



## Seed germination response to cold stratification period and thermal regime in *Phacelia secunda* (Hydrophyllaceae)

Altitudinal variation in the mediterranean Andes of central Chile

Lohengrin A. Cavieres<sup>1</sup> & Mary T. K. Arroyo<sup>2</sup>

<sup>1</sup>Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile; <sup>2</sup>Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile (e-mail: lcaviere@udec.cl)

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### Abstract

The ability to germinate under a variety of environmental conditions is essential for plant species inhabiting a wide range of altitudes and latitudes. *Phacelia secunda* J. F. Gmel. (Hydrophyllaceae) is a perennial herb with wide latitudinal and altitudinal distributional ranges. In the central Chilean Andes (33 °S) *P. secunda* can be found from 1600 m sealevel up to the vegetation limit at 3400 m. It has been suggested that seeds from populations encountering long periods with snow cover and adverse winter conditions would require longer periods of cold stratification for germination than those from populations exposed to milder winters. Given that the snow-free period decreases with elevation, seeds from high elevation populations could require longer period of cold stratification to germinate. Moreover, it has been shown that seeds from arctic and higher elevations environments are adapted to germinate better under high temperature conditions. Germination response with increasing periods of cold stratification (0–6 mo.) and under two contrasting thermoperiods (20 °/10 °C; 10 °/5 °C; 12 h day/night), were studied for 4 populations of *P. secunda* located at 1600, 2100, 2900 and 3400 m a.s.l. Initiation of germination required increasingly longer periods of stratification with elevation, and proportionately fewer seeds germinated for any one stratification treatment at the higher elevations. Seeds from higher elevations germinated to a higher percentage under the high than the low temperature thermoperiods. These results illustrates a significant variation in germination characteristics over a spatially short environmental gradient.

### Introduction

The transition from seed to seedling is a high-risk period in the life cycle of many plants (Harper 1977). Therefore, mechanisms regulating the timing of this transition would be under strong selective pressure (Meyer et al. 1997). If seed germination is an adaptive process, natural selection should favor seed germination patterns that reduce the probability of finding environmental conditions that are not appropriate for seedling establishment (cf. Angevine & Chabot 1979). Under this scenario seed germination patterns should vary depending on the environmental characteristics and their associated risks of mortality for seedlings.

Most studies relating germination patterns to habitat characteristics have been based on interspecific comparisons (e.g. Grime et al. 1981). A powerful approach is to examine the variation in seed germination among populations of a single species in different habitats. This approach holds other aspects of species life history relatively constant, making habitat-related differences easier to detect (Venable 1984). Such an approach has been used in several recent studies (Barclay & Crawford 1984; Borghetti et al. 1989; Inoue & Washitani 1989; Meyer et al. 1989; Meyer & Monsen 1991; Meyer & Kitchen 1994; Meyer et al. 1997; Schütz & Milberg 1997).

*Phacelia secunda* J. F. Gmel. (Hydrophyllaceae) is a perennial herb distributed from the south of the

United States to southern South America (Deginani 1982). In Chile, this herb shows a wide latitudinal and altitudinal range. Latitudinally, populations are found from tropical (18 °S) to subantarctic latitudes (54 °S). Altitudinally, it occurs from near sea level up to the upper limit of vegetation in the central Chilean Andes (3400 m a.s.l.), inhabiting zones with different climatic conditions such as the coastal zone, mediterranean-type climate scrublands, montane forest and alpine communities. Seed germination under a range of environmental conditions is essential for plant species inhabiting wide altitudinal and latitudinal ranges and this combination of conditions is powerful for detecting selective effects on seed germination patterns.

It is well known that environmental conditions change in predictable ways along altitudinal gradients (Billings 1974; Bliss 1985). One of the most evident changes is the increasing duration of snow cover with elevation. Meyer & Mosen (1991) suggest that populations normally encountering long periods with snow cover and adverse winter conditions would require longer periods of cold stratification for germination than those exposed to milder winters. Their reasoning was that such a function would allow seeds to sense the time spent under winter conditions, thereby timing germination to a period appropriate for seedling survival and establishment. Under natural conditions, the stratification requirement prevents precocious germination under autumn conditions, where optimum moisture and temperature regimes to complete seed germination and seedling establishment are not likely to persist more than a few days, allowing germination to occur only after seeds have experienced low winter temperatures. This also prevents young seedlings from being damaged by freezing temperatures (Billings & Mooney 1968).

