ENVIRONMENTAL REGULATION OF DORMANCY LOSS IN SEEDS OF *LOMATIUM DISSECTUM*

by

Melissa Dawn Scholten

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Melissa Dawn Scholten

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The following individuals read and discussed the thesis submitted by student Melissa Dawn Scholten, and they evaluated her presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

Marcelo Serpe, Ph.D.	Chair, Supervisory Committee
Nancy Shaw, Ph.D.	Member, Supervisory Committee
Marcia Wicklow-Howard, Ph.D.	Member, Supervisory Committee

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ABSTRACT

Lomatium dissectum (Nutt.) Mathias & Constance is a perennial plant found across much of western North America. For disturbed lands within this range, there is interest in using *L. dissectum* in restoration. A problem in the propagation of *L. dissectum* is that at the time of dispersal the seeds are dormant. Thus, prior to usage in restoration projects the type of dormancy and the procedures necessary to release dormancy in these seeds must be determined. Determining the type of dormancy and the treatments necessary to break dormancy in *L. dissectum* seeds was the primary focus of this study. To approach this problem I examined the effect of different constant and alternating temperature regimes on embryo growth and germination. I also evaluated the effect of plant hormones on seeds. Embryo growth and germination of seeds under field conditions was monitored and the soil temperature and moisture conditions correlated to changes in embryo elongation and seed germination. Finally, I explored seed dormancy in *L. dissectum* seeds collected over an elevation gradient. Exposure to 5°C stimulated embryo growth and germination. Exposure for more than 12 weeks was necessary for maximum embryo growth and germination. Exposure to 10°C and alternating between 4°C and 14°C did not result in better embryo growth or germination than exposure to only cold, moist conditions. Hormones did not enhance embryo growth or germination, nor did they serve as a substitute for seed exposure to cold, moist conditions. Based on these laboratory results, I determined that *Lomatium dissectum* seeds have deep complex

morphophysiological dormancy. Seeds placed in the field supported the laboratory findings as the best embryo growth and germination occurred after exposure to the cold, moist temperatures experienced in winter. Seeds collected at different elevations varied in the period of moist, cold temperature required to break dormancy. However, the differences were not consistent between the two years tested. Consequently, these differences may not reflect environmental adaptations to elevations but may be the result of the environmental conditions at each site during seed development.

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INTRODUCTION

The USDA Forest Service Native Plant Selection and Increase Project and the USDI Bureau of Land Management Great Basin Restoration Initiative selected *Lomatium dissectum* (Nutt.) Mathias & Constance (Apiaceae: fernleaf biscuitroot) as a species to be studied for potential use in restoration (Shaw and Pellant 2003). Expanding the availability of forbs such as *L. dissectum* for restoration and revegetation projects is vital to the future of public lands in the Great Basin. Forbs are needed to create diverse communities, which tend to be more resilient to future disturbances. Additionally, communities rich in forbs provide high quality habitat for many species of the Great Basin, including the declining *Centrocercus urophasianus* (sage-grouse) (Walker and Shaw 2005). Such healthy and diverse communities are declining in part due to overgrazing, exotic weed invasion, and altered fire regimes (Monsen 2001).

Lomatium dissectum is a perennial plant common throughout much of the western United States and Canada, often growing on dry, rocky soils. These plants are adapted to regions characterized by a short spring growing season followed by a dry summer (Schlessman 1982). It is important to note that this range was found to be defined more by an inability of seeds to disperse to suitable locations outside its current range rather than the lack of environmentally suitable sites (Marsico and Hellmann 2009). The robust plants produce thick taproots and large, highly dissected fern-like leaves that emerge directly from the rootcrown (Davis 1952; Hitchcock and Cronquist 1973; Schlessman

1982). Plants flower in late spring or early summer (Thompson 1998; USDA Plants Database 2010) and seeds mature in early to mid-summer (Davis 1952; USDA Plants Database 2010). The compound umbels may produce only staminate flowers or they may be comprised of both staminate and perfect flowers (Schlessman 1982; Thompson 1998). The flowers are capable of self-fertilization as well as out-crossing through insect pollination (Schelessman 1982). The most common pollinators of *Lomatiums* are flies and bees of the Diptera, Hymenoptera, and Coleoptera Orders (Schelessman 1982). Once fertilized, only one of the two ovules produces a brown schizocarp (fruit), the other ovule aborts (Schelessman 1982). After seed set, the plants die back to ground level (Thompson 1998).

The two varieties of this plant are *Lomatium dissectum* var. *dissectum (*fernleaf biscuitroot) and *Lomatium dissectum* var. *multifidum* (Nutt.) Mathias & Constance (carrotleaf biscuitroot) (Davis 1952; Hitchcock and Cronquist 1973; USDA Plants Database 2010). While the distribution of both varieties extend from southern Canada to the U.S.-Mexico border, the width of their distribution ranges does differ. *Lomatium dissectum* var. *multifidum* has a wider distribution overall occurring from the Pacific coast inland to the western edge of the Midwestern states. *Lomatium dissectum* var. *dissectum* is found only west of the Cascade Mountain range and in northern Idaho (USDA Plants Database 2010).

Lomatium spp. are valuable components of communities where they occur. A study by Barnett and Crawford (1994) found that prior to laying eggs, female sage-grouse rely heavily on *Lomatium* species, including *L. dissectum,* for food. This was attributed to the high crude protein content of the *Lomatium* species that leads to better egg

production and higher reproduction success. Additionally, *Lomatium* species are an important component in the diet of sage grouse chicks from the time the chicks are 2 weeks to 3 months old (Drut et al. 1994).

Lomatium dissectum is also an important medicinal and survival plant. The large taproot is edible and can be dried and ground into flour. This flour was used by the Blackfoot Indians to make large bread loaves (Willard 1992). These bread loaves were specially designed with a hole in the middle, which allowed them to be attached to a pack to provide food during travel. In the spring, many of the plant's vegetative parts are also edible and the dried seeds can be consumed in the fall (Willard 1992). This plant has a wide range of medicinal uses but is most commonly used for respiratory problems. It contains resins that act as expectorants and can kill many microbes including those that attack the lungs. It was successfully used in the influenza epidemic of 1920-1922 to lower the death toll (Willard 1992; Moore 1993).

Although there are a few species in the Apiaceae family that can reproduce via ramets, including *Cryptotaenia canadensis* (L.) DC. (Canadian honewort), *Anthriscus sylvestris* (L.) Hoffm. (wild chervil), and *Peucedanum palustre* (L.) Moerch (hogfennel) (Baskin and Baskin 1988; Hawkins et al. 2005), *Lomatium dissectum* and the majority of Apiaceae species reproduce by seed. At the time of dispersal, the embryos of *L. dissectum* are small and underdeveloped despite the large seed size. The remainder of the seed is mostly endosperm. Seeds with this type of embryo to endosperm ratio are considered evolutionarily primitive (Baskin et al. 1992; Forbis et al. 2002).

Germination is defined as the series of events that occur between the imbibition of the dried seed and radical emergence (Bewley 1997; Kucera et al. 2005). The seeds of *L.*

dissectum are dormant at the time of dispersal and fail to germinate when exposed to conditions favorable to germination (Bewley 1997; Baskin and Baskin 1998; Baskin and Baskin 2004; Bradford 2005; Kucera et al. 2005). Dormancy in seeds is not uncommon and is thought to have developed to aid in plant, population, and species survival. Dormancy often ensures that seeds will germinate at a time when conditions are conducive to seedling establishment (Rolston 1978; Bewley 1997; Baskin and Baskin 1998; Ekstam et al. 1999; Forbis et al. 2002; Baskin and Baskin 2004; Bradford 2005; Finch-Savage and Leubner-Metzger 2006). Dormancy also influences the development of seedbanks, which allows for long-term survival of a species, especially in highly variable environments (Rolston 1978; Thompson and Grime 1979; Murdoch and Ellis 1992; Forbis et al. 2002; Hawkins et al. 2007).

The dormancy classification system developed by Nikolaeva (1977) and expanded by Baskin and Baskin (1998, 2004) identifies five types of dormancy: physical (PY), morphological (MD), physiological (PD), morphophysiological (MPD), and combinatorial dormancy (PY +PD). These classifications are based on characteristics of the seeds and the treatment regimes required by the seeds to break dormancy. While a species may exhibit one dormancy type, the exact requirements for dormancy loss can vary among and within populations (Bewley 1997; Cavieres and Arroyo 2000; Cruz et al. 2003; Baskin and Baskin 2004). Variation among populations is associated with local climate and site conditions. Within population variation serves to spread germination over time and to reduce intraspecific competition among seedlings (Allen and Meyer 1998; Bradford 2002). This intraspecific variation creates a range for each required environmental condition. When the environmental conditions are within this range, seeds

will be released from dormancy. These ranges are defined by critical threshold points above and below which there is no effect on breaking seed dormancy (Bradford 2002). Treatments for dormancy release can include the environmental factors of temperature, temperature fluctuation, and light, as well as exposure to smoke and the external application of phytohormones important in promoting germination processes. These include gibberellic acid, ethylene, cytokinins, brassinosteroids, and auxins (Bewley 1997; Baskin and Baskin 1998; Ekstam et al. 1999; Matilla, 2000; Batlla et al. 2003; Baskin and Baskin, 2004; Kucera et al. 2005). The germination enhancing phyotohormones work in opposition to the phytohormone abscisic acid, which plays a role in inducing and maintaining dormancy (Kucera et al. 2005).

Based on the classification developed by Baskin and Baskin (2004), seeds with physical dormancy (PY) are impermeable to water due to a structural layer in the seed that must be penetrated in order for imbibition and subsequent germination to occur. Seeds with morphological dormancy (MD) contain underdeveloped embryos that must grow and differentiate before germination can occur. In seeds with physiological dormancy (PD), germination is blocked by a barrier to a physiological pathway such as the production of a specific hormone. There are three levels of physiological dormancy, non-deep, intermediate, and deep, which are defined by the requirements needed to release the seeds from dormancy and the ability of excised embryos to grow into normal seedlings. Seeds with non-deep PD are released from dormancy by exposure to warm or cold stratification. Germination can be enhanced by dry after-ripening, scarification, or treatment with gibberellic acid. Intermediate PD requires that seeds be exposed to cold, moist conditions for up to 3 months. Dry after-ripening and treatment with gibberellic

acid may or may not enhance germination. Embryos removed from seeds with non-deep and intermediate levels of dormancy can produce normal seedlings. Seeds exhibiting deep physiological dormancy require extended periods of exposure to cold stratification to germinate and do not respond to after-ripening or gibberellic acid treatments. Excised embryos will not form normal seedlings without treatment.

Morphophysiological dormancy (MPD) describes seeds with physiological dormancy as well as the underdeveloped embryos of morphological dormancy. There are many levels of this dormancy that are determined by the temperature regime required to break dormancy, the temperature required for embryo growth and the effectiveness of gibberellic acid treatments on breaking dormancy (Baskin and Baskin, 1998). The temperature regimes used to break dormancy require seed imbibition. Non-deep simple MPD requires that seeds be treated with warm, moist conditions before dormancy can be broken. This type of dormancy can also be broken by treating seeds with gibberellic acid. Intermediate simple MPD requires that seeds be treated with warm, moist conditions, then cold, moist stratification with embryo growth occurring during the warm stratification. These seeds also respond to gibberellic acid treatment. When seeds have deep simple MPD, they require exposure to warm, moist conditions, then cold, moist stratification, but there must be a slight decrease in temperature at the end of the warm treatment. Embryo growth occurs during the cooler portion of the warm stratification. Gibberellic acid treatment can replace warm stratification in this type of dormancy. There is a special type of deep simple MPD called deep simple epicotyl dormancy in which the radicle and epicotyl of the seed have different requirements to break dormancy. The dormancy of the radicle is broken first during the warm treatment and the dormancy

of the epicotyl is broken following the cold treatment. With this type of dormancy, treating the seed with gibberellic acid will not break dormancy in the radicle, but it will break the dormancy of the epicotyl. Deep simple double MPD requires that seeds are exposed to cold, moist stratification twice, once before and once after they are exposed to warm, moist conditions. Seeds with this type of dormancy are often called two-year seeds as they need two winters to fulfill the requirements to break dormancy. Embryo growth for these seeds occurs during the warm treatment. It is unknown how these seeds respond to gibberellic acid. Seeds with non-deep complex MPD require exposure to warm, moist conditions, then cold, moist conditions. With these seeds, the embryos grow during the cold stratification period and the warm stratification can be replaced by gibberellic acid treatment. The final two levels of MPD, intermediate complex and deep complex, both require that seeds are exposed to cold conditions, but vary on their response to gibberellic acid. Seeds with intermediate complex MPD have a favorable response while seeds with deep complex MPD do not respond to gibberellic acid treatment. Finally, seeds with combinatorial dormancy are seeds that have physiological dormancy, usually non-deep, and physical dormancy. These seeds may not always require that the physical dormancy be removed before the physiological dormancy is broken (Baskin and Baskin 1998; Baskin and Baskin 2004).

