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EFFECT OF COLD STRATIFICATION AND GERMINATION TEMPERATURE ON SEED GERMINATION OF TWO ECOLOGICALLY DISTINCT SPECIES, *LINARIA LOESELII* AND *L. VULGARIS* (SCROPHULARIACEAE)

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Abstract. The effects of stratification at 5°C and a range of constant germination temperatures were studied in seeds of *Linaria loeselii* Schweigg., a littoral species endemic to the Baltic region, and *L. vulgaris* Mill., widespread in various open habitats throughout Europe. Seeds of both species originated from the same coastal dune habitat. For stratification treatment, seeds were incubated for 4, 12 and 20 weeks at 5°C in darkness; non-stratified seeds stored dry were the control. Seeds were germinated at eight constant temperatures ranging from 0 to 35°C. The base temperature for germination was calculated for each stratification treatment using linear regression. Stratification treatment broke dormancy in seeds of both species, reflected in a decrease of the base temperature for germination. Final germination increased from 3.3% to 83.3% after 12 weeks at 5°C in *L. loeselii*, and from 20 to 69.7% in *L. vulgaris* seeds after 20 weeks. *L. vulgaris* seeds were heterogenous in their response to cold stratification, a characteristic which may increase the success of its establishment in different habitats. In contrast, *L. loeselii* seeds are more dormant and have a narrower interval of temperatures favorable for germination; this may be an adaptation to climatic conditions.

Key words: dormancy, cold stratification, germination temperature, seed heterogeneity, *Linaria*

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INTRODUCTION

Linaria loeselii Schweigg. (Scrophulariaceae) is a coastal dune plant endemic to the Baltic region (Chater *et al.* 1972). A survey of European coastal species by van der Maarel and van Maarel-Versluys (1996) lists it as requiring attention because little information on its biology is available. As *L. loeselii* is a protected species and is listed in the *Red Data Book of Latvia* (Gavrilova 2003), the possibility of conserving it *ex situ* in plant tissue culture has been investigated at the National Botanical Garden in Salaspils, Latvia (Klavina *et al*. 2006). In contrast, *L. vulgaris* Mill. is a widespread species common in ruderal habitats and open grasslands (Chater *et al.* 1972) but also found in the coastal area. It is considered a weed species and has become invasive in parts of North America (Pauchard *et al*. 2003). *Linaria vulgaris* is a perennial plant and can reproduce vegetatively (Nadeau *et al*. 1992; Pauchard *et al*. 2003). The same is true for *L. loeselii* (pers. observ.). Nadeau *et al.* (1992) quantified the relative importance of sexual and vegetative reproduction and concluded that while populations are maintained mainly by pieces of roots and shoots, seed germination may be important in the establishment of *L. vulgaris* plants in new environments. Understanding the germination physiology of this species can help predict the conditions favorable to its further spread.

Seed dormancy, a characteristic of many temperate plant species, restricts germination of mature seeds to a narrow range of temperatures or inhibits germination completely (Vleeshouwers *et al*. 1995). During dormancy breaking,

the minimum germination temperature decreases or maximum germination temperature increases; in some cases both processes can occur (Baskin & Baskin 1998). Dormancy in the genus *Linaria* is broken by a period of three or more months of incubation of imbibed seeds at *ca* 5°C (Nikolaeva *et al*. 1985). Seeds of *L. vugaris* are dormant upon maturation; while some seeds can germinate immediately after dispersal, incubation at low positive temperature (cold stratification) enhances germination (Grime *et al*. 1981). In a previous study, seeds of both species collected in Latvia were found to be dormant (Necajeva & Ievinsh 2008). Information about the germination biology of these species is needed in planning conservation of *L. loeselii*, and should prove useful in further research on the ecology of both species.

In this study we investigated the effect of cold stratification and constant germination temperatures on seed germination in the two species. We considered the observed differences in the range of temperatures favorable for germination and germination time in relation to the species' habitats and possible differences in their germination strategies.

Material and methods

Seeds of *L. loeselii* and *L. vulgaris* were collected in Kolka on the Baltic Sea shore (NW Latvia) in September 2009. Initial germination tests were made with undried seeds within a week of collection at 22°C under a 16 h photoperiod (Narva Luminofluor 58 W fluorescent tubes, avg. photon flux density 53 ± 2 μmol m^{-2} s^{-1}). Seeds were incubated in Petri dishes on 0.6% agar, and germinated seeds were counted and removed every 3 days. *Linaria loeselii* seeds were also put to germinate on agar containing 1.0 mM gibberellic acid (GA_3) to determine seed viability, because previous work showed that dormant seeds do not germinate without pretreatment (Necajeva & Ievinsh 2008).