Lower ambient temperature with elevation is another well-known characteristic of altitudinal gradients. If germination pattern changes in response to habitat conditions it could be expected that seeds from higher elevations should be adapted to germinate better under low temperature conditions; however, many studies report that optimum germination temperatures in many tundra species are surprisingly high (20 °C–30 °C) in comparison with ambient temperatures (Sayer & Ward 1966; Billing & Mooney 1968).

In this study, we test the effects of stratification period and two thermal regimes on seed germination patterns in *P. secunda* under laboratory conditions. Seeds

originated from different populations along an altitudinal gradient in the mediterranean Andes of central Chile. We expected that seeds from higher elevations would require longer periods of cold stratification, and germinate better at high temperature conditions, than those from lower elevations.

## Materials and methods

### Seed sources

Seeds were obtained from four populations located in successive vegetation belts found immediately below and above the treeline up to the upper vegetation limit in the Andes of central Chile (33 °S). Population, were located in south-facing slopes of the Rio Molina valley and its upper tributaries (Estero del Cepo) on route and above the Valle Nevado ski complex, 80 km east of Santiago.

### Population 1

This was located in The Santuario de la Naturaleza Yerba Loca at 1600 m elevation, 50 km east of Santiago below the treeline. Snow cover persists from 1–3 months (Cavieres 1999). Vegetation is a montane sclerophyllous forest dominated by *Kageneckia angustifolia* D. Don (Rosaceae). *K. angustifolia* formed an open woodland formation with a 5–10 m tall tree stratum and a discontinuous shrub layer interspersed with a rich flora of annual and perennial herbs (Arroyo & Uslar 1993). At this site, *P. secunda* populations are found in the inter-tree spaces, especially along ephemeral spring water courses. The individual rosettes are ca. 10 cm in diameter, normally with no more than 2–3 erect inflorescences, and around 25 cm tall (Cavieres 2000).

### Population 2

This was located at 2200 m elevation in the subandean scrub belt dominated by *Chuquirage oppositifolia* D. Don (Compositae). Subandean scrub belt in Rio Molina valley is found from 2100–2500 m, immediately above the discontinuous *Kageneckia angustifolia* treeline. The vegetation is comprised of low rounded shrubs interspersed with numerous species of perennial herbs, including bulbous geophytes, and a number of annual species (see Arroyo et al. 1981; Cavieres, in press). Here *P. secunda* is found in the open spaces between shrubs. Individual rosettes are slightly larger than in population 1 and had more but shorter inflorescences (Cavieres 2000).

### Population 3

This was located at 2900 m elevation in the lower andean belt dominated by cushion plants such as *Laretia acaulis* (Cav.) Gill. et Hook. (Umbelliferae) (Cavieres et al. 1998). The lower andean belt in the Rio Molina valley extends from 2600–3100 m. The dominant cushion species vary as to cover and are interspersed with numerous species of perennial herbs, a few geophytes and still some annuals (e.g. *Chaetanthera euphrasioides* (DC) Meigen, *Mecrosteris gracilis* (Dougl. ex Hook.) Greene (Cavieres, in press) are present. In this vegetation belt, *P. secunda* is one of the most abundant perennial species. Basal rosettes are about 30 cm diameter and support around 11 inflorescences, which are considerably shorter than those at Population 2 (Cavieres 2000).

### Population 4

This was located at 3400 m elevation in the high andean belt dominated by a mixture of cushion plants such as *Azorella madreporica* and *Nassauvia pyramidalis* Meyen (Compositae). The high andean belt located in the upper tributaries of the Rio Molina valley extends from 3200–3700 m (Cavieres, in press). Vegetation is low and sparse, with many species of perennial herbs interspersed among the dominants. In this vegetation belt, scattered but dense patches of *P. secunda* are found on finer and often reworked soil. At this elevation, rosettes are 30 cm in diameter and may have up to 25 individual semi-prostrate inflorescences (Cavieres 2000). At this elevation snow cover persists for 3 to 5 months each year (Cavieres 1999).

Fully ripened seeds were bulk harvested from over 50 individuals per population during the austral summer of 1996 (January to April, depending on site elevation) and stored in paper bags in a cool dry place under laboratory conditions until initiation of the experiments up to 3 months later.

