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Dormancy break and germination in *Lomatium dissectum* seeds

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Abstract

- *Background and aims Lomatium dissectum* (Apiaceae) is a perennial, herbaceous plant of wide distribution in Western North America. At the time of dispersal, *L. dissectum* seeds are dormant and have underdeveloped embryos. The aims of this work were to determine the requirements for dormancy break and germination, to characterize the type of seed dormancy, and to determine the effect of dehydration after embryo growth on seed viability and secondary dormancy.

- *Methods* The temperature requirements for embryo growth and germination were investigated under growth chamber and field conditions. The effect of GA_3 on embryo growth was also analyzed to determine the specific type of seed dormancy. We tested the effect of dehydration on seed viability and induction of secondary dormancy in seeds where embryos had elongated about four fold their initial length. Most experiments examining the nature of seed dormancy were conducted with seeds collected at one site in two different years. To characterize the degree of variation in dormancy breaking requirements among seed populations, we compared the stratification requirements of seeds collected at eig different sites.

- *Key results* Embryo growth prior to and during germination occurred at temperatures between 3 and 6 °C and was negligible at stratification temperatures of 0.5 and 9.1 °C. Seeds buried in the field and exposed to natural winter conditions showed similar trends. Interruption of the cold stratification period by eight weeks of dehydration decreased seed viability by about 30% and induced secondary dormancy in the remaining viable seeds. Comparison of the cold stratification requirements of different seed populations indicates that

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seeds collected from moist habitats have longer cold stratification requirements that those from semiarid environments.

- *Conclusions* Seeds of *L. dissectum* have deep complex morphophysiological dormancy. The requirements for dormancy break and germination reflect an adaptation to trigger germination in late winter.

Key words: Apiaceae, cold stratification, *Lomatium dissectum*, morphophysiological dormancy, secondary dormancy, seed germination.

INTRODUCTION

   Lomatium dissectum (Nutt.) Mathias and Constance (fernleaf biscuitroot) is a perennial plant within the Apiaceae family. This species is native to Western North America, where it has a wide distribution and occupies habitats with dissimilar characteristics (Hitchcock and Cronquist, 1973). There are two varieties of *L. dissectum*, *L. dissectum* var. *dissectum* and *L. dissectum* var. *multifidum* (Nutt.) Mathias & Constance (USDA Plants Database). *Lomatium dissectum* var. *dissectum* is more common west of the Cascade Mountains in areas with a mesic climate and average annual precipitation of more than 1000 mm. In contrast, *L. dissectum* var. *multifidum* is more frequent east of the Cascade Mountains in semiarid habitats, where it is found at elevations ranging from 800 to 2200 m. Independent of variety or location, *L. dissectum* flowers in early spring and produces fruits (schizocarps) that ripen in early summer (Hitchcock and Cronquist, 1973). At the time of dispersal, *L. dissectum* seeds (mericarps-fruits) are dormant and have underdeveloped, linear

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embryos. These embryos are approximately one eighth of the seed length and increase several-fold in length inside the seed before germination.

The presence of underdeveloped embryos is common among Apiaceae species. However, the type of dormancy and the conditions that promote embryo growth and germination vary among species within this family. Types of seed dormancy found within the Apiaceae include morphological and morphophysiological dormancy (MPD). Seeds of *Pastinaca sativa* and *Conium maculatum* exhibit morphological dormancy (Baskin and Baskin, 1979; 1990b). In these species, favourable conditions of moisture and temperature lead directly to embryo growth and ultimately to germination (Baskin and Baskin, 2004). More often species with underdeveloped embryos have additional requirements to break dormancy. These species are considered to have MPD; which is divided into eight types depending on the temperature requirements for dormancy break and embryo growth, and the ability of gibberellic acid (GA_3) to overcome dormancy (Baskin and Baskin, 2004).

At least, four types of MPD have been reported in the Apiaceae. Seeds of *Chaerophyllum tainturieri* and *C. procumbens* have nondeep simple MPD (Baskin and Baskin, 1990a; Baskin *et al*., 2004). Warm moist conditions during summer first break physiological dormancy, while warm moist conditions during the fall are associated with embryo growth and germination. Among species that germinate during the spring, some such as *Osmorhiza longistylis* and *O. claytonii* have nondeep complex MPD; they require warm stratification followed by cold stratification to break dormancy (Baskin and Baskin, 1984; 1991). Other species including *Osmorhiza depauperata* and *Chaerophyllum temulum* have deep complex MPD; they only require cold stratification (Walck and Hidayati, 2004; Vandelook *et al*., 2007a). In species with deep complex MPD, dormancy loss and embryo

growth occur during cold stratification and GA$_3$ cannot replace the requirement for cold stratification (Baskin and Baskin, 2004). Recently, non-deep simple MPD that is broken by cold stratification has also been reported within the Apiaceae (Vandelook *et al*., 2007*b*). Vandelook *et al* (2007*b*) observed that seeds of *Selinum carvifolia* and *Angelica sylvestris* required cold stratification to break physiological dormancy, while embryo growth and germination occurred under mild temperatures.