Although there are three genera, *Apium*, *Conium*, and *Pastinaca,* of the Apiaceae family that have only MD, the majority of Apiaceae that have been studied exhibit some form of MPD dormancy. Seeds of *Osmorhiza claytonii* (Michx) C. B. Clarke (Clayton's sweetroot) and *Thaspium pinnatifidum* (Buckl.) A.Gray (cutleaf meadowparsnip) were determined to have non-deep complex MPD (Baskin and Baskin 1991), while *Osmorhiza*

depauperata Phil. (bluntseed sweetroot) and *Aegopodium podagraria* (bishop's goutweed) seeds have deep complex MPD (Baskin et al. 1992; Walck and Hidayati 2004). Seed of *Chaerophyllum temulum* L. (rough chervil), an European member of the Apiaceae family, was found to have two types of dormancy. Most seeds exhibit deep complex MPD, but some seeds have intermediate complex MPD (Vandelook et al. 2007a). Two related annual species found in the United States, *C. procumbens* (L.) Crantz (spreading chervil) and *C. tainturieri* Hook. (hairyfruit chervil), have non-deep complex MPD. There are two known occurrences of non-deep simple MPD in the Apiaceace family: *Angelica sylvestris* L. (woodland angelica) and *Selinum carvifolia* (L.) L. (little-leaf angelica) (Vandelook et al. 2007b). Experiments involving *Lomatium triternatum* (Pursh) J.M. Coult. & Rose (nineleaf biscitroot) and *L. utriculatum* (Nutt. ex Torr. & A. Gray) J.M. Coult. & Rose (common Lomatium) indicated that they germinated well following cold, moist exposure (Drake and Ewing 1997). *Osmorhiza longistyis* (Torr.) DC. (longstyle sweetroot) was found to require warm exposure followed by cold conditions to emerge from dormancy. The response of the seeds to gibberellic acid was not studied in this experiment. It was determined that the embryo growth began during the warm treatment and continued into the cold treatment at which time the rate of growth increased (Baskin and Baskin 1984).

The experiments in this study were designed to determine the nature of dormancy in *Lomatium dissectum* seeds and the requirements for releasing that dormancy. For these experiments, we exposed seed to conditions that the seeds would naturally encounter from the time of dispersal to germination. One or more of those conditions likely play a role in releasing seed dormancy. To further understand the dynamics of this

dormancy, we explored the impact of plant hormones and duration of dry storage on *L. dissectum* germination. Laboratory results were then compared to those obtained under field conditions. Finally, we explored variations in germination requirements for seeds collected at different sites varying in elevation and annual precipitation.

METHODS AND MATERIALS

Seed Collection and Preparation

In all experiments, seeds (mericarps) were collected and processed in the following manner. Umbels were clipped from the plant by hand and stored in paper bags until dry. Dry seeds were removed from the umbels by rubbing them over 1.3 cm wire grids. The seeds were further cleaned using sieves and removing the remaining debris by hand. Cleaned seeds were sorted over a light table and empty seeds were discarded. Filled seeds were rinsed in running water for about 6 hours, then surface sterilized by soaking them in 70% ethanol for 30-60 seconds. They were then soaked in a 0.5% sodium hypochlorite solution for 30 min, drained, and rinsed with de-ionized water. Rinsed seeds were air dried for 24 - 48 hours. Seeds collected in 2005 and 2007 were coated with the powered fungicide Captan. The Captan and seeds were placed in a white plastic bottle and the bottle was manually shaken for approximately 1 min. Excess Captan was removed with a sieve Seeds collected in 2006 were not coated with Captan. Some treated seeds were used immediately in the embryo growth and germination experiments. The remaining seeds were stored at room temperature in opaque brown glass bottles.

Seeds were prepared for germination experiments by placing them into 10 cm x 10 cm x 4 cm clear plastic germination boxes (Seedburo Equipment Company, Des Plaines, IL) covered with tight fitting lids. Each germination box was lined with one

piece of 10 x 10 cm blotter paper (Seedburo Equipment Company, Des Plaines, IL). In all experiments, save the hormone experiment, the blotter paper was moisten with de-ionized water and kept moist throughout the treatments. In the hormone study, the blotter paper was moistened with the hormone solution. Approximately 5 ml of fluid was used to moisten the blotters. Germination boxes were placed in germination chambers (germinators) for treatments. All germinators were set on a 12-h light/12-h dark cycle. The florescent lights in the germinators supplied 35 µmol $m^{-2}s^{-2}$ PAR.

Effect of Temperature on Embryo Growth and Germination

Three experiments were conducted to examine the effect of incubation temperature and dry after-ripening on embryo elongation and germination. Surface sterilized seeds (LODI-41: Crowley, OR 43° 33' N, 117° 47' W, 1367 m) collected in 2005 were used for all three experiments.

The first experiment was designed to evaluate the effects of warm and cold stratification on embryo elongation. Approximately 2 weeks following collection, seeds were placed in germination boxes. Each of the germination boxes received 60 seeds imbibed with de-ionized water. Treatments were assigned in a completely randomized design of warm stratification (20°C for 0, 2, 4, or 6 weeks) followed by cold stratification (5°C for 0, 2, 4, 6, 8, 10, or 12 weeks). Three additional moist, cold treatments of 15, 18, and 22 weeks were also tested to examine their impact of embryo growth. Four boxes were assigned to each treatment combination.

After treatment, the embryos were extracted from non-germinated seeds using a dissecting scope, forceps, and razor blades. Seeds were cut just off the longitudinal

center line to expose the embryo. Embryos were then removed from the seeds with forceps and measured.

The second experiment evaluated the effects of dry after-ripening temperature on the germination of seeds receiving subsequent warm and cold stratification. Dry seeds were separated into germination boxes lined with one dry blotter. Each germination box received 60 seeds. Dry after-ripening treatments were conducted for 0, 2, 4, or 6 weeks followed by warm stratification for 0, 2, 4, and 6 weeks and cold stratification for 0, 2, 4, 6, 8, 10, and 12 weeks. Three additional treatments (15, 18, and 22 weeks) of cold, moist stratification alone were added to test the effects of prolonged cold stratification. After-ripening treatments were conducted by placing the boxes in a germinator set at 20°C. Seeds were then imbibed and warm and cold stratification treatments were conducted as described previously. Treatments were applied in a completely randomized 4 x 6 x 6 factorial combination. Five treatment boxes were assigned to each treatment combination

Following treatment, the germination boxes were transferred to a germinator set at 15°C for 2 weeks. Boxes were then removed from the germinator, and all germinated and non-germinated seed were counted and total germination determined. Germination was defined as radical emergence of at least 1 mm. Intact seeds with no radical emergence were considered non-germinated. Moldy or degraded seeds were recorded as a separate category and were not included in the total germination calculations. Total germination was calculated by dividing the number of germinated seeds by the total number of firm seeds. This germinator also had a 12 hr light/dark cycle.

In a third experiment, the effects of incubation at 10°C on embryo growth and germination were examined. Surface sterilized seed after-ripened for 6 months in opaque

brown glass bottles. Groups of approximately 60 imbibed seeds were placed in germination boxes in a germinator set at 10°C. Treatment lengths were 6, 8, 10, 12, 14, 16, 18, and 20 weeks. After treatment, the boxes were removed and 10 seeds were removed for embryo measurement. The embryos of these seeds were extracted and measured as described previously. The remaining seeds were placed in a germinator set at 15°C for 2 weeks. Germinated and non-germinated seeds were then counted and the total germination for each box determined.

Alternative Temperature Regimes

Seeds of *L. dissectum* var. *dissectum* collected at Adair (44° 48’ N, 123° 14’ W; 81 m elevation), and Site 99 (44° 40’ N, 123° 13’ W; 86 m). For each site, we prepared ten boxes with 60 seeds per box. The boxes were incubated at 4 °C or on a 12hr/12hr 4/15°C cycle. Germination was measured at weekly intervals for a period of 28 weeks.

Effect of Storage on Embryo Growth and Germination

The following experiment was designed to explore the effect of long-term storage on embryo elongation and seed germination. Seeds collected in 2005 (Crowley, OR: LODI-41, 43° 33' N, 117° 47' W, 1367 m) were surface sterilized, then placed in opaque brown glass bottles and stored at room temperature for 1 year. Groups of 60 imbibed seeds were then placed in germination boxes. Each germination box was assigned a treatment and each treatment was represented by 5 boxes. Treatments were 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 weeks in a germinator set at 5ºC. Following the treatment, germinated seeds were counted and removed and the germination boxes were transferred to a germinator set at 15°C for 2 weeks. After 2 weeks, the boxes were removed and

germinated and non-germinated seeds were counted. Total germination was considered the sum of the two counts.

Effect of Gibberellic Acid on Embryo Growth

To ascertain whether the seeds have deep complex MPD, I tested whether GA_3 can substitute for the cold stratification requirement. The effect of GA_3 on embryo growth was investigated using seeds collected in 2007, which had been in storage for approximately 3 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 de-ionized water and the pH adjusted to 6.5 with 0.1-1.0 mM KOH. Fifty seeds were placed on germination paper inside plastic boxes and 15 ml of water or GA_3 solution were added per box. Each treatment was replicated four times. The boxes were covered with lids to reduce water loss and, if needed, de-ionized water was added to the papers. Embryo lengths of 10 seeds from each box were measured after 4, 6, 8, 10, and 12 weeks.

Effect of Plant Hormones on Germination

The impact of plant hormone treatments on germination was tested by providing seeds with 12 weeks of cold stratification in a germinator set at 5°C, then treating them with selected plant hormones or hormone combinations. Surface sterilized seed (Crowley, OR: LODI-41, 43° 33' N, 117° 47' W, 1367 m) collected in 2005 were stored for 6 months at room temperature in brown opaque glass bottles. Groups of 60 imbibed seeds were then placed in germination boxes. The hormones and concentration tested were benzyl amino purine (BAP, 250 and 500 ppm), chloroethyl phosphonic acid (CP,

100 and 500 ppm), naphtaleneacetic acid (NAA, 50 and 100 ppm), and gibberellic acid (GA, 250 and 500 ppm). Additionally, combinations of hormones were used. These combination treatments were GA and CP (each at 250 ppm); GA, BAP, and CP (each at 250 ppm); GA and BAP (each at 250 ppm); CP and BAP (each at 250 ppm); and, BAP (250 ppm) and NAA (50 ppm). The liquid hormone treatments were added to the germination boxes for 24 - 48 hours, then drained. After treatment, the germination boxes were placed in a germinator set at 15°C for 14 weeks. Each hormone treatment was assigned to a total of 5 germination boxes. After 14 weeks, the germination boxes were removed and the germinated and non-germinated seeds were recorded and total germination calculated.

Embryo Growth and Germination under Field Conditions

We conducted a field experiment to examine embryo growth and germination under natural conditions. The study site was located near the Idaho Botanical Gardens in northeast Boise, ID (43° 35' N 116° 9' W, 846 m). According to http://www.climate-zone.com/climate/united-states/idaho/boise/ (Climate–zone, 2007), this site has an average annual precipitation of 31.5 cm, an average maximum temperature of 17°C, and an average minimum temperature of 3.6°C. The majority of the precipitation comes during the winter. The soil is a sandy loam. The study site was surrounded by a 10 ft fence to exclude large herbivores. Smaller grazers such as *Thomomys* species (gophers) were controlled by chemical methods. Prior to establishment the vegetation, comprised primarily of weedy species such as skeletonweed (*Chondrilla juncea* L.) and cheatgrass (*Bromus tectorm* L.), was removed from the study site and a 2 m border area with a hoe.

Within the study site, ten $1m^2$ plots were laid out in two parallel rows of five running north and south, creating five sets of paired plots. The distance between the pairs of plots was less than 5 cm. One randomly selected plot of each pair was surrounded by black plastic garden edging approximately 10 cm in width.

Surface sterilized seed (Crowley, OR: 2005 collection, 43° 33' N, 117° 47' W, 1367 m) was stored for 4 months at room temperature in brown opaque glass bottles. Sixty seeds were then placed in each of 260 bags made from fine black chiffon. The seed bags were buried at the field site in November 2005. Twenty-six seed bags were buried 2 cm deep in each of the 10 plots. Two moisture probes (Echo 20, Decagon Devices, Inc., Pullman, WA) were buried at depths of 2cm, one in each of the two rows. Additionally, 6 thermocouples were buried at 2cm depths in three plots of each row. The probes were connected to a data logger (21X Campbell Scientific, Logan, UT) that recorded soil moisture and temperature every hour. Once a month from December 2005 to April 2006 a seed bag was recovered from each plot and taken to the laboratory. The collection for February 2006 was delayed until early March 2006 due to frozen soil. During the summer bags were collected in June and July. The final collection date was in October 2006. This study was originally to run for 2 years, but seed degradation shortened the experiment.

Following collection from the field, 10 seeds were removed from each bag for embryo measurement. The remaining seeds were placed in germination boxes and imbibed. Any seeds that had germinated in the field were counted and removed. As the length of residence in the field increased, determination of field germination became more difficult as seedlings began to separate from their seed coats. At this point,

germination was determined by examining seeds for indicators of germination. Extended embryo cavities and swollen seed coats from embryo growth as well as slits in the seed coats from radical emergence were all used as germination indicators. Remaining non-germinated seeds in the box were placed in a germinator set at 15°C. After approximately 14 days, the boxes were removed and the germinated and non-germinated seeds were counted and total germination calculated.

Stratification Requirements of Different Seed Populations

An experiment was designed to determine if incubation requirements for embryo growth and germination varied depending on the site at which the seed develops. In 2006, seed of *L. dissectum* var. *dissectum* (LODID) and *L. dissectum* var. *multifidum* (LODIM) were collected from 5 populations representing a range of elevations: Moores Mountain (LODIM-86: 43° 47' N, 116° 5' W, Ada County, ID, 2200 m), Black's Creek (LODIM-18: 43° 31' N, 115° 58' W, Elmore County, ID, 1287 m), Bitner (LODI-61M: 43° 36' N, 116° 48' W, Canyon County, ID, 797 m), Willamette Valley 1 (LODID-100: 44° 48' N, 123° 14' W, Polk County, OR, 81 m), and Willamette Valley 2 (LODID-102: 44° 40' N, 123° 13' W, Benton County, OR, 86 m). Once cleaned and sterilized, seeds from these sites were placed in germination boxes and imbibed. Due to poor seed production and high levels of insect damage, the number of seeds per box and number of replications varied by site. There were five replications for the *L. dissectum* var. *multifidum* collections with 40 seeds per box for Moore's Mountain and 60 seeds per box for Black's Creek and Bitner collections. There were four replications for the *L. dissectum* var. *dissectum* collections with 30 seeds per box. All seed boxes were then assigned a treatment. Treatments were 0, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 weeks of

exposure to 5°C in germinators with a 12-h light/12-h dark cycle. The Moore's Mountain site had an additional treatment of 28 weeks of cold stratification. Following treatment, the boxes were removed from the germinator and approximately 5-10 seeds were removed from each box for measurements of embryo elongation. The remaining seeds were placed in a germinator set at 15°C with a 12 -h light/12-h dark cycle. After 14 days, germinated and non-germinated seeds were counted.