As a preliminary experiment conducted to observe the germination time of *L. loeselii* in conditions close to those in the field, its seeds collected in 2006 near the Irbe River estuary were sown in pots in October 2008 and the pots were stored outdoors through the winter in a covered trench. Emerged seedlings were counted in April and May 2009. There were three replicates of 20 seeds each.

For cold stratification, seeds were stored on agar plates at 5°C in darkness for 4, 12 and 20 weeks before the germination tests. Seeds stratified for 20 weeks were put on agar plates for stratification first; meanwhile other seeds were dried at 17°C and 9% RH before the beginning of stratification treatment eight and sixteen weeks later. Control seeds were dried and from March 2010 stored in an air-tight container at –20°C.

Seeds from each stratification treatment were put to germinate on 1% agar at 0, 5, 10, 15, 20, 25, 30 or 35°C, except for one treatment of *L. loeselii* seeds (4 weeks at 5°C) which was germinated at temperatures in the 0–25°C range because there were not enough seeds for the whole range, and one treatment of *L. vulgaris* seeds (12 weeks at 5°C) germinated at 0–30°C. Each Petri dish contained 30 seeds, one Petri dish for each treatment. Germinated seeds were scored twice a week. Germination tests lasted 73 days. Tetrazolium tests were performed after the germination tests and the number of empty seeds was subtracted from the total number of seeds sown in each treatment. Viability of the non-germinated seeds was assessed as follows: seeds were cut transversely and incubated in 1% tetrazolium chloride solution at 30°C in darkness for 48 h before evaluation.

To estimate the base germination temperature (T_b), linear regressions were fitted to curves obtained by plotting germination rate against germination temperature and T_b determined as the intercept of the regression line on the X axis. Maximum (ceiling) germination temperature (T_c) was estimated in the same way. Average values of T_b and T_c at 10, 20, 30, 40 and 50% germinated seeds were calculated. T_{50} was determined as the time (day) at which 50% of the seeds sown germinated. The calculations used Microsoft Excel.

Results

In freshly collected seeds, final germination of seeds incubated with 1.0 mM GA_3 was 90% for *Linaria loeselii* and 47% for *L. vulgaris*, but none of *L. loeselii* germinated at 22°C without GA_3.

Only 3.3% of the seeds of *L. loeselii* germinated without stratification (control) and only at high temperatures, while in *L. vulgaris* up to 20% germinated in the control treatment and the range of favorable temperatures was wider (Fig. 1). The final germination percentage was highest in *L. loeselii* seeds after 12 weeks of cold stratification (83.3%), and in *L. vulgaris* seeds after 20 weeks of cold stratification (69.7%) (Fig. 1).

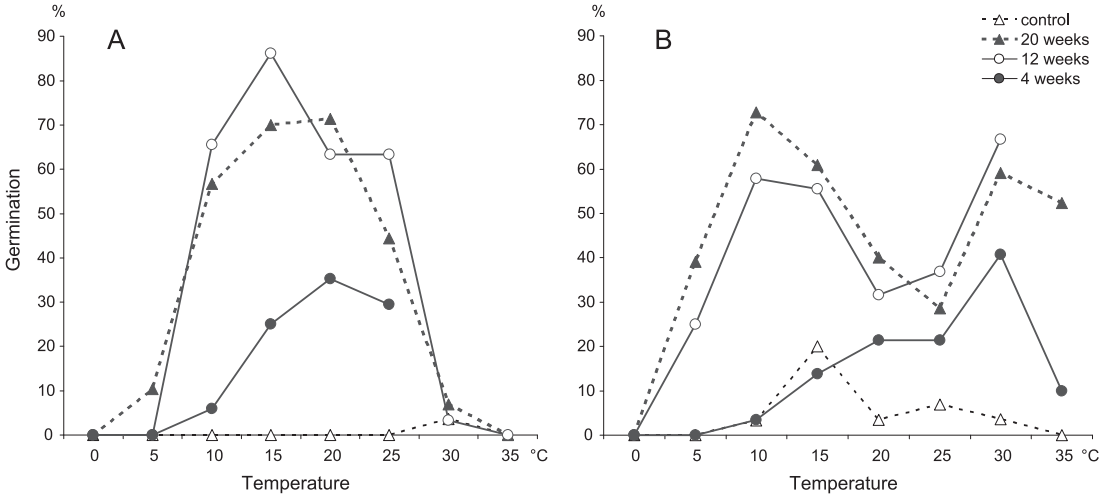

Fig. 1. Effects of a range of constant temperatures on germination of *Linaria loeselii* Schweigg. (A) and *L. vulgaris* Mill. (B) seeds after different periods (0, 4, 12 and 20 weeks) of cold stratification at 5°C in darkness.