Various environmental conditions may play a role in regulating dormancy of *L. dissectum* seeds. Under natural conditions, these seeds are exposed to dry and warm environments during the summer, mild temperatures and moist conditions during the fall and chilling temperatures during the winter. Seeds then germinate during the late winter or early spring. The effects that the various environmental conditions have on seed dormancy are unclear. The dry summer period could contribute to dormancy loss by a process known as dry after-ripening (Allen and Meyer, 1998; Baker *et al*., 2005). In addition, the mild and moist conditions during the fall could be considered a form of warm stratification that may favour embryo growth (Baskin and Baskin, 1998). Alternatively, summer and fall conditions may not affect the dormancy status of the seeds. Under this scenario, embryo growth and dormancy loss would occur only during the winter.

Nondormant seeds of some species can re-enter dormancy if environmental conditions are unfavourable for germination (Hilhorst, 1998; Krebreab and Murdoch, 1999). This type of dormancy is known as secondary dormancy and it is important in the formation of persistent seed banks (Baskin and Baskin, 1998). Depending on the species, several factors can induce secondary dormancy, including temperature extremes, hypoxia, and water stress (Baskin and Baskin, 1980; Kebreab and Murdoch, 1999; Momoh *et al.*, 2002; Baker *et*

al., 2005). In *L. dissectum*, seeds may not complete their stratification requirements during the winter or they may experience unfavourable germination conditions in the spring. These seeds, particularly those in semiarid regions, would then encounter the dry summer period. The fate of these seeds is unclear; dehydration may induce secondary dormancy or alternatively reduce seed viability. Although few studies indicate that desiccation induces secondary dormancy, this effect was observed in *Carica papaya* and *Panicum virgatum* seeds (Wood *et al.*, 2000; Shen *et al.*, 2001). Moreover, for *Panicum virgatum* desiccation reversed the effect of cold stratification on breaking dormancy. Also, in *Anemone nemorosa*, a species with morphological dormancy, loss of desiccation tolerance occurs at late stages of embryo growth inside the seed (Ali *et al.*, 2007). Similar phenomena may occur in *L. dissectum*.

The aims of this study were to determine the type of seed dormancy in *L. dissectum*, to analyze the temperature requirements for embryo growth and germination, and to investigate the effect of dehydration on the induction of secondary dormancy. We conducted most of the experiments with seeds of *L. dissectum* var. *multifidum* collected at one site. In addition, we investigated the cold stratification requirements of three other populations of var. *multifidum* and four populations of *L. dissectum* var. *dissectum*. Analysis of these populations allowed us to gain an insight into the variation in dormancy breaking requirements of plants growing in different habitats (Allen and Meyer, 1998).

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MATERIALS AND METHODS

Seed collection and preparation

Unless otherwise indicated, seeds (mericarps) of *L. dissectum* var. *multifidum* were

collected near Harper, Oregon, USA (43° 33' N, 117° 47' W) during June 2005 and 2007.

We collected the seeds at the point of natural dispersal, when they were dry on the plants.

Seeds were rinsed with running water and surface sterilized by soaking them in 70% ethanol

for 1 min and 0.5% sodium hypochlorite for 30 min. Subsequently, we rinsed the seeds with

deionised water and dried them to a water content of about 8%. The dry seeds were stored in

dark bottles at room temperature (about 21 °C) until used. The experiments were conducted

with seeds that were in storage from two weeks to four and half months. For a particular

experiment, however, the seeds were of the same age. Preliminary experiments suggested

that storage for up to one year does not affect seed dormancy status. Seeds that were cold

stratified two weeks after harvest showed a time course of embryo growth similar to that of

seeds stored for one year (data not shown). Prior to use, the seeds were treated with the

fungicide Captan at approximately 0.01 g Captan g^{-1} of seeds.

Temperature requirements for embryo growth and germination

The effect of temperature on embryo growth and germination was analyzed in three

separate experiments using seeds collected in 2005. The first experiment was aimed at

determining the effects of warm and cold stratification on embryo growth. Approximately

two weeks after harvest, the seeds were placed on germination paper (grade 632, Hoffman

Manufacturing Inc., Jefferson, OR) moistened with deionised water inside clear plastic boxes

(10 x 10 x 4 cm). We placed fifty seeds in each box, covered the boxes with lids, and

maintained the paper moist throughout the stratification. The experiment consisted of a

completely randomized 4 x 7 factorial combination of warm stratification at 20 °C

(exposures times of 0, 2, 4, and 6 weeks) followed by cold stratification at 5 °C (exposures

times of 0, 2, 4, 6, 8, 10, and 12 weeks). Four boxes were prepared for each treatment

combination. The growth chambers provided a 12 h photoperiod with fluorescent lamps that

supplied 35 μmol m^{-2} s^{-2} PAR. At the end of each treatment, we excised the embryos from

the seeds and measured their lengths through a dissecting microscope. We only measured

the embryo length of ungerminated seeds. Following germination, the embryos elongated

very rapidly. Inclusion of those measurements would have overestimated the embryo growth

that occurred inside the seed. In situations where the embryos just appeared to emerge from

the seeds, their lengths ranged between 8 and 10 mm.