Using seeds collected in the spring of 2007, the experiment described above was repeated with four populations of *L. dissectum* var. *multifidum* and four populations of *L. dissectum* var. *dissectum.* Seeds of *L. dissectum* var. *multifidum* were collected at Prairie, Idaho (43° 31' N, 115° 58' W, Elmore county, 1287 m); Harper, Oregon (43° 33' N, 117° 47' W, Malheur county, 1347 m); Fairfield, Idaho (43° 10' N, 114° 40' W, Gooding county, 1700 m); and, Moore's Mountain, Idaho (43° 47' N, 116° 5' W, Ada county, 2200 m). Seeds of *L. dissectum* var. *dissectum* were collected at: Adair 1 (44° 48' N, 123° 14' W, Polk County, 81 m), Adair 2 (44° 40' N, 123° 13' W, Benton County, 86 m), Buell 1 (45° 2' N, 123° 28' W, Polk County, 117 m), and Buell 2 (45° 1' N, 123° 24' W, Polk County, 178 m). For each site, we prepared 10 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. To compare differences in stratification requirements among populations, we estimated their mean germination time. 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 (Hartman and Kester 1983).

Statistical Analysis

The data for all experiments were analyzed with JMP software. The effects of warm and cold laboratory stratification on embryo growth and germination, hormone treatments on germination, embryo elongation and germination in the field, and collection site elevation on embryo growth and germination were analyzed with One-way and Two-way Analyses of Variance (ANOVA) tests. Means were separated via the Tukey test. T-tests were used to analyze data on the effects of incubating seed at 10°C on embryo growth and germination, and the effect of storage on embryo elongation and germination. The critical p-value for all experiments was 0.05. The figures and tables were created with Microsoft Excel software.

RESULTS

Effect of Temperature on Embryo Growth and Germination Embryo Growth

The embryos of *Lomatium dissectum* seeds grew best when exposed to cold, moist conditions alone, with greater embryo lengths resulting from longer treatment durations (Fig. 1). The majority of the significant increases in embryo length occurred at treatment periods shorter than 8 weeks. The embryos continued to elongate at the longer treatment lengths; however, the changes were less significant (Tables 1, 2; Fig. 2; $p < 0.0000$).

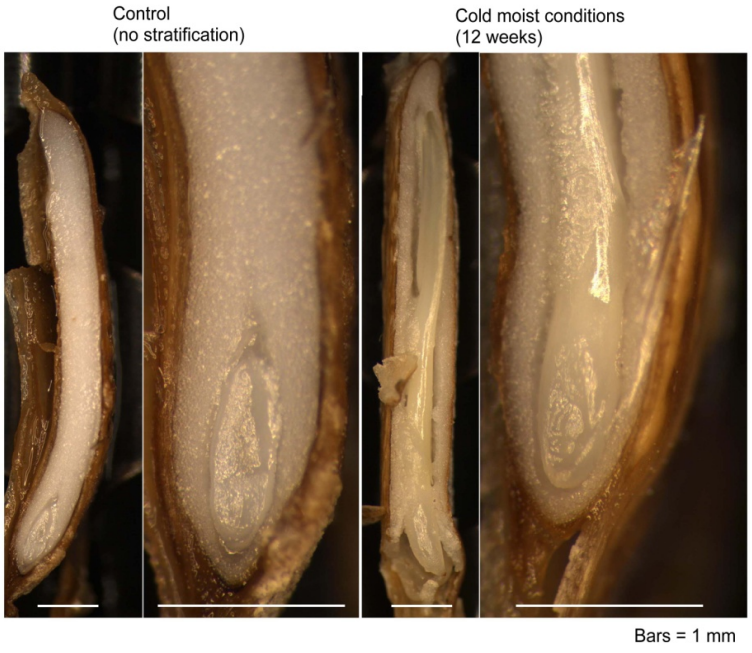

Figure 1. Photograph of an Imbibed *Lomatium dissectum* Embryo Before Exposure to Any Treatment (a) and an Embryo After Exposure to 12 Weeks of 5°C (b).

Table 1. ANOVA Table Comparing Embryo Lengths of Imbibed *Lomatium dissectum* Seeds After Exposure to 5°C for 0-22 Weeks. There Were 4 Replications of 60 Seeds Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	9	127.06777	14.1186	27.2675	<0.0001
Error	22	11.39122	0.5178		
C. Total	31	138.45899			

Table 2. Embryo Lengths of Imbibed *Lomatium dissectum* Seeds Exposed to 5°C ($p < 0.05$; Tukey test).

Stratification period (weeks)	Embryo length (mm)	
0	1.19	A
2	2.22	AB
4	3.66	BC
6	4.71	CD
8	5.31	CD
10	5.88	DE
12	5.72	DE
15	6.92	DE
18	7.44	DE
22	8.18	E

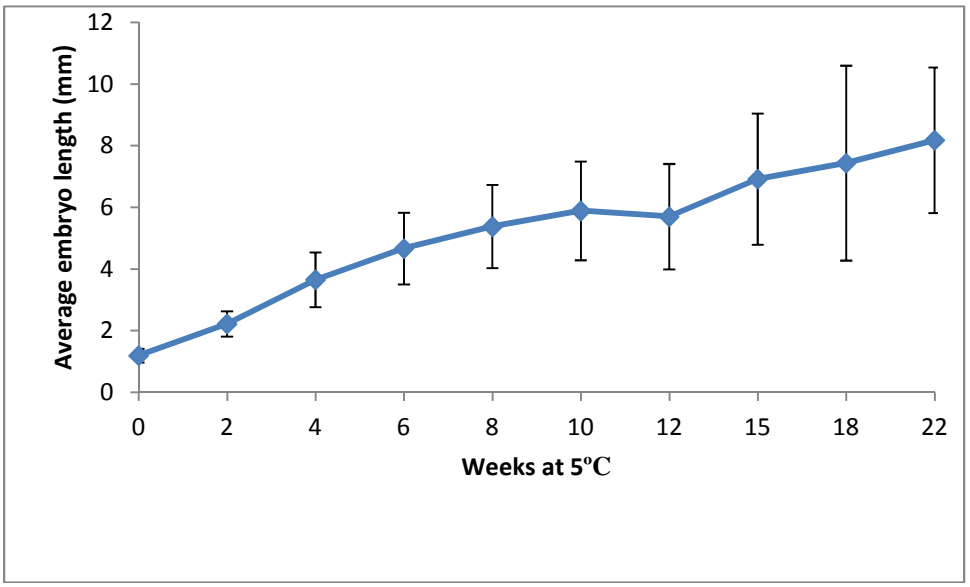

Figure 2. ***Lomatium dissectum*** **Embryo Length (± standard deviation) After Exposure to 5°C for 0-22 Weeks. There Were 6 Replications for the Control Treatment and 4 Replications for the Remaining Treatments Except for 2 Weeks of Cold, Which Had Only 3 Replications.**

When seeds were exposed only to warm, moist conditions (20°C), embryo lengths varied with treatment duration ($p = 0.0108$). All treatments increased embryo length over that of the control. However, 2 weeks of exposure was significantly more effective in increasing length than the 4 or 6 week treatments (Tables 3, 4: Fig. 3).

Table 3. ANOVA Table Comparing Embryo Lengths of Imbibed, Non-germinated Lomatium dissectum Seeds After Exposure to 20°C for 0-6 Weeks. There Were 4 Replications of 60 Seeds Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	3	0.22004755	0.073349	5.4452	0.0108
Error	14	0.18858736	0.013471		
C. Total	17	0.40863491			

Table 4. Embryo Lengths of Imbibed, Non-germinated *Lomatium dissectum* Seeds Exposed to 20°C. Embryo Lengths Followed by the Same Letter are not significantly different ($p < 0.05$; Tukey test).

Stratification period (weeks)	Embryo length (mm)
0	1.19 A
2	1.49 B
4	1.36 AB
6	1.37 AB

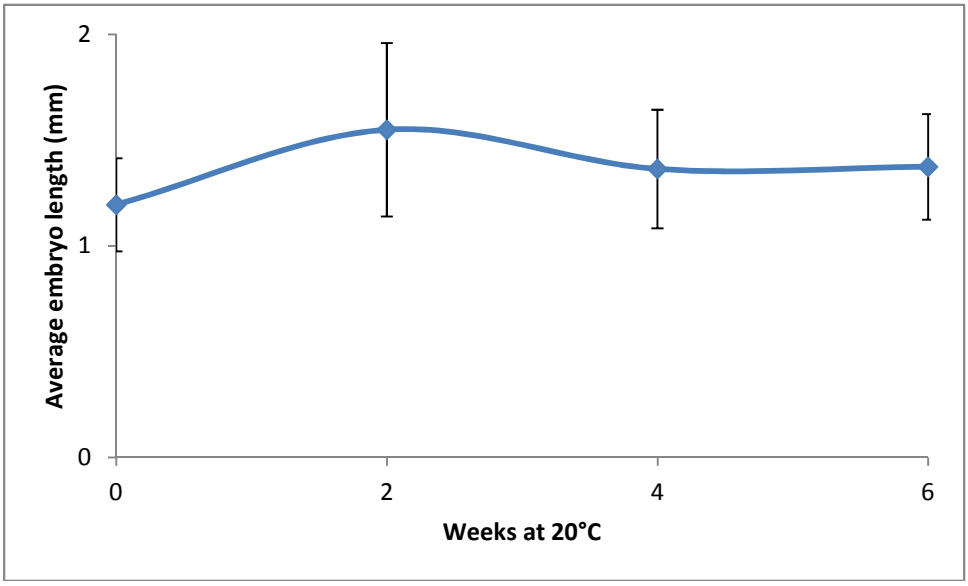

Figure 3. *Lomatium dissectum* Embryo Length (± standard deviation) After Exposure to 20°C for 0-6 Weeks. There Were 4 Replications Per Treatment.

When warm and cold, moist conditions were combined, the two-way ANOVA test indicates that there was no interaction between the two treatment components (Table 5; $p = 0.8145$). The cold treatment was significant ($p < 0.0001$) and the warm treatment was not significant ($p = 0.8145$).

Table 5. Two-way ANOVA Table Comparing Embryo Lengths of Imbibed *Lomatium dissectum* Seeds After Exposure to 20°C for 0-6 Weeks, Followed by Exposure to 5°C, for 6-12 Weeks. There Were 4 Replications of 60 Seeds Per Treatment.

Source	Degrees of freedom	Sum of squares	F ratio	Prob > F
Warm treatment	1	0.02166	0.0554	0.8145
Cold treatment	1	278.56134	712.0188	<0.0001
Interaction	1	0.02133	0.0545	0.8145

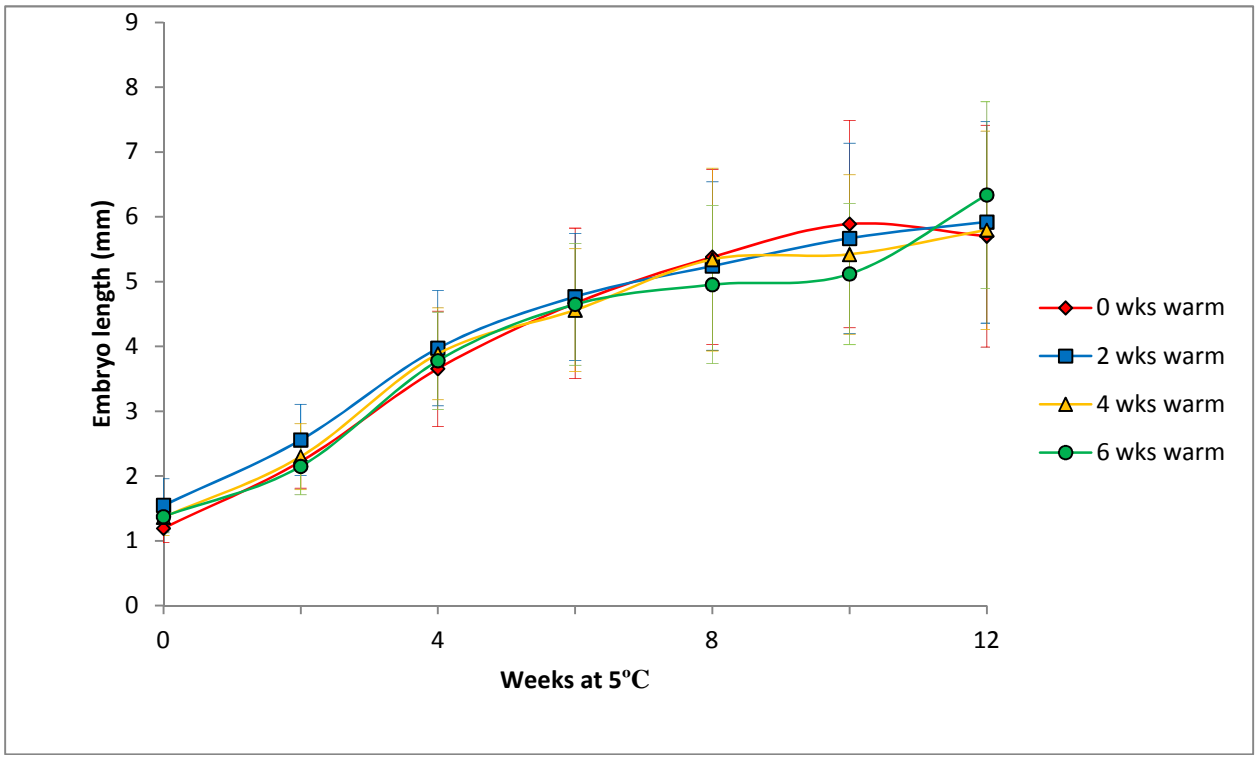

Figure 4. *Lomatium dissectum* Embryo Length (± standard deviation) After Exposure to 20°C for 0-6 Weeks Followed by Exposure to 5°C for 0-12 Weeks. There Were 4 Replications Per Treatment.