### Seed viability

Initial seed viability was assessed with the Tetrazolium test (Hendry & Grime 1993) before the initiation of experiments. Four replicates of 50 seeds each, from each population, were placed on moist filter paper at room temperature for 24 h and then sliced along the longitudinal axis with the aid of a scalpel. Both seed sections were incubated in a 0.1% aqueous solution of tetrazolium chloride for 24 h at 25 °C in darkness. Seeds showing a strong red-stained embryo were considered viable.

### Stratification

After bulk collection, about 6000 seeds from each population were stored in humid and dark conditions at 4 °C in a laboratory refrigerator (Hendry & Grime 1993). To maintain humidity seeds were wrapped in white paper towels which were kept wet throughout the stratification period. Seeds were touching the wet surface of paper towels. To ensure darkness, petri dishes were wrapped in aluminum foil. Seeds were maintained in those conditions for 1 to 6 months. As a control (thereafter unstratified seeds), around 6000 seeds from each population were stored in a cool-dry place at ambient temperature in paper bags in the laboratory, and maintained in those conditions for 1 to 6 months.

To assess any loss of viability with treatment (stratified or unstratified seeds), seeds were tested at the beginning of the experimental period, once each month for 6 months.

### Thermoperiod

Two growth chambers with a controlled thermoperiod and photoperiod (12:12 h light/dark) were used for germination assays. One chamber was set for a 20 °C day and 10 °C night while a second was set for a 10 °C day and 5 °C night. The thermal and particular light regime were selected in representation of field conditions in early spring at the two extremes of the elevation range over which the seeds of *P. secunda* were derived. Chambers were illuminated with cool-white fluorescent light with an average photosynthetic photon flux density of 25  $\mu\text{mol m}^{-2}\text{s}^{-1}$ .

### Germination assays

Each month, for a total period of 6 months (June, July, August, September, October, November), 400 seeds per population were retrieved from the refrigerator and placed between two pieces of wet white paper towels in 8 petri dishes containing 50 seeds each. Additionally, a parallel set of 8 petri dishes with 50 seeds each was taken from the bulk collections maintained under dry laboratory conditions.

Both for stratification and control, one set of 4 petri dishes of each population was placed in each growth chamber. Germinated seeds were recorded and removed from the petri dishes every 2 or 3 days for a period of 1 month since the initiation of the experiment. Visible radicle profusion was the criterion for

recording germination (Hendry & Grime 1993). After the 1 month period, all non-germinated seeds were tested for viability by the Tetrazolium test as explained earlier.

### Statistical analyses

The effects of stratification and thermoperiod on final germination percentages of seed collected from different populations were assessed by Multifactorial ANOVAs (Potvin 1994). The model considered stratification treatment and control nested on a thermal regime. The dependent variable was the final germination percentage arcsin transformed (Steel & Torrie 1981).

## Results

### Seed viability

Initial average viability of *Phacelia secunda* seeds was very high at all sites: Population 1 (1600 m): 98.8%; Population 2 (2200 m): 100%; Population 3 (2900 m): 99.3% and Population 4 (3400 m): 97.5%. There were no significant differences in seed viability with altitude ( $F_{3,12} = 1.921$ , n.s.). Throughout the entire experimental period, seed viability remained high, with no significant differences between seeds placed to germinate at different times after collection ( $F_{3,24} = 3.038$ , n.s.).

### Germination of unstratified seeds

Not a single seed of *P. secunda* germinated in the dark and light treatments under the two thermal regimes considered in freshly collected seeds, although many seeds remain viable as determined by the Tetrazolium test.

Unstratified seeds from all populations began germination after 3 months stored in the laboratory, reaching very low percentage of germination (< 10%). Low germination occurred at the two thermal regimes and both in light and dark conditions (Figure 1).

However, under the two thermal regimes, the elapsed time stored in the laboratory in all seeds sources significantly increased the final percentage of germination (Table 1). This suggests that there is an effect of seed age on the final germination percentage without stratification.

Table 1. F values of a multifactorial ANOVA to detect the effect of period of storage and thermoperiod on final percentage of germination on unstratified *Phacelia secunda* seeds, from different elevations in the Andes of central Chile (33 °S).