A second experiment was conducted to analyze the distribution of embryo growth

during cold stratification. Three months after harvest, we prepared five boxes with 100 seeds

per box. After 0, 2, 4, 6, 8, 10, 12, and 14 weeks of cold stratification at 4 °C, ten

ungerminated seeds were taken from each of the five replicate boxes. We used these seeds to

measure the lengths of the whole embryo, the embryo axis and cotyledons, and then calculate

the embryo axis: cotyledon ratio.

The third experiment was aimed at determining the range of temperatures under

which embryo growth and germination can occur. Four months after harvest, we prepared 60

boxes with 60 seeds per box. Fifteen boxes were placed in each of four germination

chambers at temperatures of 0.5 ± 1.5 (mean ± s.d.), 3.4 ± 0.4, 5.5 ± 0.2, and 9.1 ± 0.7 °C.

Five boxes were used to measure embryo growth and ten to measure germination. For each

temperature and retrieval time (4, 6, 8, 10, and 12 weeks), ten ungerminated seeds were taken

from each of the five replicate boxes and the embryo length measured as described previously. Similarly for each temperature and at weekly intervals, we counted and discarded germinated seeds in each of ten replicate boxes. Germination was recorded at radicle emergence (~2 mm) and was monitored for a period of 30 weeks. The cumulative germination time courses were used to estimate the final germination and the mean germination time (MGT). The values of final germination simply represent the number of seeds that germinated during the 30 weeks in the growth chambers. The mean germination time was estimated as $MGT = \sum_1^i n_i.t_i / N$; where n_i is the number of seeds that germinated within consecutive intervals of time, t_i is the time between the beginning of the test and the end of a particular interval of measurement, and N is the total number of seeds that germinated (Hartmann and Kester, 1983).

Effect of GA_3 on embryo growth

The effect of GA_3 on embryo growth was investigated in seeds collected in 2007, which have been in storage for approximately three months.   We tested four concentrations of GA_3 (0, 0.03, 0.3, and 3 mM) at two temperatures (4 and 12 °C) for a total of eight treatments. Gibberellic acid was dissolved in deionised water and the pH adjusted to 6.5 with 0.1-1.0 mM KOH. Fifty seeds were placed on germination paper and 15 ml of water or GA_3 solution were added per box. Each treatment was replicated four times. Embryo lengths of ten ungerminated seeds from each box were measured after 4, 6, 8, 10, and 12 weeks.

Desiccation tolerance and induction of secondary dormancy in cold stratified seeds

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At the time of dispersal, *L. dissectum* seeds have embryos that tolerate dehydration. The seeds are dry at the time of dispersal and can be stored dry for prolong periods without a decrease in viability (data not shown). After 8 to 10 weeks of cold stratification, the seeds have embryos that are several times their initial size. The effect of dehydration on seed viability and dormancy status of elongated embryos was tested using the 2007 collection with seeds that have been in storage for two months. We prepared ten boxes with 100 seeds per box. These seeds were moistened and placed in a germination chamber at 4 °C for 10 weeks. Subsequently four boxes were maintained moist at 4 °C, while the seeds in the other boxes were dried at room temperature to a water content of about 8%. The dried seeds were stored at room temperature for 8 weeks; which approximates the period that seeds may remain dry during the summer in semiarid areas of western Oregon and southern Idaho. The seeds were then rehydrated and transferred to a germination chamber at 4 °C. Germination was monitored at weekly intervals for an additional 20 weeks on six replicate boxes with 70 to 100 seeds per replication. In addition, we monitored the viability of the embryos prior to dehydration, immediately following rehydration, and at the end of the experiment in the same batch of seeds used to measure germination. Viability was determined by the tetrazolium chloride test. Ten ungerminated seeds were taken from each of the six replicate boxes. Embryos were removed from the seeds and incubated for 12 h in solutions containing 0.1% tetrazolium chloride in phosphate buffer saline (Hartmann and Kester, 1983). We incubated the embryos in the dark at room temperature. The development of red colour throughout the embryo was taken as an indication that the seeds were viable.

Embryo growth and germination under field conditions

To analyze embryo growth and germination under natural conditions, we conducted two field experiments. The first was started in November 2005 and the second in November 2007 using seeds collected during the previous spring. The seeds were placed in the field in November rather than after harvest to avoid seed molding during early fall. Seeds were cleaned as described earlier and placed into nylon mesh bags. Sixty seeds were placed into each bag at the bags were buried at a depth of 2-5 cm in experimental plots at the Idaho Botanical Garden (Boise, Idaho, USA, 43° 36' N, 116° 13' W). Twenty-four bags were buried into one of ten plots, which were separated by plastic garden dividers. Thermocouples and moisture probes (Echo 5 and Echo 20, Decagon Devices, Inc. Pullman, WA) were buried at the plots at a depth of 2 –7 cm. Temperature and soil moisture was monitored at hourly intervals. To relate the values of soil moisture to the water potential of the soil, we determined the moisture release curve of the soil as described by Kursar *et al*., 2005. The moisture release curve was estimated from the average of five soil samples. Once a month, one seed bag from each plot was collected and taken to the laboratory. From each bag, 10 ungerminated seeds were used to measure embryo length. Germination that occurred in situ was determined by counting the number of seedlings or the number of seeds that were empty and had a slit at the site of radicle emergence. This second approach was followed because seedlings left in the bags during late spring dried out and it was difficult to determine germination by counting the number of seedlings. For the experiment started in 2005, some seeds were left in the field over the summer. On June 29, July 25, and October 1, 2006, we collected ten bags of seeds and determined the viability of the ungerminated seeds by the tetrazolium chloride test as previously described.

