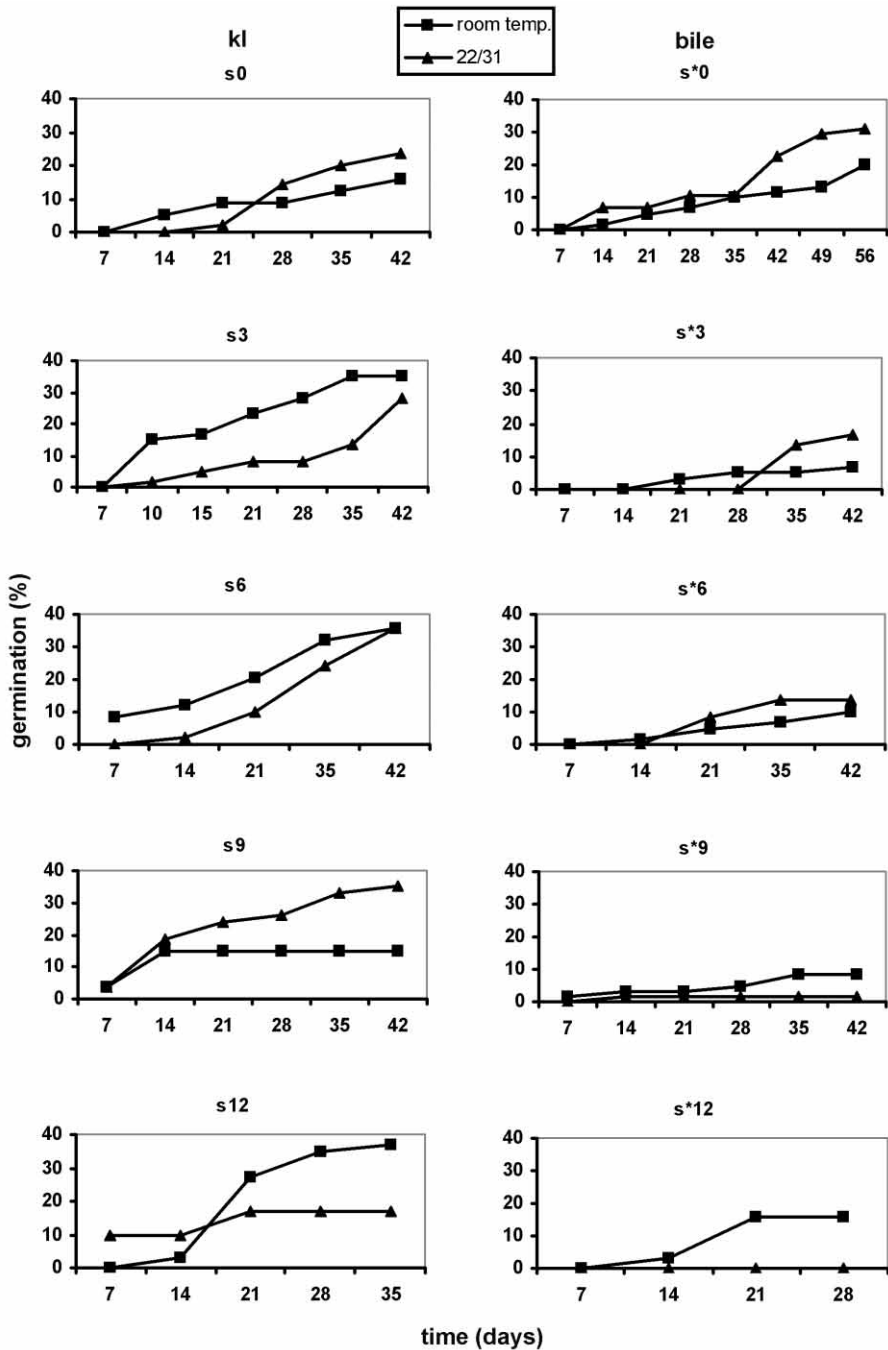


Fig. 5. Germination of *Degenia velebitica* seeds collected in the Botanical Garden, Faculty of Science, University of Zagreb and the Bile natural habitat after 0, 3, 6, 9 and 12 weeks of cold stratification at 5 °C. Seeds were incubated on moist filter paper at room temperature (24 °C) and at the temperature of a hot greenhouse (22/31 °C) over 14-hr photoperiod for 42 days.