Seed source (m.a.s.l.)	Storage period $F_{3,72}$	Thermoperiod $F_{1,72}$	Interaction
1.600	10.424*	3.423	1.850
2.200	11.751*	3.599	0.454
2.900	14.945*	7.349*	0.176
3.400	5.292*	8.221*	0.878

\* $P < 0.01$ .

### Effect of stratification

For all seed populations, at each stratification period, final germination with stratification was significantly higher than germination without it ( $F_{1,144} = 7238.4$ ,  $p \ll 0.001$ ).

Under the two thermal regimes, the stratification period necessary to initiate seed germination as well as to reach the maximum germination, varied according to the seed source (Figure 2). Stratification significantly increased the final percentage of germination from all the populations (Table 2).

Seeds from lower elevations (1600 and 2200 m) needed shorter period of stratification to begin germination and to reach germination higher than 80% (Figure 2). Seeds from higher elevations (2900 and 3400 m) require longer periods of stratification to begin germination and to reach a maximum lower than 60% of germination (Figure 2). This percentage of maximum germination was significantly lower than those obtained for seeds from lower elevations (Table 2). The percentage of total variation explained with stratification decreased with elevation of seed source (Table 3), while the percentage explained by the elapsed time (with or without stratification) increased (Table 3). According to these results, seeds from sites at increasing elevation tend to require longer periods of stratification to initiate as well as to reach maximum number of seeds germinated. However, elapsed time has an increasing importance on germination with elevation of seed source.

### Effect of the thermal regime

Thermoperiod had a significant effect on germination of stratified seeds from all sites (Table 2), however, except for those from 3400 m, the proportion of explained variance is low (Table 3). For unstratified

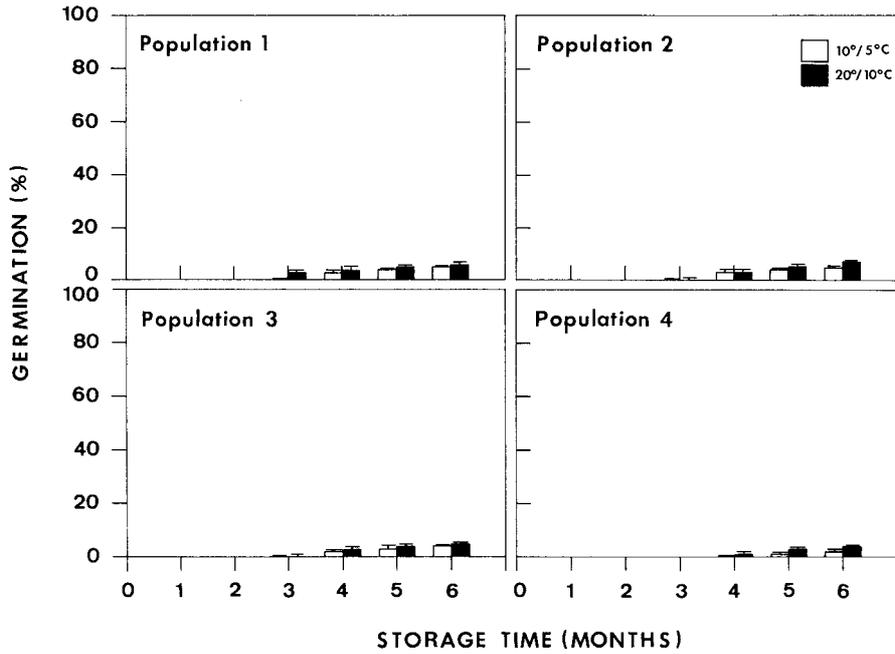


Figure 1. Final germination in seeds of *Phacelia secunda* ( $\pm 2$  E.E.) collected at different elevations in the mediterranean Andes of central Chile and maintained for different time intervals at laboratory conditions (ca. 25 °C, dark). Number in upper right of each graph, indicates the time of storage in laboratory (months). Seeds were germinated for 30 days at two thermal regimes (10 °/5 °C and 20 °/10 °C) in light and dark conditions. After 1 and 2 months of storage no seed germinated.