Germination

Lomatium dissectum seeds had high germination after being treated with prolonged cold, moist conditions. Seeds began to germinate after 8 weeks of stratification at 5°C and slowly increased to 21.2% at 12 weeks of exposure. Following

12 weeks of exposure, the germination rapidly increased to 81% at 15 weeks of exposure. Germination exceeded 80% for the remaining treatment lengths. The ANOVA test comparing these treatments showed that there were significant differences (Tables 6, 7; Fig. 5; $p < 0.0001$).

Table 6. ANOVA Table Comparing Average Percent Germination of Imbibed *Lomatium dissectium* Seeds After Exposure to 5°C for 0-22 Weeks Followed by Incubation at 15°C for 2 Weeks. There Were 5 Replications of 60 Seeds Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	7	57923.890	8274.84	179.3581	< 0.0001
Error	32	1476.348	46.14		
C. Total	39	59400.230			

Table 7. Germination of Imbibed *Lomatium dissectum* Seeds Exposed to 5°C for 0-22 Weeks. Means Followed by the Same Letter Do Not Differ ($p < 0.05$; Tukey test).

Stratification period (weeks)	Germination (percent)
0	0 A
6	0 A
8	4 AB
10	17 BC
12	21 C
15	81 D
18	88 D
22	87 D

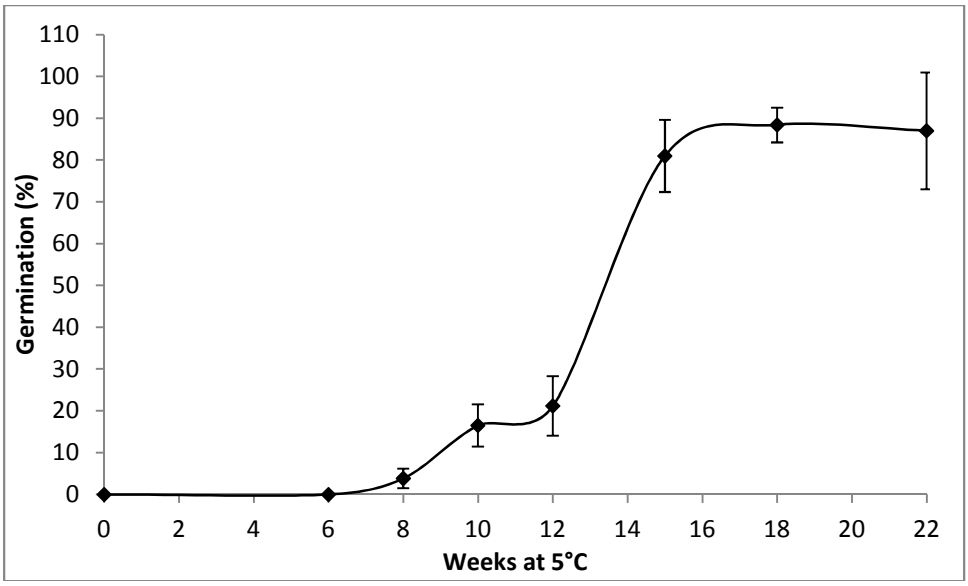

Figure 5. Percent Germination (± standard deviation) of *Lomatium dissectum* Seeds After Exposure to 5°C for 0-22 weeks. There Were 5 Replications Per Treatment.

Seeds treated only with after-ripening conditions failed to germinate at any treatment length. This failure to germinate occurred despite treatment lengths up to 10 weeks.

When after-ripening conditions preceded the cold, moist treatment, the two-way ANOVA test revealed a significant interaction between the two treatment components (Table 8; $p = 0.0003$). This interaction can also be seen in Figure 6 as the lines of the graph cross. The Tukey test results showed that the greatest germination was obtained when the cold exposure lengths were longer than 10 weeks and preceded by either 6 weeks of after-ripening or no after-ripening (Table 9).

Table 8. Two-way ANOVA Table Comparing Percent Germination of *Lomatium dissectum* Seeds Allowed to Dry After-ripen at 20°C, for 0-6 weeks, Prior to Imbibition and Exposure to 5°C for 8-12 Weeks. There Were 5 Replications of 60 Seeds Per Treatment.

Source	Degrees of freedom	Sum of squares	F ratio	Prob > F
After-ripening treatment	2	1075.8603	15.7983	<0.0001
Cold treatment	3	2405.0387	23.5442	< 0.0001
Interactions	6	1073.3143	5.2536	0.0003

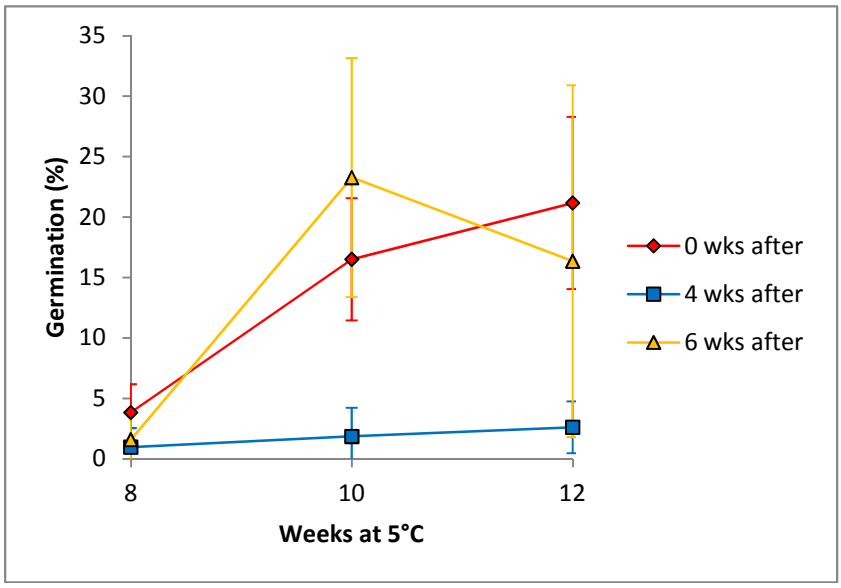

Figure 6. Percent Germination (± standard deviation) of *Lomatium dissectum* Seeds Exposed to 20°C for 0-6 Weeks Prior to Imbibition and Exposure to 5°C for 8-12 Weeks. There Were 5 Replications Per Treatment.

Table 9. Germination of *Lomatium dissectum* Seeds Dry After-ripened at 20°C Followed by Imbibition and Exposure to Cold, Moist Stratification at 5°C for 8-12 Weeks. Means Followed by the Same Number Do Not Differ ($p < 0.05$; Tukey test).

Stratification treatment	Germination (percent)	
6 wks after – 10 wks cold	23	A
0 wks after – 12 wks cold	21	A
0 wks after – 10 wks cold	17	A
6 wks after – 12 wks cold	16	AB
0 wks after – 8 wks cold	4	BC
4 wks after – 12 wks cold	3	C
4 wks after – 10 wks cold	2	C
6 wks after – 8 wks cold	2	C
4 wks after – 8 wks cold	1	C
0 wks after – 0 wks cold	0	C
6 wks after – 0 wks cold	0	C
4 wks after – 0 wks cold	0	C

Warm, moist conditions were also combined with cold, moist conditions. In these treatments, 2, 4, or 6 weeks of warm, moist conditions preceded exposure to 8, 10, or 12 weeks of cold, moist conditions. Warm stratification decreased germination under cold conditions (Fig. 7). However, there is an interaction between the two treatment components. This interaction is confirmed as significant with the two-way ANOVA test results, p-value of 0.0462 (Table 10). The Tukey test shows that the interaction seems to be a weak negative interaction with the longer lengths of warm, moist exposure resulting in lower germination. At the shorter cold exposure lengths, there is no interaction between the treatment components, most likely reflecting that for 8 weeks of cold there is very little germination for any of the warm stratification treatments (Table 11).

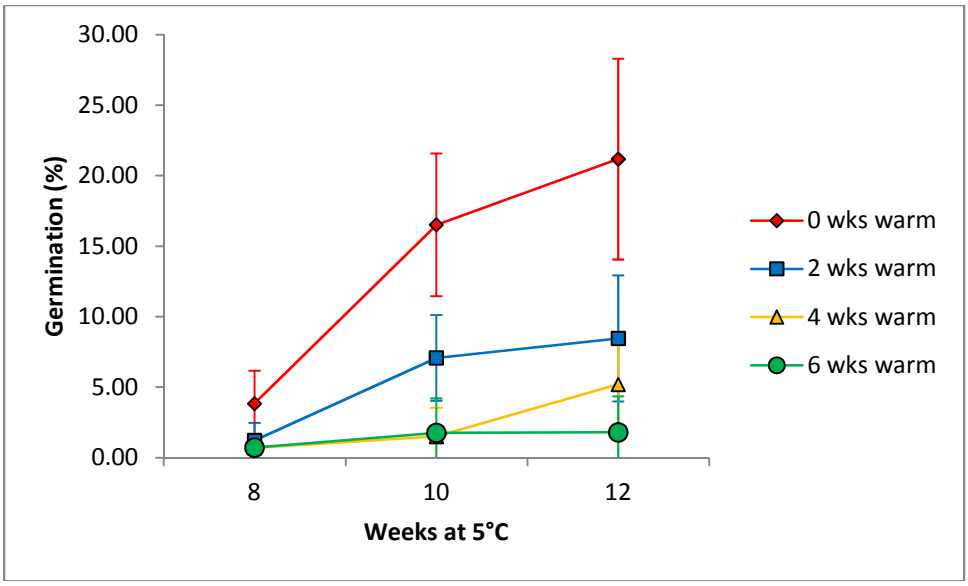

Figure 7. Percent Germination (± standard deviation) of Imbibed *Lomatium dissectum* Seeds Exposed to 20°C for 0-6 Weeks Prior to Exposure to 5°C for 8-12 Weeks. There Were 5 Replications Per Treatment.

Table 10. Two-way ANOVA Table Comparing Percent Germination of Imbibed *Lomatium dissectium* Seeds Exposed to 20°C for 0-6 Weeks, Then Exposed to 5°C for 8-12 Weeks. There Were 5 Replications of 60 Seeds Per Treatment.

Source	Degrees of freedom	Sum of squares	F ratio	Prob > F
Warm treatment	2	140.60800	10.7678	0.0002
Cold treatment	2	138.80933	10.6300	0.0002
Interaction	4	70.34267	2.6934	0.0462

Table 11. Germination of Imbibed *Lomatium dissectum* Seeds Exposed to Warm, Moist Conditions at 20°C for 0-6 Weeks Followed by Exposure to Cold, Moist Stratification at 5°C for 8-12 Weeks. Means Followed Bby the Same Number Do Not Differ ($p < 0.05$; Tukey test).

Stratification treatment	Germination (percent)	
2 wks warm – 12 wks cold	8	A
2 wks warm – 10 wks cold	7	AB
4 wks warm – 12 wks cold	5	ABC
6 wks warm – 12 wks cold	2	BC
6 wks warm – 10 wks cold	2	BC
4 wks warm – 10 wks cold	2	C
2 wks warm – 8 wks cold	1	C
6 wks warm – 8 wks cold	1	C
4 wks warm – 8 wks cold	1	C

Embryo Growth and Germination Following Incubation at 10°C

When imbibed seeds were exposed to 10°C for 6, 8, 10, 12, 14, 16, 18, or 20 weeks, the seeds exhibited very little embryo growth. The embryo lengths of the seeds incubated at 5°C were compared to those of seed incubated at 10°C at corresponding lengths. In each comparison, the embryos of the seeds at 5°C were always longer than their counterparts (Fig. 8). However, when each of these treatment pairs were compared with T-tests, the differences were never significant. Table 12 shows the ANOVA tables comparing embryo growth at these two temperatures.