T_b decreased after cold stratification in both species, but the decrease was greater in *L. vulgaris*, *ca* 6°C as compared to *ca* 2°C for *L. loeselii* after 4 weeks of stratification (Table 1). T_c could only be determined for *L. loeselii*: average 30±1°C for all stratification treatments.

The non-germinated *L. loeselii* seeds incubated at temperatures below 15°C remained highly viable; viability decreased at higher temperatures (Fig. 2A). In *L. vulgaris* the viability of seeds was slightly lower but there was no notable decrease at higher temperatures (Fig. 2B). Estimated viability was 100% at 0°C in *L. loeselii* seeds in all stratification treatments, but in *L. vulgaris* seeds it was higher in control seeds (Fig. 2). The viability of *L. vulgaris* seeds stored dry was 95%.

Table 1. Effects of cold stratification on base germination temperature (T_b) estimates for *Linaria loeselii* Schweigg. and *L. vulgaris* Mill. after linear regression of the relationship between rate of germination ($1/T_{50}$) and temperature.

Weeks of 5°C stratification	T_b,°C ± se	
	Linaria loeselii	*Linaria vulgaris*
0 (control)	–	5.8 ± 0.03
4	5.3 ± 1.00	6.6 ± 0.10
12	4.3 ± 0.01	1.3 ± 0.20
20	3.1 ± 0.20	0.7 ± 0.35

DISCUSSION

A reduction of the base temperature for germination after cold stratification reflects dormancy loss (Pritchard *et al.* 1999). In both species the final germination percentage and the range of favorable temperatures increased substantially after stratification. Lowering of T_b in response to a dormancy-breaking treatment (cold stratification) corresponds to type II physiological dormancy (Baskin & Baskin 1998); in this way germination is prevented after seed dispersal in autumn and is programmed to occur in the spring after dormancy is broken by low winter temperatures. This germination strategy is evident in both species of *Linaria* but particularly in *L. loeselii*, whose dormancy was deeper at the time of dispersal. *L. loeselii* seeds sown in pots in October did not germinate until late April of the following year.

The population of *L. vulgaris* seeds was heterogeneous: a fraction of the seeds were dormant at maturity, while some seeds germinated at relatively low temperature (15°C). Maximum germination was lower in *L. vulgaris* than in *L. loeselii*, possibly because seeds that did not respond to stratification either lost viability or entered secondary dormancy; this lack of response to chilling can also be due to seed heterogeneity.

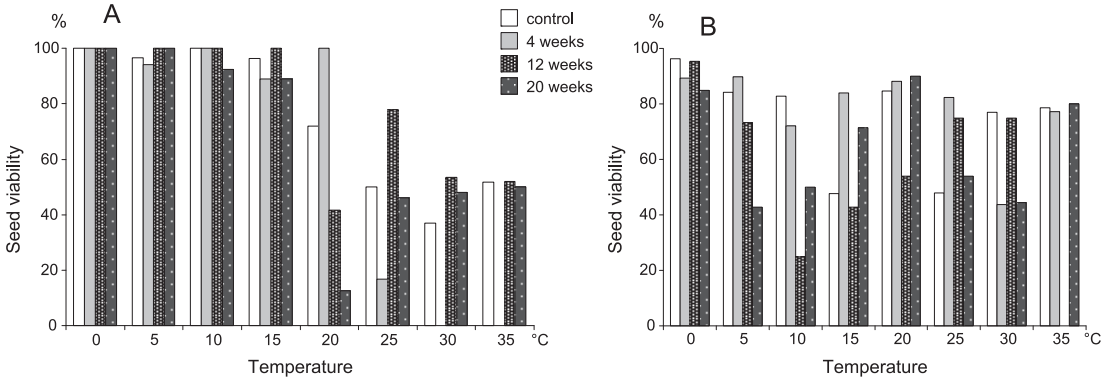

Fig. 2. Viability (%) of non-germinated seeds of *Linaria loeselii* Schweigg. (A) and *L. vulgaris* Mill. (B) seeds assessed using the tetrazolium test. Seed were germinated at 0–35°C for 73 d after different periods (0, 4, 12 and 20 weeks) of cold stratification at 5°C.