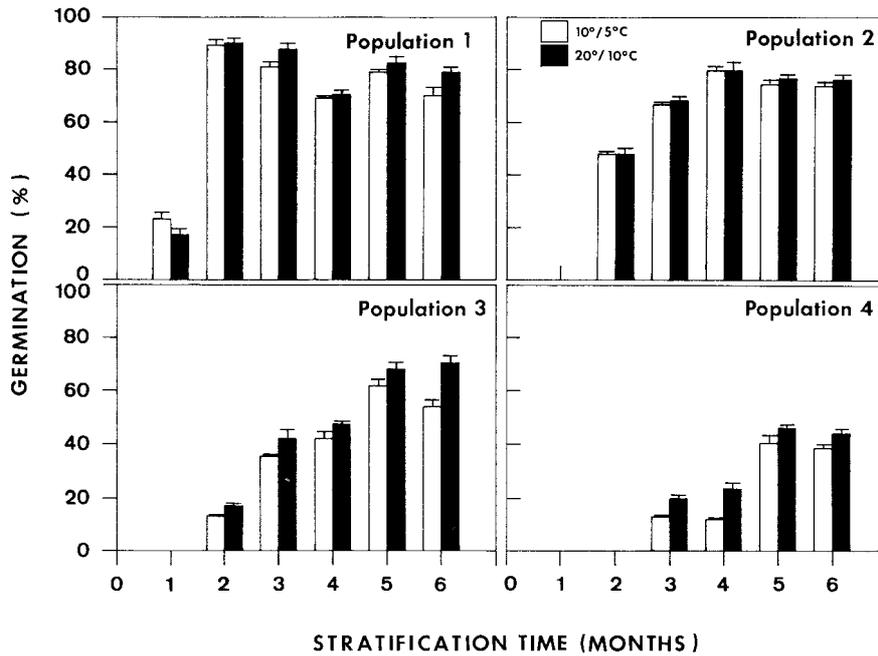


Figure 2. Final germination in seeds of *Phacelia secunda* ( $\pm 2$  E.E.) collected at different elevations in the mediterranean Andes of central Chile and maintained for different time intervals in stratification (4 °C, dark). Number in upper right of each graph indicates the months of storage in laboratory. Seed were germinated for 30 days at two thermal regimes (10 °/5 °C and 20 °/10 °C) in light and dark conditions. No bars indicate no germination.

Table 2. F values of a multifactor ANOVA to detect the effect of treatment (stratification and control) on final percentage of germination on *Phacelia secunda* seeds, collected at different elevations in the Andes of central Chile (33 °S).

Seed source (m.a.s.l.)	Factors			Interactions	
	1 Treatment	2 Storage period	3 Thermoperiod	1*2	2*3
1.600	3469.414*	180.627*	14.939*	54.967*	4.093*
2.200	3253.092*	377.927*	6.362*	133.378*	1.627
2.900	1907.216*	497.478*	45.523*	89.925*	4.290*
3.400	419.659*	186.761*	32.780*	47.675*	4.498*

\*  $P < 0.01$ .

Table 3. Percentage of explained variance of factors considered in Table 2, that show significant effect on final percentage of germination on *Phacelia secunda* seeds from different elevations in the Andes of central Chile (33 °S).

Seed source (m.a.s.l.)	Treatment	Months	Thermoperiod	1*2	2*3	error
1.600	92.6	4.9	0.4	1.4	0.1	0.1
2.200	79.5	16.4	0.1	3.2	–	0.1
2.900	71.9	22.3	1.7	3.4	0.1	0.2
3.400	62.1	25.5	4.0	5.9	0.4	0.5

seeds, thermoperiod had a significant effect only on seeds from higher elevation sites (2900–3400, Table 2), with higher germination at 20 °/ 10 °C (Figure 2). For stratified seeds, thermoperiod had a significant effect on final percentages of germination, except in seeds from 2200 m (Table 1). In seeds from the 1600 m site, after 1 month of stratification, germination was higher at 10 °/ 5 °C than at 20 °/10 °C (Figure 2). In seeds from higher elevation sites (2900 or 3400 m), germination was higher at 20 °/10 °C (Figure 2).

## Discussion

For many plant species, cold stratification is known to increase germination (Bewley & Black 1982). In *Phacelia secunda* populations from the Andes of central Chile, independently of the collection elevation, seeds do not germinate immediately after being dispersed by the parent plant. They require a minimum period of cold stratification or dry storage at room temperature to acquire the capacity to germinate. Cold stratification is needed to initiate early and to reach maximum germination rates.