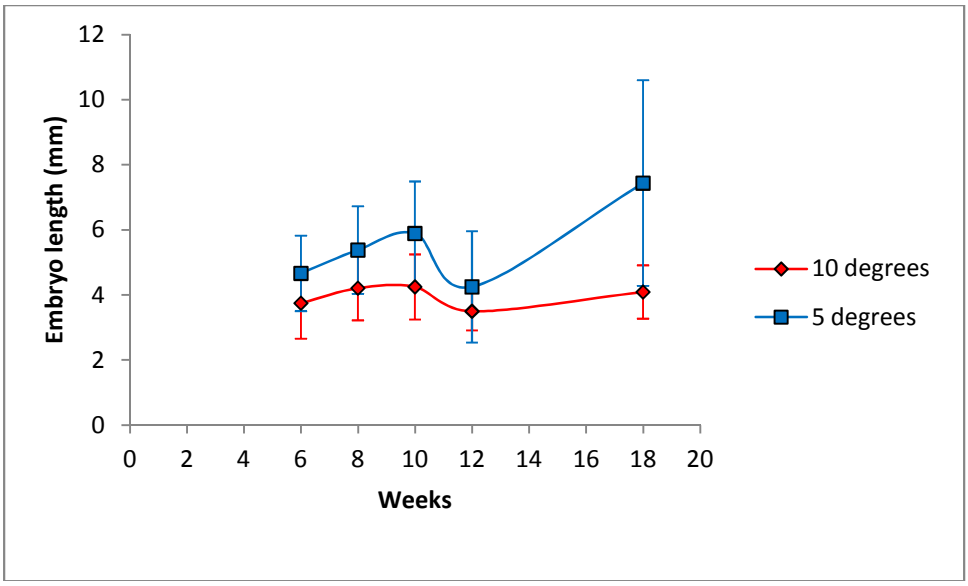

Figure 8. ***Lomatium dissectum*** **Embryo Lengths (± standard deviations) from Imbibed Seeds Exposed to 10°C for 6-18 Weeks Compared to the Length of Embryos from Imbibed** ***Lomatium dissectum*** **Seeds Exposed to 5°C for 6-18 Weeks. There Were 5 Replications Per Treatment.**

Table 12. ANOVA Table Comparing the Embryo Length of Imbibed ***Lomatium dissectum*** **Seeds Exposed to 5°C or 10°C for 6-8 Weeks. There Were 5 Replications of 60 Seeds Per Treatment.**

Ti me	Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
6 weeks	Treatment	1	1.8611753	1.86118	3.4053	0.1143
	Error	6	3.2793297	0.54655		
	C. Total	7	5.1405051			
8 weeks	Treatment	1	2.4080580	2.40806	4.4734	0.0788
	Error	6	3.2298160	0.5383		
	C. Total	7	5.6378740			
10 weeks	Treatment	1	5.2901251	5.29013	9.5736	0.0213
	Error	6	3.3154443	0.55257		
	C. Total	7	8.6055694			
12 weeks	Treatment	1	7.582752	7.58275	13.4619	0.0105
	Error	6	3.379663	0.56328		
	C. Total	7	10.962415			
18 weeks	Treatment	1	7.470176	7.47018	3824.73	0.0103
	Error	1	0.0019531	0.00195		
	C. Total	2	7.4721291			

Seeds treated at 10°C had very poor germination. Germination did not exceed 2% for any treatment.

Alternative Temperature Regimes

The seeds from the Adair and Side 99 sites were treated with two other temperature regimes to explore the possibility of other better dormancy breaking procedures. When imbibed seeds from the two sites were incubated at 4°C, the average percent germination increased as the length of the stratification increased throughout the entire 28 weeks. However, with both sites, around the 15th and 16th weeks there was a decrease in the rate at which the embryo lengths increased. When seeds were treated for 12 weeks at 4 °C and then transferred to the 5/15 °C alternating regime, the average percent germination increased until the 13th or 14th weeks where it seemed to level out. These seeds exposed to alternating temperatures also reached a much lower average percent germination than those exposed only to 4°C (Fig. 9).

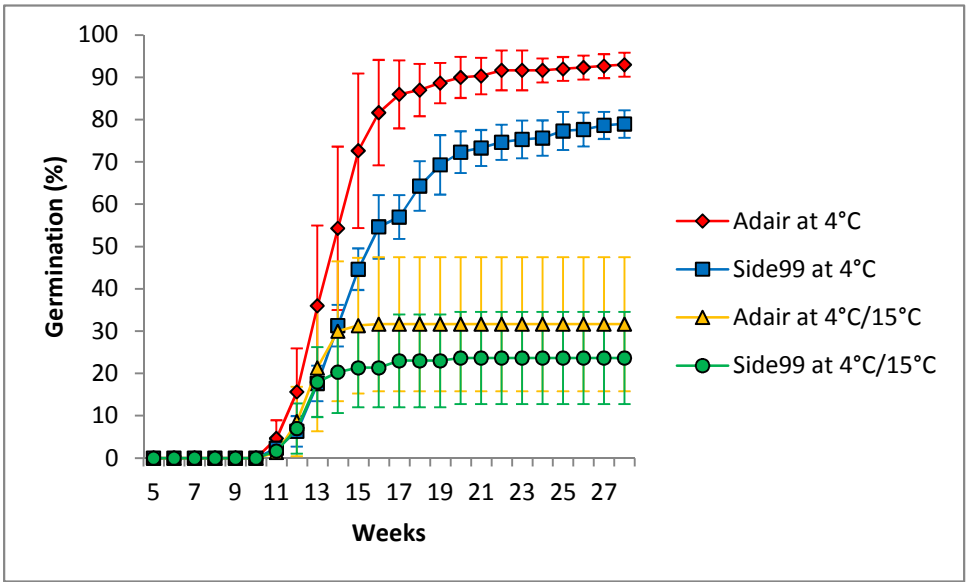

Figure 9. Percent Germination (± standard deviation) of Imbibed *Lomatium dissectum* Seeds from Two Locations After Exposure to Two Temperature Regimes.

Effect of Storage on Embryo Growth and Germination

When *Lomatium dissectum* seeds were stored at room temperature for one year then exposed to cold, moist conditions, embryo growth did occur. Embryo lengths gradually increased up to 12 weeks after which time no significant growth occurred. After the 12 weeks treatment length, the embryo lengths leveled out, fluctuating between 7 and 8 mm for the remaining treatment lengths. This pattern of growth resembled the embryo growth of fresh seed until 10 weeks. After 10 weeks, the embryo length of the stored seeds remained slightly higher than those of the fresh seeds until the final treatment length (22 weeks) when the roles reversed (Fig. 10). When the embryo lengths were compared at each treatment length, there was no significant difference between the stored and fresh seeds (Table 13).

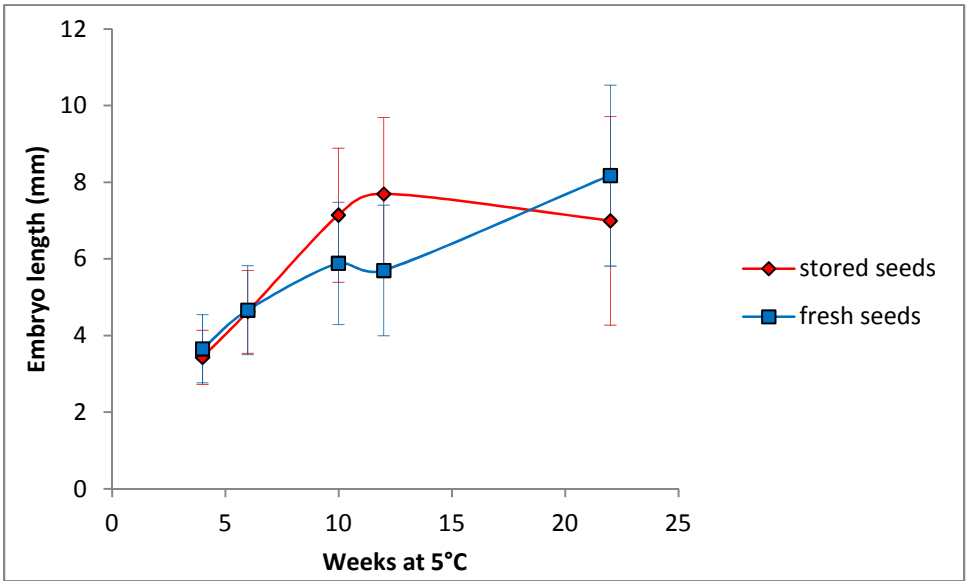

Figure 10. ***Lomatium dissectum*** **Embryo Lengths (± standard deviation) from Seeds Stored Dry for 1 Year Prior to Imbibition and Exposure to 5°C for 4-22 Weeks Compared to Embryo Lengths (± standard deviation) of Fresh** ***Lomatium dissectum*** **Seeds Imbibed and Exposed to 5°C for 4-22 Weeks. There Were 5 Replications Per Treatment.**

Table 13. ANOVA Table Comparing the Embryo Length of *Lomatium dissectum* Seeds Stored Dry for 1 Year, Then Imbibed and Exposed to 5°C for 4-22 Weeks, and the Embryo Length of Fresh *Lomatium dissectum* Seeds Imbibed and Exposed to 5°C for 8-22 Weeks. There Were 5 Replications of 60 Seeds Per Treatment.

Time	Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
4 weeks	Treatment	1	0.1143159	0.114316	0.5593	0.4828
	Error	6	1.2262645	0.204377		
	C. Total	7	1.3405804			
6 weeks	Treatment	1	0.0127832	0.012783	0.0289	0.8706
	Error	6	2.6554640	0.442577		
	C. Total	7	2.6682472			
10 weeks	Treatment	1	3.2442931	3.24429	4.8598	0.0697
	Error	6	4.0054443	0.66757		
	C. Total	7	7.2491374			
12 weeks	Treatment	1	7.856815	7.85681	12.9310	0.0114
	Error	6	3.645565	0.60759		
	C. Total	7	11.502380			
22 weeks	Treatment	1	1.3144463	1.31445	2.8167	0.1919
	Error	3	1.4000000	0.46667		
	C. Total	4	2.7144463			

The average germination of stored seeds remained relatively low until 12 weeks when it increased rapidly to 61% (Fig. 11). After 16 weeks, of exposure the germination increased again, establishing a plateau, ranging from 80% to 86%, which extended throughout the remaining treatments. At all treatment lengths except the final treatment, the percent germination of the stored seeds was higher than those of the fresh seeds (Fig. 11). The germination of the stored and fresh seeds was compared at each treatment length. The first treatment length with any germination was 8 weeks, which resulted in a significantly higher germination for the stored seeds (Table 14; $p = 0.0003$). After 10 weeks, the stored and fresh seeds had similar germination rates that were not significantly different (Table 14). The ANOVA test of the germination rates after 12 weeks of treatment showed that the stored seeds had a significantly higher germination, 61.3%,

than the fresh seeds (Table 14; p = 0.0004). The final treatment, 22 weeks, resulted in the two treatments having germination that were not significantly different (Table 14).

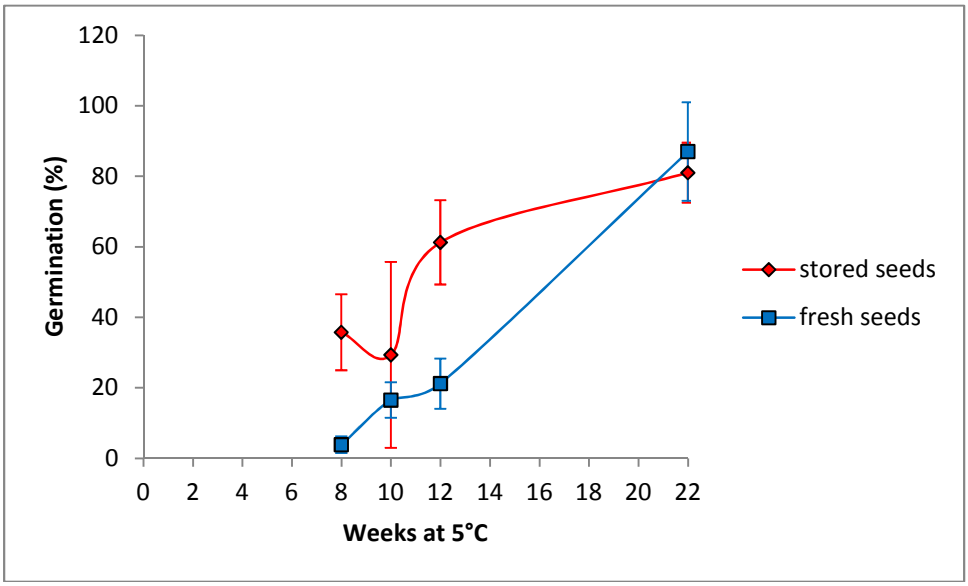

Figure 11. Percent Germination (± standard deviation) of Imbibed *Lomatium dissectum* Seeds Stored Dry for 1 Year Prior to Imbibitions and Exposure to 5°C for 8-22 Weeks Compared to Percent Germination of Imbibed *Lomatium dissectum* Seeds Immediately Exposed to 5°C for 8-22 Weeks. There Were 5 Replications Per Treatment.

Table 14. ANOVA Table Comparing the Percent Germination of *Lomatium dissectum* Seeds Stored Dry for 1 Year, Then Imbibed and Exposed to 5°C for 4-22 Weeks, and the Percent Germination of Fresh *Lomatium dissectum* Seeds Imbibed and Exposed to 5°C for 8-22 Weeks. There Were 5 Replications of 60 Seeds Per Treatment.

Time	Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
8 weeks	Treatment	1	2262.7736	2262.77	42.7305	0.0003
	Error	7	370.682	52.95		
	C. Total	8	2633.4556			
10 weeks	Treatment	1	307.8403	307.84	1.2387	0.3083
	Error	6	1491.0547	248.509		
	C. Total	7	1798.895			
12 weeks	Treatment	1	3568.0109	3568.01	39.5455	0.0004
	Error	7	631.578	90.23		
	C. Total	8	4199.5889			
22 weeks	Treatment	1	80	80	0.56	0.4786
	Error	7	1000	142.857		
	C. Total	8	1080			

Effect of Gibberellic Acid on Embryo Growth

Embryo growth was higher at 4°C than at 12°C, and GA_3 did not substitute the requirement for low temperature. At 12°C, embryo elongation was minimal and no differences were observed between seeds incubated in water and those incubated in GA_3 (Fig. 12). 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.