Heterogeneity of seeds is important in plants growing in unpredictable habitats. For example, in flax and *Penstemon* seeds, cold stratification released dormancy in one fraction of seeds and enhanced it in another fraction, with the non-germinated seeds forming a persistent seed bank (Allen & Meyer 1998). *Linaria vulgaris* has been reported to form a large and persistent seed bank (Roberts 1986), but the persistence of a soil seed bank in a particular habitat has yet to be investigated.

The seeds' response to chilling differed between the two species. In *L. loeselii*, T_b could not be estimated for control seeds due to insufficient data, but the main effect of cold stratification occurred during the first 4 weeks, when temperatures favorable for germination dropped from above 25°C to 5.3°C. In *L. vulgaris* there was a more substantial change: after 20 weeks the difference in T_b between control and stratified seeds reached 5°C (Table 1). In *L. vulgaris* seeds the final germination percentage and the germination rate increased after cold stratification within a wide range of temperatures both above and below the temperatures optimal for control seeds (25–30°C and 5–15°C, respectively). Consequently, while *L. loeselii* is highly unlikely to germinate before the spring following seed dispersal when dormancy is released, *L. vulgaris* seeds potentially are able to germinate in different seasons, an ability which would maximize the possibility of successful establishment.

Figure 3 illustrates the difference between the two species with respect to the favorable temperature range for germination after dormancy is broken by five-month cold stratification. Besides genetic variation, another factor that increases heterogeneity within a seed population is variation of seed maturity due to heterogeneity of flowering, because seed dormancy can be determined by environmental conditions experienced by the mother plant (Fenner & Thompson 2005). The flowering period is from June to September for *L. vulgaris*, and shorter for *L. loeselii* (July/August), which

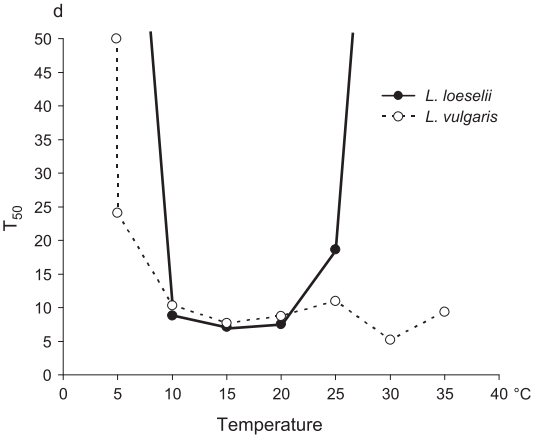

Fig. 3. Time to 50% (T_{50}) of maximum germination over a range of constant temperatures in seeds of *Linaria loeselii* Schweigg. and *L. vulgaris* Mill. after 20 weeks stratification at 5°C. d – days.

may explain the observed more uniform response to chilling.

In this work we showed that *L. vulgaris* seeds are able to germinate at low temperatures, which would allow its seedlings to establish earlier than species that germinate later. This is one of the traits that contribute to the spread of a non-native species, for example *Heracleum mantegazzianum* (Pyšek *et al*. 2007). In the case of *L. vulgaris* the importance of seed germination in colonizing new habitats still needs to be investigated. Colonization of a new habitat or expansion of an existing population are often more influenced by low success of seedling establishment than by germination itself, as well as by the presence of highly competitive dominant species (Turnbull *et al*. 2000). Seedling mortality is generally high in sand dunes (Maun 2009); this is likely to prevent *L. vulgaris* from spreading in this habitat. In suitable conditions, however, its ability to germinate at a wide range of temperatures and possibly immediately after dispersal may assist its establishment.

Linaria loeselii is adapted to the climatic conditions of its habitat (selection pressure for germination to occur in the spring so that seedlings avoid the harsh winter temperatures). Both *L. vulgaris* and *L. loeselii* grow in open habitats, but the latter only grows in coastal dunes. The similar distribution of *L. thymifolia* and several other species endemic to coastal dunes in the southwest France was explained by the hypothesis that these species, preferring open habitats, were restricted to the coast when forest areas increased after the end of glaciation and evolved as separate species (or subspecies) tolerant to the environmental conditions of the dunes but avoiding competition (Berghen 1964).