Stratification requirements of different seed populations

We collected seeds for four populations of *L. dissectum* var. *dissectum* and for four populations of *L. dissectum* var. *multifidum* during the spring of 2007. Seeds of *L. dissectum* var. *dissectum* were collected at four sites at the Willamette Valley, Oregon, USA. The four sites were: Adair *1* (44° 48' N, 123° 14' W; 81 m elevation), Adair *2* (44° 40' N, 123° 13' W; 86 m), Buell *1* (45° 2' N, 123° 28' W; 117 m), and Buell *2* (45° 1' N, 123° 24' W; 178 m). Seeds of *L. dissectum* var. *multifidum* were collected at Prairie, Oregon (43° 31' N, 115° 58' W; 1287 m elevation); Harper, Oregon (43° 33' N, 117° 47' W; 1347 m); Fairfield, Idaho (43° 10' N, 114° 40' W, 1700 m); and Moore's Mountain, Idaho (43° 47' N, 116° 5' W; 2200 m). For each site, we prepared ten boxes with 60 seeds per box. The boxes were incubated at 4 °C and germination was measured at weekly intervals for a period of 28 weeks. With the seeds collected at the lowest elevations, Adair *1* and Adair *2*, we also tested germination at higher temperature. These seeds were first placed at 4 °C for 12 weeks and then some seeds were kept at this temperature while others were exposed to a daily (12/12 h) alternating temperature regimen of 5/15 °C.

Statistical analysis

All experiments were analyzed using the MIXED procedure in SAS 9.1 (SAS Institute Inc., Cary, NC). When needed, different variances were modelled into the MIXED procedure to allow for unequal variance between treatments (Littell *et al*., 1996). For the experiment involving the analysis of warm and cold stratification on embryo growth, fixed factors in the analysis were warm and cold stratification, and the interaction of these factors. In the experiment to test the effect of GA_3 on embryo growth, fixed factors were GA_3,

temperature, and the interaction between GA_3 and temperature. All the other experiments

had only one fixed factor: site for the comparison of the cold stratification requirements of

different populations or temperature for the analysis of the effect of various temperatures on

embryo growth and percent germination. For the field experiment, we also compared

changes in embryo length and percent germination over time. In this case, time was the fixed

factor. The significance of pairwise differences between treatment means was evaluated at P

< 0.05, using a Tukey-Kramer adjustment for multiple comparisons.

RESULTS

Temperature requirements for embryo growth and germination

Prior to stratification, the average embryo length was 1.35 ±0.03 mm, approximately 15% of the seed length. Neither warm stratification at 20 °C nor the interaction between warm and cold stratification had an effect on embryo growth. In contrast, cold stratification at 5 °C had a significant effect on embryo length ($P < 0.0001$); this was not affected by prior warm stratification periods (Fig. 1).

During 14 weeks of cold stratification, the embryo length increased approximately seven fold to 7.4 ± 0.4 mm (Fig. 2a) due to elongation of both the embryo axis and the cotyledons. Elongation, however, was more rapid in the cotyledons, which lead to a decrease in the embryo-axis to cotyledon ratio from 0.67 ± 0.04 to 0.35 ± 0.04 during the 14 weeks of stratification (Fig. 2 a, b).

Further analysis of the effect of temperatures below 10 °C on embryo length indicated that small differences in temperature can markedly affect embryo growth. After 6 weeks of incubation, seeds at 0.5 or 9.1 °C had embryos that were shorter than those at 3.4 and 5.5 °C (Fig. 3). This difference remained after 12 weeks of stratification (Fig. 3) when seeds at 0.5 and 9.1 °C had embryo lengths of 4.0 ±0.8 and 3.7 ±0.6 mm, respectively compared with 7.5 mm at 3.4 or 5.5 °C.

Continued incubation at 3.4 and 5.5 °C resulted in germination, albeit to different extents. The final germination was higher at 3.4 °C (87%) than at 5.5 °C (64%) (Fig. 4). In addition, the MGT at 3.4 °C (13.0 weeks) was shorter than at 5.5 °C (15.5 weeks). Thus, germination occurred more rapidly at 3.4 than at 5.5 °C, even though embryo elongation was

similar at these temperatures. Germination at 3.4 and 5.5 °C was also higher than at 0.5 and

9.1 °C where few seeds germinated (Fig. 4).