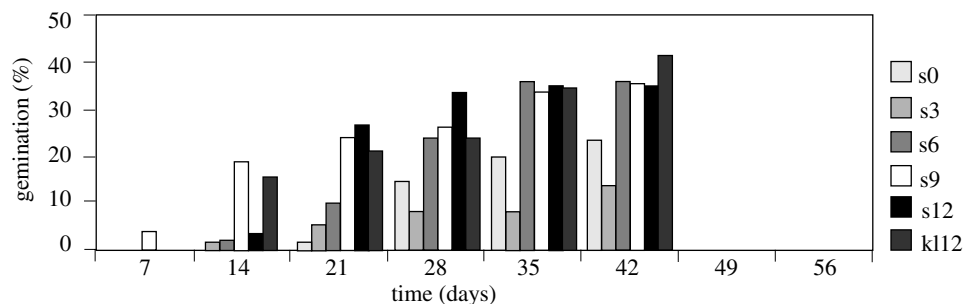


Fig. 6. Germination of *Degenia velebitica* seeds after 0, 3, 6, 9 and 12 weeks of cold stratification at 5 °C and after 12 weeks of dry storage at room temperature. Seeds were incubated on moist filter paper at hot greenhouse temperature (22/31 °C) over a 14-hr photoperiod for 42 days.

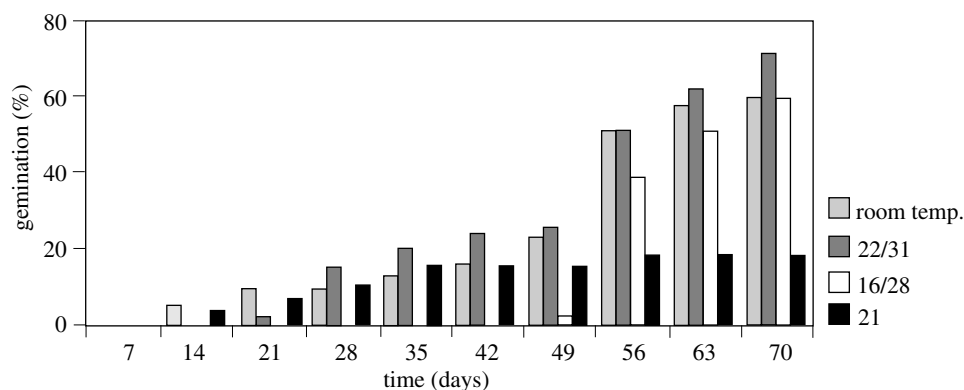


Fig. 7. Germination of *Degenia velebitica* seeds at room temperature (24 °C), the temperature of a hot greenhouse (22/31 °C), the temperature of natural conditions (16/28 °C) and 21 °C, incubated on moist filter paper over a 14-hr photoperiod for 70 days.

Acknowledgements

Many thanks to Prof. Carol Baskin the University of Kentucky for advice and support, Biserka Juretić MSc, the head of the Botanical Garden, Faculty of Science, University of Zagreb, who supported this project, Renata Kralj for technical help, Svjetlana Lupret-Obradović for collecting seeds and Iva Dobrović for her useful comments on the manuscript and for preparing the map.

References

- BASKIN, C. C., BASKIN, J. M., 1988: Germination ecophysiology of herbaceous plant species in a temperate region. *Am. J. Bot.* 75, 286–305.
- BASKIN, C. C., BASKIN, J. M., 1998: *Seeds Ecology, Biogeography, and Evolution of Dormancy and Germination*. School of Biological Science, University of Kentucky, Lexington, Kentucky.

- BLOOM, T. C., BASKIN, J. M., BASKIN, C. C., 2002: Ecological life history of facultativ woodland biennial *Arabis laevigata* variety *laevigata* (Brassicaceae): seed dispersal. J. Torrey Bot. Soc. 129, 21–28.
- COTTREL, H. J., 1947: Tetrazolium salt as a seed germination indicator. Nature 159, 748.
- GRIME, J. P., MASON, G., CURTIS, A. V., RODMAN, J., BAND, S. R., MOWFORTH, M. A. G., NEAL, A. M., SHAW, S., 1981: A comparative study germination characteristics in local flora. J. Ecol. 69, 1017–1059.
- MARTIN, A. C., 1946: The comparative internal morphology of seeds. Am. Midl. Nat. 36, 513–660.
- MAYER, E., 1981: *Degenia velebitica* (Deg.) Hay. and *Fibigia triquetra* (DC.) Boiss. Morfološko-taksonomska paralela. Acta Biokov. 1, 283–290.
- PEVALEK-KOZLINA, B., PAVLICA, M., VUJEVIĆ, M., 1999: Micropropagation od *Degenia velebitica* (Deg.) Hay., a Croatian endemic plant species. Phytion (Austria) 39, 293–296.
- TOOLE, E. H., TOOLE, V. K., 1941: Progress of germination of seed of *Digitaria* as influenced by germination temperature and other factors. J. Agric. Res. 63, 65–90.