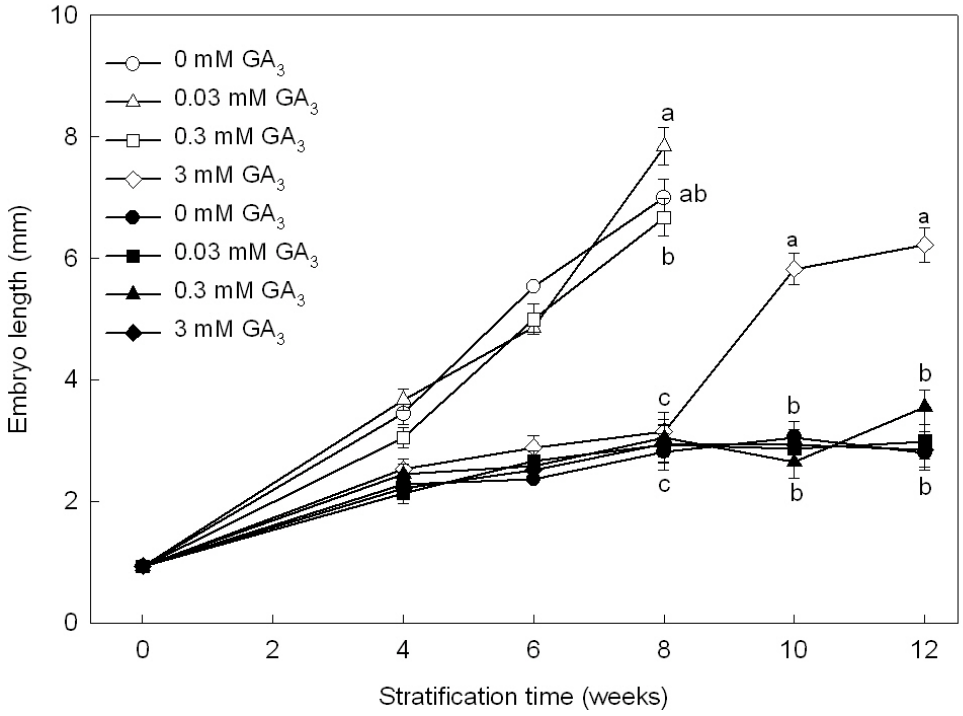

Figure 12. Embryo Elongation of Imbibed *Lomatium dissectum* Seeds Incubated at 4°C (white symbols) or 12 °C (black symbols) in Various Concentrations of GA_3. Means (± s.e.) of Four Replications with 10 Embryos Per Replication. For a Particular Week, Means Not Labeled with the Same Letter are Significantly Different ($p < 0.05$) Based on Tukey-Kramer Least-Square Means Test.

Effect of Plant Hormones on Germination

When seeds, already exposed to 12 weeks of cold, moist conditions, were treated with various hormones at selected concentrations, then exposed to 15°C for 14 weeks the resulting germination was similar (Fig. 13). The percent germination of the treatments ranged from 39.5 to 52.8%. Seeds exposed to only cold, moist conditions for 12 weeks served as the control treatment. The variations in germination from treatment to treatment were not significant according to the ANOVA test (Table 15; p = 0.9457).

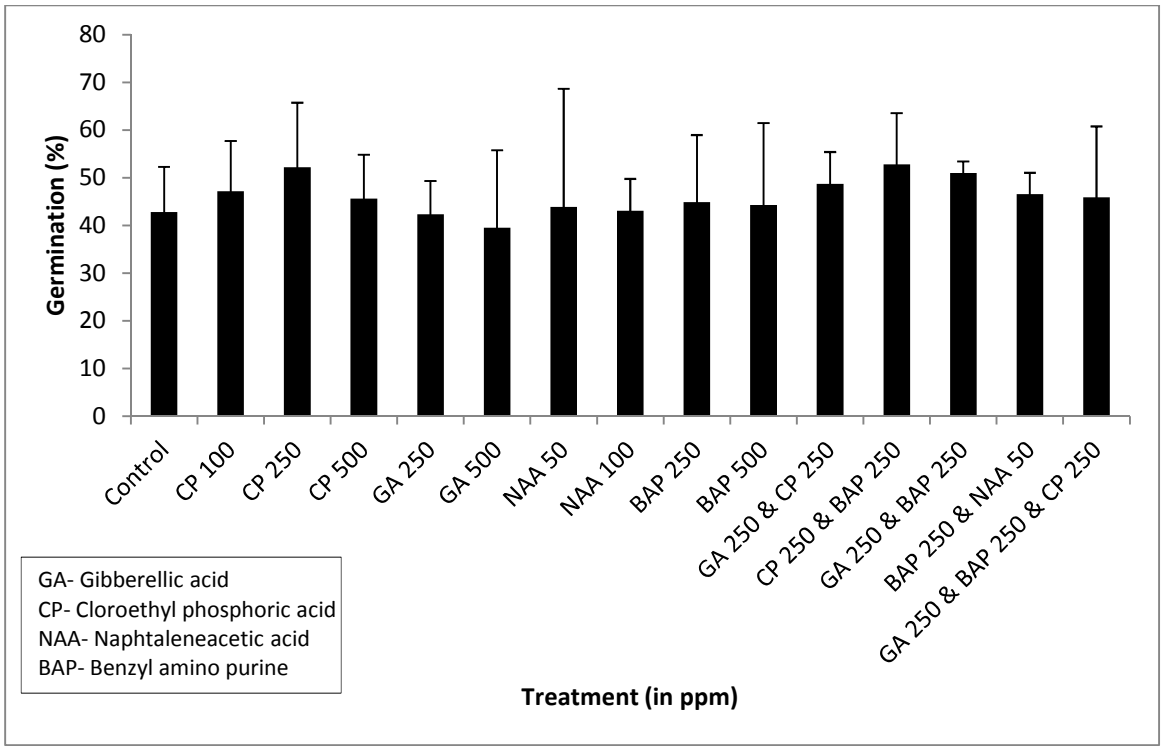

Figure 13. Percent Germination (± standard deviation) of Imbibed *Lomatium dissectum* Seeds Exposed to 5°C for 12 Weeks Then Treated with Various Concentrations of Hormones and Returned to 5°C for 14 Weeks with 5 Replications Per Treatment.

Table 15. ANOVA Table Comparing the Percent Germination of Imbibed *Lomatium dissectum* Seeds Exposed to 5°C for 12 Weeks, Then Treated with Various Concentrations of Hormones and Returned to 5°C for 14 Weeks. There Were 5 Replications of 50 Seeds Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	14	1005.474	71.82	0.4591	0.9457
Error	60	9386.847	156.447		
C. Total	74	10392.321			

Embryo Growth and Germination under Field Conditions

When seeds were placed in field conditions, embryos reached their longest length in early March of 2006 with an average length of 6.7 mm. Upon reaching this length, the

seeds had been exposed to the equivalent of 12.5 weeks of temperatures at or below 5°C. Soil moisture prior to this sampling date averaged .08 volume of water: volume of soil (Fig. 14). When the embryo lengths from each collection date were compared with an ANOVA test, the embryo lengths were significantly different (Table 16; $p < 0.0001$). Embryo lengths increased from Dec through early March, but no increase in length was seen thereafter (Table 17) (Fig. 14).

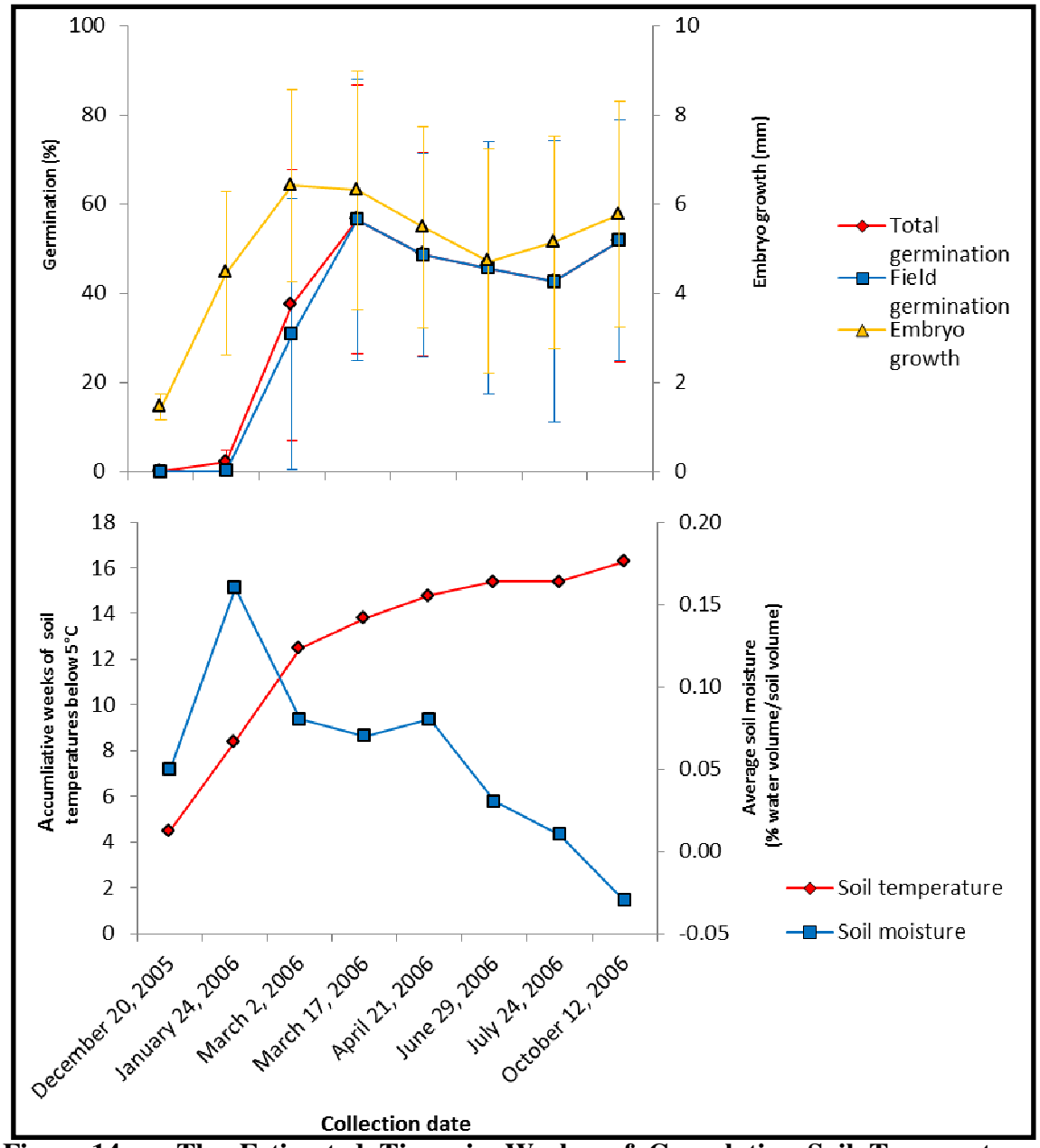

Figure 14. The Estimated Time, in Weeks, of Cumulative Soil Temperatures Below 5°C, Soil Moisture, and the Corresponding Embryo Length and Field and Total Percent Germination (± standard deviation) of *Lomatium dissectum* Seeds Removed from the Field on Selected Dates. There Were 10 Replications Per Date.

Table 16. ANOVA Table Comparing Embryo Lengths of *Lomatium dissectum* Seeds Buried in the Field Then Collected After Various Lengths of Time. There Were 10 Replications of 10 Embryos Per Collection Date.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	6	184.39369	30.7323	25.5026	<0.0001
Error	56	67.48362	1.2051		
C. Total	62	251.87731			

Table 17. Embryo Lengths of *Lomatium dissectum* Seeds Buried in the Field Then Collected After Various Lengths of Time. Embryo Lengths Followed by the Same Number Do Not Differ (p < 0.05; Tukey test).

Collection date	Embryo length (mm)	
December 20, 2005	1.44	A
January 24,2006	4.49	B
March 2, 2006	6.71	C
March 17, 2006	6.56	C
April 21, 2006	5.46	B C
June 29,2006	4.76	B
July 24, 2006	5.14	B C
October 12, 2006	5.89	BC

Total germination was used to analyze field germination. Total germination included germination occurring in the field prior to collection and any germination that occurred at 15°C in the 2 weeks following collection. Most germination occurred in the field prior to collection. At only two collection dates, Jan 24 and Mar 2, did the total and field germinations differ. The difference between field and total germinations was barely significant for the Jan 24 collection date (Table 18; p = 0.0497). Peak germination occurred in mid-March of 2006 with a percentage germination of 58% (Fig 14). By this sampling date, the seeds had been exposed to the equivalent of 13.8 weeks of temperatures at or below 5°C. The average soil moisture prior to this germination peak was .07 volume of water: soil volume. Following the mid-March germination peak, the

percent germination was relatively stable remaining above 40% (Fig. 14). When compared with an ANOVA test, the germination were significantly different (Table 19; $p < 0.0001$). In this case, the first two collection dates had significantly lower germination than all the other collection dates (Table 20).

Table 18. ANOVA Table Comparing the Field Percent Germination to the Total Percent Germination of *Lomatium dissectum* Seeds Buried in the Field at One Collection Date. There Were 10 Replications of 60 Seeds Per Collection Date.

Date	Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Jan 24,2006	Treatment	1	18.030591	18.0306	4.4281	0.0497
	Error	18	73.293909	4.0719		
	C. Total	19	91.324500			

Table 19. ANOVA Table Comparing Percent Germination of *Lomatium dissectum* Seeds Buried in the Field Then Collected After Various Lengths of Time. There Were 10 Replications of 60 Seeds Per Collection Date.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	7	33787.022	4826.72	8.1066	< 0.0001
Error	69	41083.029	595.41		
C. Total	76	74870.051			

Table 20. Germination of *Lomatium dissectum* Seeds Buried in the Field Then Collected After Various Lengths of Time. Embryo Lengths Followed by the Same Number Do Not Differ ($p < 0.05$; Tukey test).