Length of the stratification period required for germination changes for populations from different elevations. At higher elevation, seeds of *Phacelia* require longer periods of cold stratification to initiate germination and to reach their maximum percentage of germination. For example, seeds from the 1600 m site only need 1 month of cold stratification to begin germination, while seeds from the 3400 m site need 3 months. The stratification periods necessary for germination has also been found to increase with elevation of seed source in other plant species growing along altitudinal gradients (Billings & Mooney 1968; Dorne 1981; Borghetti et al. 1989). Hence, this may be a typical pattern for species with wide altitudinal distribution, and an adaptation for the increase of duration of snow cover with elevation.

Due to differences in reproductive phenology among populations (Arroyo et al. 1981) seeds from lower elevation were 3–4 months older than seed from higher elevation. Seed germination was higher in seeds from higher elevation stored for longer time, suggesting that in addition to stratification, seeds also needed time to ripe to germinate (dry storage after-ripening). This could suggest that differences in germination between low and high elevation seeds were related with

age differences. However, even in seeds from higher elevations, germination of unstratified seeds was significantly lower than cold stratified seeds, and the proportion of total variance explained by treatment is higher than explained by the elapsed time (Table 3), suggesting that cold stratification is a more important factor on seed germination in *P. secunda*.

If longer stratification requirement to initiate seed germination is a response to longer snow cover, one might expect to find almost universal seed dormancy as a protective mechanism against premature germination in alpine seeds. In an early review of germination requirements in alpine species (Amen 1966), it was established that only about 40% of 60 species present dormancy, but few of them require stratification for germination. However, with more recent information, stratification improved germination performance in many arctic and alpine species (Chabot & Billings 1972; Bell & Bliss 1980; Marchand & Roach 1980; Reynolds 1984; see Baskin & Baskin 1998).

The short stratification period needed by seeds of population of *Phacelia* in montane sclerophyllous forest (1600 m) also seems to be adaptive. In this habitat winters are relatively mild, with short snow cover. However, risks of seedling death from drought increases rapidly as summer progress (Peñaloza 1996). Seeds that germinate as soon as snow melt, have seedlings that grow and develop extensive root systems that enable them to successfully overcome the drought stress of late summer.

Seeds from higher elevation sites (2900 and 3400 m) shows significantly higher germination at 20 °/10 °C than at 10 °/5 °C. Billings & Mooney (1968) report that optimum germination temperatures in many tundra species are surprisingly high (20 °–30 °C). Similar patterns have been reported by Sayers & Ward (1966) for alpine species at the Rocky Mountains, Chabot & Billings (1972) for alpine species in the White Mountains, Marchand & Roach (1980) for alpine pioneer species in the White Mountains, and by Kibe & Mazusawa (1994) for *Carex doenizzi* seeds from the alpine zone of Mt. Fuji. All these authors suggest that this germination response favors germination late in spring, under higher temperatures and lower probability of late season frosts that could damage the new seedlings. In contrast, seeds from lower elevation sites (1600 and 2200 m) do not show differences in germination between the two thermal regimes assayed, although seeds from the lower site (1600) germinate higher at lower temperatures could result in earlier seedling establishment allowing the development of a

root system appropriate to successfully overcome the summer stress conditions (Peñaloza 1996).

After 6 months of stratification, seeds from higher elevation site reached the lowest percentages of germination (< 50%), and many ungerminated seeds remained viable. This suggests that seeds from higher elevations have a stronger dormancy than those from lower altitudes. Because this dormancy is not completely broken by stratification in the laboratory or in the field (Cavieres 1999), we propose that seeds have deep physiological dormancy (*sensu* Nikolaeva 1969). The germination response to gibberellic acid would be needed to support or refute our suggestion.

We observed considerable between population variation in germination responses. This variation may be adaptive since it was strongly correlated with the duration of snow cover at different sites. Currently, there is much discussion in the literature about the adaptive value of germination strategies (including dormancy mechanisms) (Meyer & Monsen 1991). Species with broad distributions have shown variation in dormancy mechanisms (Billings & Mooney 1968; Meyer & Monsen 1991) which suggests that dormancy mechanisms can be under selection by physical environment in which species grow (Meyer et al. 1989).

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