Effect of GA$_3$ on embryo growth at 4 and 12 °C

Embryo growth was higher at 4 than at 12 °C and GA$_3$ did not substitute the

requirement for low temperatures. At 12 °C, embryo elongation was minimal and no

differences were observed between seeds incubated in water and those incubated in GA$_3$

(Fig. 5). At 4 °C, the elongation of the embryo was similar in water and in 0.03 and 0.3 mM

GA$_3$, while 3 mM GA$_3$ delayed embryo growth. Furthermore, seeds incubated in 0 to 0.3

mM GA$_3$ at 4 °C had germination percentages of 45 ±5.3 and 72 ±5.2% after 10 and 12

weeks, respectively. Seeds incubated in 3 mM GA$_3$ at 4 °C showed much lower germination,

0 and 12 ± 5 % after 10 and 12 weeks, respectively. No germination was observed in seeds

incubated at 12 °C.

Desiccation tolerance and induction of secondary dormancy in cold stratified seeds

For seeds that were cold stratified for 10 weeks, dehydration caused a decrease in

seed viability from 96 ± 3 to 61 ± 7 % (Fig. 6). The seeds that survived the dehydration

treatment did not germinate after rehydration, but began to germinate after 7 weeks of cold-

moist conditions. The final germination was, however, much lower than in the control seeds;

33 ±3 and 97 ±3 % for the dried and control seeds, respectively (Fig. 6). The lower

germination of the dried seeds was not entirely due to a loss of viability. Of the seeds that

had not germinated, 29 ±9 % remained viable. These seeds appeared to have entered a very

deep dormancy that was not broken by 22 weeks of cold stratification.

Embryo growth and germination under field conditions

For seeds buried on November 18, 2005, minimal embryo growth occurred during the first month in the field (Fig. 7a). This coincided with a period when average daily soil temperatures largely remained between -6 and 2 °C (Fig. 7b). The second month in the field was the period with the largest increase in embryo length; the embryos grew from 1.44 ±0.03 to 4.5 ±0.4 mm (Fig. 7a). During this time, the predominant temperatures were between 0 and 6 °C and the soil had high moisture content, in general above 10% (-0.12 MPa; Fig. 7b). Growth of the embryo continued until March 2, 2006, when the average embryo length was 6.4 ±0.2 mm. At this time, 31 ±10 % of the seeds had germinated (Fig. 7a). We also recorded germination on March 17, April 21, and June 2, 2006; the values recorded were 48 ±8, 60 ±12, and 50 ±8 %, respectively (Fig. 7a). Although these percents were somewhat higher than that recorded on March 2, the differences were not statistically significant. This suggests that little germination occurred during April and May. Similarly, no increase in germination was observed for the seeds that were collected on June 29, July 25, and October 1; which had germination percentages ranging from 42 to 52 %. Of the seeds that did not germinate by the beginning of the summer (June 29, 2006), 81 ± 3 % were viable. One month later this percent declined to 52 ±7 % and further declined to 30 ±7% by the beginning of the fall.

For the second field experiment that started on November 2007, the results were overall similar to those described above. Embryo growth and germination occurred while the average daily soil temperatures were below 5°C (Fig. 8). There were, however, some differences in the time course of embryo growth and germination. In the 2007 experiment,

the embryos reached lengths of about 6 mm one month earlier than in the 2005 experiment. Similarly, germination occurred sooner in the 2007 experiment and the final germination was about 30% higher than in the 2005 experiment.

Stratification requirements of different seed populations

Like the seeds collected at Harper-Oregon, seeds collected at other sites germinated at a low temperature (~ 4 °C). However, there were differences in the cold stratification requirements of the various populations. Seeds of *L. disssectum* var. *multifidum* from the intermediate elevations (1287, 1347, and 1700 m) began to germinate after 8 weeks (Fig. 9). In contrast, seeds from Moore's Mountain (2200 m) and seeds of *L. dissectum* var. *dissectum* began to germinate after 10 weeks. The MGT for seeds of *L. dissectum* var. *multifidum* collected at intermediate elevations were between 10.5 and 11 weeks, which were significantly shorter than those of *L. dissectum* var. *dissectum* seeds (14.4 to 16 weeks). The seeds collected at the highest elevation (2200 m) had an intermediate MGT of about 13 weeks. The longer time to germination for *L. dissectum* var. *dissectum* seeds did not appear to be attributed to a negative effect of cold temperatures on germination. Seeds of *L. dissectum* var. *dissectum* collected at Adair *1* and Adair *2* were placed at 4 °C for 12 weeks and then some seeds were kept at this temperature while others were exposed to a daily (12/12 h) alternating temperature regimen of 5/15 °C. The final germination of seeds exposed to the alternating temperatures was 55 to 60% lower than those maintained at a constant temperature of 4 °C.

DISCUSSION

At the time of dispersal, *L. dissectum* seeds have underdeveloped embryos with an embryo to seed length ratio of about 0.15. Before germination, the embryos elongated between five and seven fold over several weeks of cold stratification. Low temperatures also favour germination, since we observed the highest rate of germination in seeds maintained at 3.4 °C. In contrast, germination was negligible for seeds incubated at 9.1 °C. Warm stratification breaks dormancy in many species with morphological or MP dormancy (Baskin and Baskin, 2004). This phenomenon, however, was not observed in *L. dissectum*. The requirement for a long period of cold conditions for embryo growth and dormancy break indicates that *L. dissectum* seeds have complex MPD. Furthermore, GA_3 did not replace the cold stratification requirement demonstrating that the seeds had deep complex MPD (Baskin and Baskin, 2004). To our knowledge, this is the first report describing the type of dormancy in a species in the *Lomatium* genus, a genus of 81 species all native to middle and western North America (USDA Plants Database).