Collection date	Germination (percent)	
December 20, 2005	0	A
January 24, 2006	2	A
March 2, 2006	37	B
March 17, 2006	58	B
April 21, 2006	49	B
June 29, 2006	46	B
July 25, 2006	43	B
October 12, 2006	52	B

Stratification Requirements of Different Seed Populations

Prior to any treatment, the 2006 seeds from the different collection sites had significantly different embryos lengths (Table 21; $p < 0.0080$). The embryos from LODID-102 had significantly shorter embryos than the seeds from the other, relatively similar sites (Table 22). Following exposure to cold, moist conditions, the embryos from all sites increased in lengths, as can be seen in Figures 15 and 16. However, the rate at which the embryos grew were different. After 6 weeks of treatment, the differences in the embryo length between sites were significant (Table 23; $p < 0.0001$). The Tukey test showed that embryos of LODIM-86 were significantly longer than all other embryos (Table 22). Seeds from LODIM-86 continued to have the longest average embryo length, 6.1 mm, after 10 weeks of treatment. However, the ANOVA test shows no significant differences between the embryo lengths of the sites (Table 24; $p = 0.0699$). After 14 weeks, the seeds from LODID-102 became too degraded and this site was removed from the remainder of the experiment. The embryos from the four remaining sites at this treatment length visually segregated into two groupings with the embryos from LODID-100 and LODID-86 forming a longer embryo group and the embryos from LODI-18 and LODIM-61 forming a shorter embryo group. The embryos from these four sites were significantly different (Table 25; $p < 0.0001$). The Tukey test showed that LODID-100 and LODIM-86 did have significantly longer embryos than the other two sites (Table 22). For the final treatment length, 18 weeks, LODID-100 had to be removed from the experiment due to seed degradation. For this treatment length, the embryo lengths for each site remained relatively un-changed and continued to be significantly different (Table 26; $p <0.0001$). The Tukey test showed that LODIM-86 embryos remained significantly longer than embryos from the other two sites (Table 22).

Table 21. ANOVA Table of Embryo Length of Imbibed *Lomatium dissectum* Seeds Over An Elevation Gradient. Replications Varied Between 4 and 5 Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	4	0.44869466	0.112174	5.3320	0.0080
Error	14	0.29452974	0.021038		
C. Total	18	0.74322440			

Table 22. Embryo Length of Imbibed *Lomatium dissectum* Seeds Collected at Different Sites and Exposed to 5°C for Various Lengths of Time. Embryo Lengths Followed by the Same Number Do Not Differ ($p < 0.05$; Tukey test).

Site	Embryo length (mm) prior to treatment	Embryo length (mm) after 6 weeks of exposure to 5°C	Embryo length (mm) after 14 weeks of exposure to 5°C	Embryo length (mm) after 18 weeks of exposure to 5°C
LODID-86	1.25 A	5.05 A	7.18 A	6.46 A
LODID-61	1.20 A	3.26 B	3.30 B	3.92 B
LODID-18	1.11 AB	3.51 B	3.20 B	3.06 B
LODIM-100	0.93 AB	3.09 B	7.33 A	
LODIM-102	0.84 B	3.23 B		

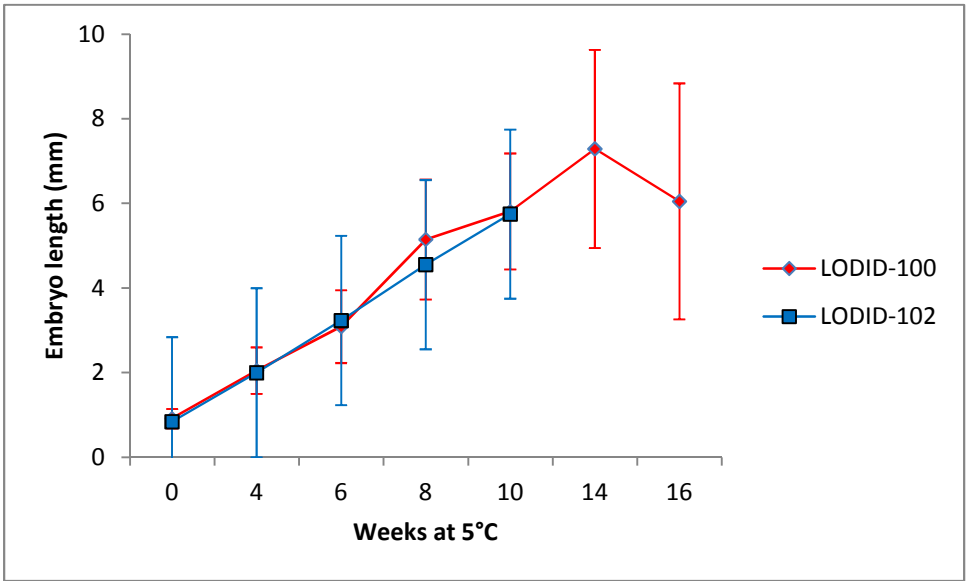

Figure 15. *Lomatium dissectum* var. *dissectum* Embryo Lengths (± standard deviation) Collected Over an Elevation Gradient. There Were 4-5 Replications Per Treatment.

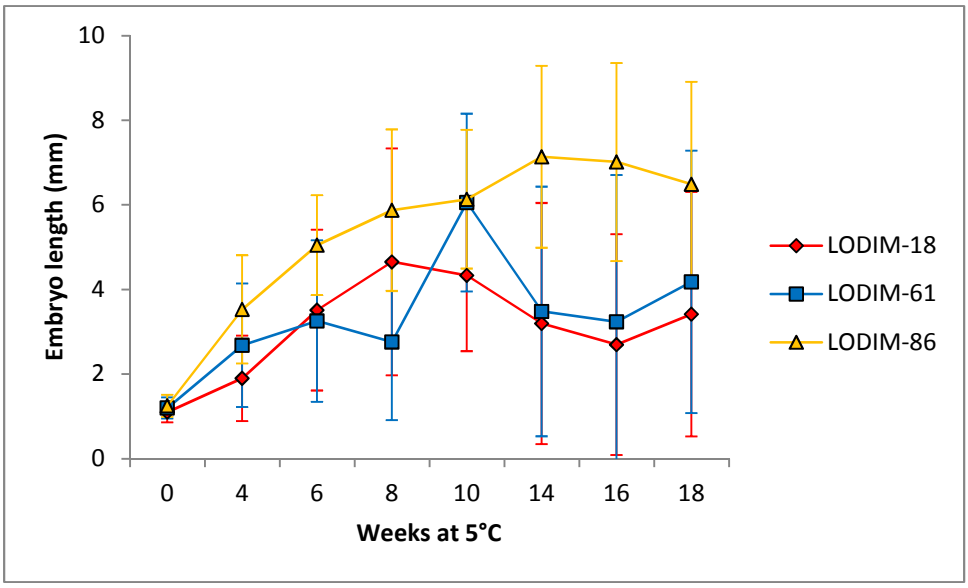

Figure 16. ***Lomatium dissectum*** **var.** ***multifidium*** **Embryo Lengths (± standard deviation) Collected Over an Elevation Gradient. There Were 4-5 Replications Per Treatment.**

Table 23. ANOVA Table of Embryo Length of Imbibed *Lomatium dissectum* Seeds Collected Over An Elevation Gradient and Exposed to 5°C for 6 Weeks. Replications Varied Between 4 and 5 Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	4	12.616441	3.15411	29.3828	<0.0001
Error	18	1.932216	0.10735		
C. Total	22	14.548657			

Table 24. ANOVA Table of Average Embryo Length of Imbibed *Lomatium dissectum* Seeds Collected Over An Elevational Gradient and Exposed to 5°C for 10 Weeks. Replications Varied Between 4 and 5 Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	4	12.811436	3.20286	2.6759	0.0699
Error	16	19.151130	1.19695		
C. Total	20	31.962566			

Table 25. ANOVA Table of Average Embryo Length of Imbibed *Lomatium dissectum* Seeds Collected Over An Elevation Gradient and Exposed to 5°C for 14 Weeks. Replications Varied Between 4 and 5 Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	3	75.662933	25.2210	16.8683	<0.0001
Error	15	22.427569	1.4952		
C. Total	18	98.090503			

Table 26. ANOVA Table of Average Embryo Length of Imbibed *Lomatium dissectum* Seeds Collected Over An Elevation Gradient and Exposed to 5°C for 18 Weeks. Replications Varied Between 4 and 5 Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	2	58.23293	29.1165	16.1666	<0.0001
Error	25	45.02578	1.8010		
C. Total	27	103.25871			

The percent germination of all seed collections increased as the length of exposure to cold, moist conditions increased (Figs. 17 and 18). After 6 weeks of cold, moist treatment only, LODID-18 and LODID-61 exhibited any germination. The germination totals of both sites were quite low, 0.4 and 2.5%, respectively, and consequently there were no significant differences between the sites that germinated and those that did not (Table 27; $p = 0.1521$). After 12 weeks, all seed collections had begun to germinate. However, at this time, there was no representative from LODID-86 due to a shortage of seeds, due to poor seed production, and high level of insect damage at that site in 2006 (Figs. 17 and 18). The ANOVA test showed a significant difference between the sites (Table 28; $p = 0.008$). At this treatment length, the percent germination of LODID-102 seeds was significantly higher than those for LODIM-18 and LODIM-61 (Table 29). The percent germination of LODID-102 and LODID-100 continued to increase after 14 weeks to 88.8 and 61.8%, respectively (Fig. 17). The percent

germination of LODIM-86 also increased to 35.6%, while the remaining two sites exhibited only moderate increases (Fig. 18). When tested with an ANOVA test, the differences were significant (Table 30; $p < 0.0001$). The Tukey test showed that LODID-100 and LODID-102 differed significantly from each other as well as from the other three sites (Table 29). At 18 weeks, LODID-100 increased to 80%, nearing the relatively stable percent germination of LODID-102 (Fig. 17). The remaining sites continued to experience only slight changes in their germination (Fig. 18). The ANOVA test of the data showed there were significant differences between the sites (Table 31; $p < 0.0001$). The lower elevational sites, LODID-102 and LODID-100, experienced significantly higher germination than the other sites (Table 29). At 26 weeks, there was little change in germination except for the increasing percent germination from LODIM-86 and LODIM-18 (Fig. 18). The percent germination of all sites were significantly different (Table 32; $p < 0.0001$). The Tukey test showed that LODID-100 and LODID-102 had a significantly higher percent germination than the other sites and LODIM-86 had significantly higher germination rates than LODIM-18 and LODIM-61 (Table 29).

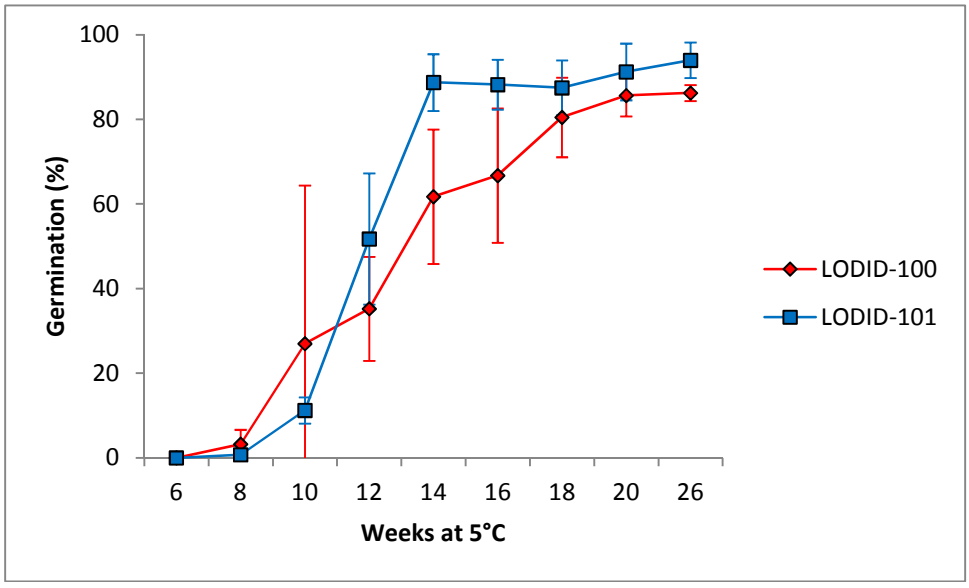

Figure 17. Percent Germination (± standard deviation) of *Lomatium dissectum* var. *dissectum* Collected Over an Elevation Gradient and Exposed to 5°C for 6-26 Weeks. There Were 4-5 Replications Per Treatment.

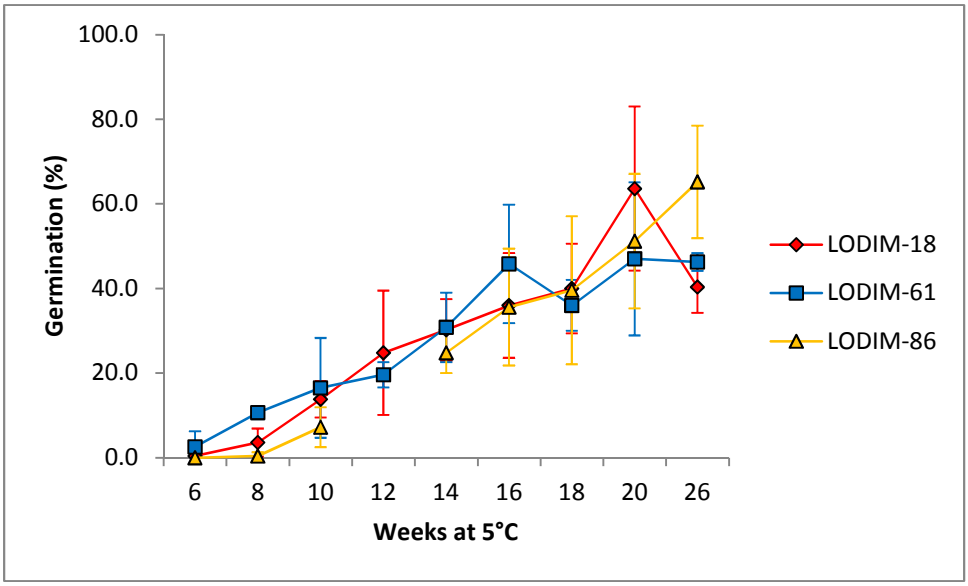

Figure 18. Percent Germination (± standard deviation) of *Lomatium dissectum* var. *multifidium* Collected Over an Elevation Gradient and Exposed to 5°C for 6-26 Weeks. There Were 4-5 Replications Per Treatment.