In the case of *L. vulgaris*, different germination characteristics can be expected in populations growing in different climatic conditions. The flowering period and the length of the vegetation season can influence these differences, as can natural selection for germination timing. Germination characteristics may remain similar if a species is spread mainly by humans (Thompson 1970). These possibilities pose questions requiring further research on the germination characteristics of different populations of *L. vulgaris*, especially where the species is non-native.

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REFERENCES

ALLEN P. S. & MEYER S. E. 1998. Ecological aspects of seed dormancy loss. *Seed Science Research* **8**: 183–191.

BASKIN C. C. & BASKIN J. M. 1998. Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, London.

BERGHEN C. V. 1964. Notes sur la végétation du sud-ouest de la France I: La végétation des dunes mobiles. *Bull. Jard. Bot. État Bruxelles* **34**: 519–525.

CHATER A. O., VALDÉS B. & WEBB D. A. 1972. *Linaria* Miller. In: T. G. TUTIN, V. H. HEYWOOD, N. A. BURGES, D. M. MOORE, D. H. VALENTINE, S. M. WALTERS & D. A. WEBB D. A. (eds), *Flora Europaea*. **3**: 226–236. Cambridge University Press, Cambridge.

FENNER M. & THOMPSON K. 2005. The ecology of seeds. Cambridge University Press, Cambridge.

GAVRILOVA G. 2003 *Linaria loeselii* Schweigg. In: G. ANDRUSAITIS (ed.), *Red Data Book of Latvia: rare and threatened plants and animals*. **3**: 618–619. LU Bioloģijas Institūts, Rīga (in Latvian with English summary).

GRIME J. P., MASON G., CURTIS A. V., RODMAN J. & BAND S. R. 1981. A comparative study of germination characteristics in a local flora. *J. Ecol*. **69**: 1017–1059.

KLAVINA D., GAILITE A. & IEVINSH G. 2006. Initial responses of explants from rare and endangered coastal plant species during initiation of tissue culture. *Acta Universitatis Latviensis, Biology* **710**: 81–91.

MAAREL VAN DER E. & MAAREL-VERSLUYS VAN DER M. 1996. Distribution and conservation status of littoral vascular plant species along the European coasts. *Journal of Coastal Conservation* **2**: 73–92.

MAUN M. A. 2009. The biology of coastal sand dunes. Oxford University Press, Oxford.

NADEAU L. B., KING J. R. & HARKER K. N. 1992. Comparison of growth of seedlings and plants grown from root pieces of yellow toadflax (*Linaria vulgaris*). *Weed Sci.* **40**: 43–47.

NECAJEVA J. & IEVINSH G. 2008. Seed germination of six coastal plant species of the Baltic region: effect of salinity

and dormancy-breaking treatments. *Seed Science Research* **18**: 173–177.

NIKOLAEVA M. G., RASUMOVA M. V. & GLADKOVA V. N. 1985. Reference book on dormant seed germination. Nauka, Leningrad (in Russian with English summary).

PAUCHARD A., ALABACK P. B. & EDLUND E. G. 2003. Plant invasions in protected areas at multiple scales: *Linaria vulgaris* (Scrophulariaceae) in the West Yellowstone area. *W. N. Amer. Naturalist* **63**: 416–428.

PRITCHARD H. W., STEADMAN K. J., NASH J. V. & JONES C. 1999. Kinetics of dormancy release and the high temperature germination response in *Aesculus hippocastanum* seeds. *J. Exp. Bot*. **50**: 1507–1514.

PYŠEK P., COCK M. J. W., NENTWIG W. & RAVN H. P. 2007. Ecology and management of giant hogweed (*Heracleum mantegazzianum*). CAB International, Wallingford, UK.

ROBERTS H. A. 1986. Seed persistence in soil and seasonal emergence in plant species from different habitats. *J. Appl. Ecol*. **23**: 639–656.

THOMPSON P. A. 1970. A comparison of germination character of species of *Caryophyllaceae* collected in Central Germany. *J. Ecol*. **58**: 699–711.

TURNBULL L. A., CRAWLEY M. J. & REES M. 2000. Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* **88**: 225–238.

VLEESHOUWERS L. M., BOUWMEESTER H. J. & KARSSEN C. M. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. *J. Ecol*. **83**: 1031–1037.

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