For seeds with MPD, little is known about the effect of interruptions in embryo growth on the dormancy status of the seeds. Pioneer work by Stokes (1952) showed that in *Heracleum sphondylium* interruption of the cold stratification period by transferring the seed to room temperature does not induce secondary dormancy. In Stokes study, however, the effect of dehydration on secondary dormancy was not investigated. Various species with MPD germinate over several years (Hawkins *et al*., 2007), which suggests that they may re-enter dormancy under unfavourable conditions. We analysed this possibility in *L. dissectum* by drying the seeds after 10 weeks of cold stratification. Drying either damaged the embryo as reflected by the decrease in seed viability or significantly extended the cold stratification

period required for germination. The latter observation indicates that in some seeds dehydration triggered secondary dormancy, suggesting that *L. dissectum* seeds have the potential to form a persistent soil seed bank. In addition, under field conditions a fraction of the seeds that did not germinate during the first spring remained viable after experiencing dehydration over the summer. Thus, *L. dissectum* appears to have several mechanisms that contribute to population persistence including resprouting from their large root crowns and germination from seeds of various ages.

Among species with deep complex MPD, variations exist in the time course of embryo growth, the effect of warm stratification on embryo growth at cold temperatures, and the optimum temperatures for embryo growth and germination. For example, seeds of *Osmorhiza aristata* and *Chaerophyllum temulum* show a delay between the start of cold stratification and the initiation of embryo growth (Walck *et al.*, 2002; Vandelook *et al.*, 2007a). These embryos do not elongate during the first two weeks of cold stratification, suggesting that some release of PD is needed before embryo growth. These results contrast with those observed in *Osmorhiza depauperata* (Walck and Hidayati; 2004) and those reported here for *L. dissectum*. In both species, significant elongation of the embryo was observed during the first two weeks of cold stratification. Furthermore, *L. dissectum* embryos had the highest relative rates of embryo growth, about 30% per week, during the first two weeks of cold stratification. Thus, in *L. dissectum* the release of PD and MD appears to occur simultaneously. The possibility exists, however, that the degree of PD varies along the embryos. Although elongation occurred in the embryo axis and cotyledons, portions of the embryo such as the shoot and root apical meristems may remain quiescent. These regions and in particular the root apical meristem may require long periods of cold

stratification before the initiation of meristematic activity. Some support for this notion comes from the observation that most of the elongation of the embryo occurred toward the chalazal end of the seed. In contrast, little elongation occurred between the radicle and the micropyle until the beginning of germination.

In species that require cold stratification, the temperatures that break dormancy are most often lower than those that trigger germination (Baskin and Baskin, 1998). Exceptions to this situation, however, have been reported, particularly for species with MPD. For example, embryos of *O. depauperata* grew at 1 and 5°C, while germination only occurred at 1 °C (Walck and Hidayati, 2004). *Lomatium dissectum* showed a somewhat similar pattern. Embryo growth was similar at 3.4 and 5.5 °C, but germination was higher at 3.4 than at 5.5 °C. A possible explanation for these results is that the release from physiological dormancy occurs more rapidly at 3.4 than at 5.5 °C. As mentioned above, parts of the embryo may remain quiescent until late stages of embryo growth. The low temperature might accelerate dormancy break in these portions of the embryo resulting in earlier germination.

Overall, the results obtained under field conditions were consistent with those from the growth chambers. In the field, embryo growth and germination mainly occurred when the soil temperatures were below 5 °C. A more detailed comparison of the stratification requirements under growth chamber and field conditions reveals, however, some differences (Fig. 10). We estimated the stratification hours under field conditions from hourly measurements of soil temperature and assumed that stratification occurred at temperatures between 0 and 10 °C. This approach most likely overestimates the stratification hours because temperatures close to 0 and 10 °C are not as effective in breaking dormancy as a temperature of about 4 °C. Furthermore, under field conditions the seeds were exposed to

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periods of low moisture that may limit embryo growth. Even with this overestimation, a plot relating stratification hours to germination indicates that under field conditions the seeds required less hours of stratification than in the growth chamber (Fig. 10). This was observed for both field experiments.

The reasons for the shorter stratification requirement in the field are unclear. Seeds in the growth chamber were exposed to a 12 h photoperiod while those in the field were buried. For some species, stratification in light is less effective in releasing dormancy that stratification in darkness (Steadman, 2004). Dark stratification increases the sensitivity to light in species that require light to germinate (Steadman, 2004). *Lomatium dissectum*, however, does not appear to require light to germinate. We have observed that seeds germinate while buried in the soil and in a refrigerator under dark conditions. In addition to light, another difference between the growth chamber and the field was the extent to which the seeds were exposed to fluctuations in temperature. In some seeds, fluctuations in temperatures decrease the length of the cold stratification requirement to terminate dormancy (Ekstam *et al.*, 1999; Battla *et al.*, 2003). When we tested in the growth chamber an alternating temperatures regimen of 5/15 °C, we did not observe, however, a decrease in dormancy. On the contrary, the alternating temperature regimen significantly decreased germination. Further work is needed, however, to determine whether smaller oscillations in temperature, particularly within the range permissive for embryo growth, reduce the cold stratification period.

Differences in stratification requirements were also observed between seeds collected in 2005 and those in 2007. In both the greenhouse and field experiments, the 2005 seeds required more hours of stratification than the 2007 seeds. Seeds were collected from the

same population; consequently, it is unlikely that the differences were attributed to genetic variability among the seeds. Also from the time of harvest to the initiation of the experiments, the seeds were in storage for approximately the same period suggesting that differences in the degree of dormancy cannot be attributed to disparity in the age of the seeds. The difference in stratification requirements may reflect differences in environmental conditions during seed development, which can alter the degree of seed dormancy (Fenner, 1991; Hoyle *et al.*, 2008). Precipitation data from weather stations within a 50 km distance from the collection site (Vale-OR 43º 59' N, 117º 15 W; West Fall-OR 43º 59' N, 117º 47' W, and Owyhee Dam-OR 43º 39' N, 117º 15' W) indicates that at the time of seed development, May and June, the precipitation was higher in 2005 than in 2007, 78.2 ± 25 and 27.2 ± 10 mm, respectively (Oregon Climate Service). In species from various families, water deficits during seed development result in less dormant seeds (Steadman et al., 2004; Hoyle et al., 2008). Perhaps, this phenomenon also occurs in *L. dissectum*. Under this scenario, the seeds that developed during the spring of 2007 would require less stratification than those that developed during the moister spring of 2005, which would account for the results observed. For *L. dissectum*, embryo growth largely occurs after seed dispersal. An intriguing possibility is that mild water deficits during embryo growth after seed dispersal also reduce dormancy. This notion requires investigation, but such phenomenon would explain the shorter stratification requirements of seeds in the field with respect to those in the growth chamber.

Variation in stratification requirements was also observed among seeds collected at different sites. Seeds of *L. dissectum* var. *multifidum* collected at intermediate elevations required less stratification than those of *L. dissectum* var. *dissectum*. At present, it is not clear

whether these differences reflect genetic differences between the populations and/or differences in environmental conditions during seed development. Independent of the factors involved, the shorter stratification requirements for seeds from the drier sites may provide an ecological advantage. Seeds of variety *multifidum* were from sites where the growing season is relatively short due to the dry summer conditions. For these seeds, the shorter stratification results in early germination, which extends the growth period allowing the seedlings to photosynthesize and accumulate reserves until the dry summer. On the other hand, variety *dissectum* seeds were from sites where temperature and precipitation allow plant growth through most of the spring and summer. Under these conditions, early germination is not so critical for seedling survival.

In summary, *L. dissectum* seeds have complex MPD; embryo growth and germination occurred at temperatures between 3 and 6 °C and GA_3 did not replace the requirement for cold stratification. We observed differences in cold stratification requirements among seeds collected at different sites and between seeds from one site collected in different years. The latter is likely the result of differences in environmental conditions during seed development. In semiarid regions, seeds that do not germinate during the first year remain dehydrated for several weeks during the summer. Interruption of the cold stratification period by dehydration decreased seed viability and induced secondary dormancy. The negative effect of dehydration on seed viability suggests that under field conditions germination is mainly attributed to seeds produced during the previous spring. The seeds that enter secondary dormancy have the potential to form a persistent seed bank, which could be important for species survival following marked ecosystem disturbances (Baskin and Baskin, 1998; Wardle, 2003).

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FIGURE LEGENDS

Figure 1. Effect of warm and cold stratification on embryo growth of *Lomatium dissectum*. Seeds were exposed to 0 (●), 2 (○), 4 (▲), or 6 (◊) weeks of warm stratification at 20 °C prior to cold stratification. Arrows indicate when the seeds were transferred from warm stratification at 20 °C to cold stratification at 5 °C. Mean (± s.e) of three or four replications with 50 embryos per replication.

Figure 2. Growth of the embryo axis and cotyledons during cold stratification of *Lomatium dissectum* seeds. (**a**) Length of the whole embryo, cotyledons, and embryo axis during stratification. (**b**) Ratio of embryo axis to cotyledon length. Mean (± s.e.) of five replications with ten embryos per replication. Means not labeled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square means test.

Figure 3. Embryo growth of *Lomatium dissectum* seeds at four cold stratification temperatures. Mean (± s.e) of five replications with ten embryos per replication. Means not labeled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square means test.

Figure 4. Effect of four cold stratification temperatures on germination of *Lomatium dissectum* seeds. Mean (± s.e) of ten replications with 60 seeds per replication. Means not 0 labeled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square means test.

Figure 5. Embryo elongation in *Lomatium dissectum* seeds incubated at 4 °C (white symbols) or 12 °C (black symbols) in various concentrations of GA$_3$. Means ($\pm$ s.e.) of four replications with ten embryos per replication. For a particular week, means not labeled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square means test.
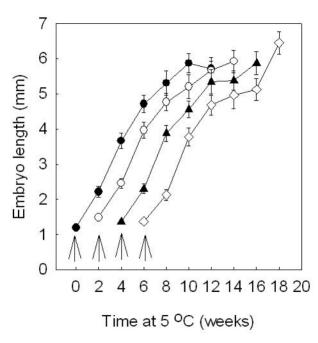
Figure 6. Effect of dehydration on seeds exposed to cold stratification at 4 °C for 10 weeks. Germination of control ($\bullet$) and dried ($\circ$) seeds; viability of dried-ungerminated seeds ($\square$) and viability plus germination of dried seeds ($\blacksquare$). Controls were maintained moist at 4 °C throughout the experiment.   Dried seeds were cold stratified for 10 weeks and then dehydrated to 8% water content and kept at room temperature for 8 weeks. At the time indicate by the arrow, the dried seeds were rewatered and returned to 4 °C. For germination, values represent mean $\pm$ s.e. of four or six replications with 70 to 100 seeds per replication. For viability of ungerminated seeds, values represent mean $\pm$ s.e. of six replications with ten seeds per replication. The total viability was also estimated based on the number of germinated seeds plus the number of viable ungerminated seeds, values represent mean $\pm$ s.e. of six replications. Means not labeled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square means test.
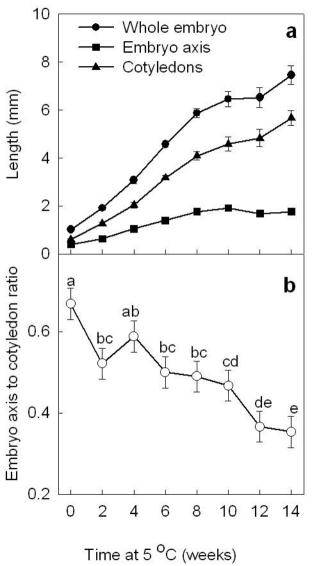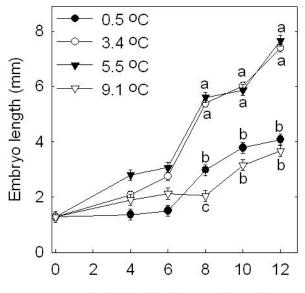
Figure 7. Embryo growth of *Lomatium dissectum* seeds exposed to field conditions. **a**, Embryo length (mean $\pm$ s.e. of ten replications with five to ten embryos per replication) and seed germination percentages (mean $\pm$ s.e. of ten replications with 60 seeds per replication). Means not labeled with the same letter are significantly different ($p < 0.05$) based on Tukey-

Kramer least square means test. **b**, daily average soil temperature and soil moisture. Insert, relationship between soil volumetric water (θ) and soil water potential (ψ_m) for the soil were the seeds were buried.

Figure 8. Embryo growth of *Lomatium dissectum* seeds exposed to field conditions. **a**, Embryo length (mean ± s.e. of ten replications with five to ten embryos per replication) and seed germination percentages (mean ± s.e. of ten replications with 60 seeds per replication). Means not labeled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square means test. **b**, daily average soil temperature and soil moisture.

Figure 9. Germination of *Lomatium dissectum* seeds collected at different sites. Mean ± s.e. of five to ten replications with 60 seeds per replication.
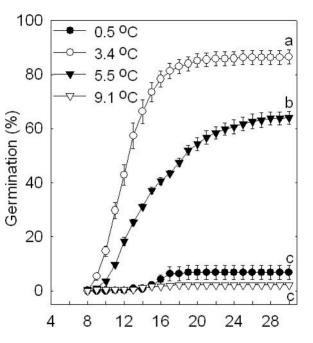
Figure 10. Relationship between stratification hours and germination under field and growth chamber conditions. (○) Field conditions 05-06; (●) growth chamber 05 seeds; (□) field conditions 07-08; (■) growth chamber 07 seeds. The stratification hours under field conditions were estimated from hourly measurements of soil temperature assuming that stratification occurred at temperatures between 0 and 10 °C. Mean ± s.e. of t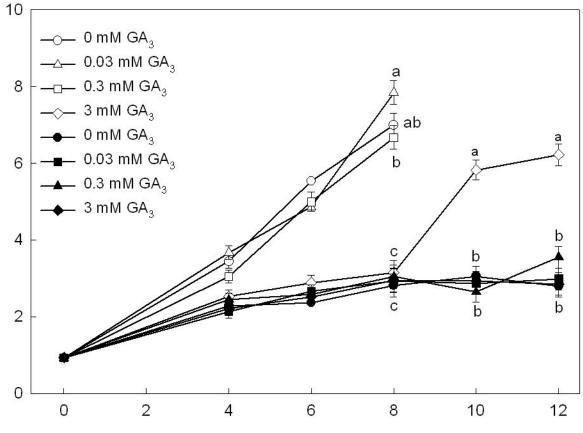en replications with 50 to 60 seeds per replication.