Table 27. ANOVA Table Comparing the Percent Germination of Imbibed *Lomatium dissectum* Seeds Collected Over An Elevation Gradient and Exposed to 5°C for 6 Weeks. Replications Varied Between 4 and 5 Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	4	24.817391	6.20435	1.9123	0.1521
Error	18	58.4	3.24444		
C. Total	22	83.217391			

Table 28. ANOVA Table Comparing the Percent Germination of Imbibed *Lomatium dissectum* Seeds Collected Over An Elevation Gradient and Exposed to 5°C for 12 Weeks. Replications Varied Between 4 and 5 Per Treatment.

Source	Degrees of	Sum of	Mean square	F ratio	Prob > F

	freedom	squares			
Treatment	3	2628.5	876.167	5.9158	0.008
Error	14	2073.5	148.107		
C. Total	17	4702			

Table 29. Germination of Imbibed *Lomatium dissectum* Seeds Collected Over An Elevation Gradient and Exposed to 5°C for Various Lengths of Time. Replications Varied Between 4 and 5 Per Treatment. Embryo Lengths Followed by the Same Number Do Not Differ ($p < 0.05$; Tukey test).

Site	Germination (percent) after exposure to 12 weeks of 5°C	Germination (percent) after exposure to 14 weeks of 5°C	Germination (percent) after exposure to 18 weeks of 5°C	Germination (percent) after exposure to 26 weeks of 5°C
LODI-102	52 A	89 A	88 A	94 A
LODI-100	35 A	62 B	81 A	86 A
LODI-86		25 C	40 B	65 B
LODI-18	25 B	30 C	40 A	46 C
LODI-61	20 B	31 C	36 A	40 C

Table 30. ANOVA Table Comparing the Percent Germination of Imbibed *Lomatium* dissectum Seeds Collected Over An Elevation Gradient and Exposed to 5°C for 14 Weeks. Replications Varied Between 4 and 5 Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	4	12921.404	3230.35	39.7745	< 0.0001
Error	18	1461.9	81.22		
C. Total	22	14383.304			

Table 31. ANOVA Table of Percent Germination of Imbibed *Lomatium dissectum* Seeds Collected Over An Elevation Gradient and Exposed to 5°C for 18 Weeks. Replications Varied Between 4 and 5 Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	4	10932.017	2733	22.2678	< 0.0001
Error	18	2209.2	122.73		
C. Total	22	13141.217			

Table 32. ANOVA Table of Percent Germination of Imbibed *Lomatium dissectum* Seeds Collected Over An Elevation Gradient and Exposed to 5°C for 26 Weeks. Replications Varied Between 4 and 5 Per Treatment.

Source	Degrees of freedom	Sum of squares	Mean square	F ratio	Prob > F
Treatment	4	8261.5833	2065.4	35.8175	< 0.0001
Error	15	864.9667	57.66		
C. Total	19	9126.55			

The stratification requirements of different populations were also tested with seeds collected in 2007. The results showed 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. 19). 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. No clear differences in the final percent germination were observed except for the seeds collected at Moores's mountain, which had lower final germination.

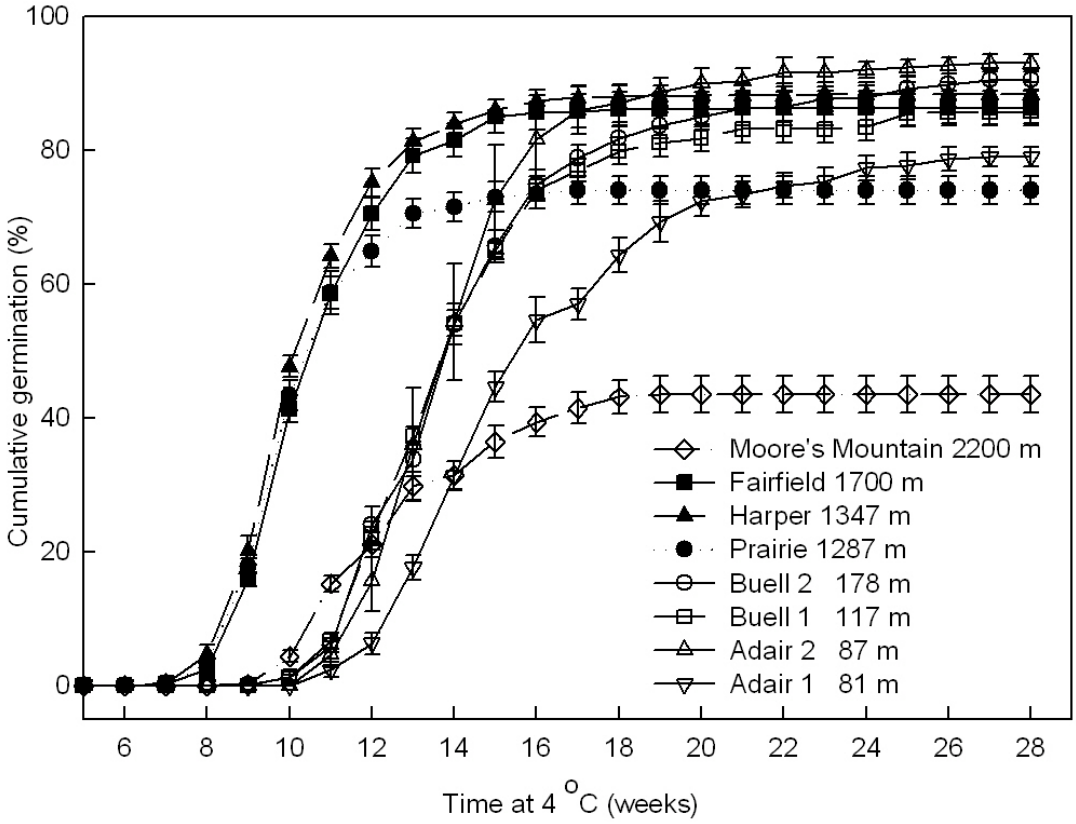

Figure 19. Germination of *Lomatium dissectum* Seeds Collected Over an Elevation Gradient. Mean ± s.e. of Five to 10 Replications with 60 Seeds Per Replication. Moore's Mountain, Fairfield, Harper, and Prairie are Populations of *L. dissectum* var. *multifidum*, and Adair 1, Adair, 2, Buell 1, and Buell 2 are Populations of *L. dissectum* var. *dissectum*.

DISCUSSION

Under laboratory conditions, the greatest embryo growth of *Lomatium dissectum* seeds resulted from exposure to cold, moist stratification. Within the scope of the treatments tested, longer exposure to cold, moist conditions resulted in greater embryo growth. Warm, moist stratification either alone or in combination with cold, moist stratification did not improve embryo growth. The stimulation of embryo growth by cold, moist stratification revealed the necessary treatments for breaking the morphological dormancy in *Lomatium dissectum.* Physiological dormancy was also broken during this treatment since seeds germinated at cold temperatures.

Prolonged exposure of the *Lomatium dissectum* seeds to cold, moist conditions also resulted in high germination. The greatest germination percentages occurred after seeds were exposed to cold, moist conditions for more than 15 weeks. Treatments combining cold, moist conditions with short-term after-ripening and warm, moist conditions resulted in limited germination but the resulting germination was not significantly improved. In fact, when the cold, moist exposure was combined with warm, moist conditions, germination was depressed. These results suggest that the complete release of physiological dormancy in *Lomatium dissectum* is mainly dependent upon prolonged exposure to cold, moist conditions. Additionally, I determined that neither short-term nor long-term after-ripening significantly facilitated dormancy breakage. I

also found that release of dormancy was not enhanced by fluctuating temperatures but is best obtained through exposure to a low, constant temperature.

Cool, moist treatments at 10°C did not stimulate embryo growth or germination to the same extent as did exposure to 5°C, but the differences were not significant. Thus, the critical upper temperature limit for the range of temperatures, as suggested by Bradford (2002), capable of stimulating dormancy release is above 10°C. As the treatment lengths increased, the p-value did near the critical value (0.05), suggesting that the upper temperature limit may be near 10°C.

Treating seeds with selected plant hormones following 12 weeks of exposure to cold, moist conditions did not enhance germination. I chose hormones and concentrations that are commonly used to release physiological seed dormancy, and despite the wide range of chemicals and concentrations tested there was no effect (Bewley 1997; Baskin and Baskin 1998; Ekstam et al. 1999; Matilla 2000; Baskin and Baskin 2004; Kucera et al. 2005). This knowledge not only allows us to define the type of dormancy in our seeds but it also allows us to speculate about the mechanisms causing the dormancy. In the case of *L. dissectum*, we can reason that the hormones we tested are not the limiting factor of germination and that the seed dormancy is the result of another germination factor such as an inability of the seed to detect the hormones or the presence of high concentrations of the dormancy promoting abscisic acid (Bewley 1997; Matilla 2000; Kucera et al. 2005)

Based on the results from the laboratory experiments, it is evident that the dormancy of the embryos was most effectively broken by exposure to cold, moist conditions for 15 weeks or longer and is not improved by any of the other treatments

tested. Prior to the beginning of this research project, I was able to determine that *L. dissectum* seeds do not have physical dormancy as they were permeable to water (data not shown). As determined from dissecting seeds prior to treatment, the embryos of the *Lomatium dissectum* seeds were underdeveloped at the time of dispersal. Based on this knowledge, it was evident that the seeds possess morphological dormancy. In addition, the delay of embryo growth after dispersal suggested the presence of some type of physiological dormancy as well, thus narrowing the possibilities to one of the forms of morphophysiological dormancy (MPD). The lack of increase in embryo growth and germination after exposure to warm, moist conditions and hormones allowed us to rule out non-deep simple, intermediate simple, and deep simple types of MPD. Because the seeds did emerge from dormancy after exposure to what would amount to only one season of cold temperatures, we could rule out deep simple double MPD. Again the lack of a requirement for exposure to warm, moist conditions and the lack of response to hormones such as gibberellic acid eliminated the possibility of the seeds having non-deep complex and intermediate MPD. The response of the seeds to long exposures to cold, moist conditions fit the requirements for the seeds for having deep complex MPD. This places *L. dissectum* in the same dormancy category as *Osmorhiza depauperata, Aegopodium podagraria*, and *Chaerophyllum temulum* (Baskin et al. 1992; Walck and Hidayati 2004; Vandelook et al. 2007a; Phartyal et al. 2009). This type of dormancy allows *L. dissectum* to germinate during a time of decreased competition and to take advantage of the improved soil moisture conditions that occur in the spring within the range of the species. As seeds of this size, germination strategy and dormancy type usually form transient seedbanks, which have short lifespans (Thompson and Grime

1979; Hawkins et al. 2007); these seeds will be unlikely to remain in the soil for more than one growing season.

Species with complex MPD show high percent germination without warm stratification (Baskin and Baskin 2004). In some cases, however, warm stratification can reduce the length of the cold stratification period (Walck and Hidayati 2004). This phenomenon was not observed in *L. dissectium*. Warm stratification for several weeks did not result in faster rates of growth under cold conditions. Similarly, warm stratification did not reduce the length of the cold stratification period required to initiate germination.

Among species with deep complex MPD, variation exists in the exposure length leading to embryo growth, the effect of warm stratification prior to cold stratification on embryo growth, and the optimum temperatures for embryo growth and germination. For example, seeds of *Osmorhiza aristata* and *Chaerophyllm temulum* show a delay between the start of cold stratification and the initiation of embryo growth (Walck and Hidayati 2004; Vandelook et al. 2007a). These embryos do not elongate during the first 2 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 2 week of cold stratification. Furthermore, *L. dissectum* embryos had the highest relative rates of embryo growth, about 30% per week, during the first 2 weeks of cold stratification. Thus, in *L. dissectum*, the release of PD and MD appears to occur immediately. The possibility exists, however, that the degree of PD varies along the embryo. 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 occurred towards the chalazal end of the seed. Very little elongation occurred between the radicle and the micropyle until the beginning of germination.

When planted in the field, the embryos gradually increased in length until early March. As expected, the maximum embryo length was less under field conditions than under laboratory conditions. This difference is due partially to the more extreme, less consistent conditions of the field as well as the shorter exposure to cold, moist conditions. After reaching peak length, the embryos began to display a decrease in embryo length. However, once embryos reach their maximum length, there should be no change in embryo length. It is likely that the decrease in embryo length is due to embryo desiccation rather than actual shrinkage in the embryos themselves.

The majority of the germination of the field seeds occurred while the seeds were experiencing the lowest field temperatures. The single exception was the Jan. 24th collection. We speculate that this difference was due to a delayed response to cold exposure, which manifested as the seeds were incubated in the lab at 15°C. This higher temperature is likely too high to stimulate germination, as suggested by our research. The highest percent germination of the seeds in the field occurred shortly after the peak in embryo length in mid-March. As with embryo growth, the peak in germination was much lower under field conditions than under laboratory conditions. Again, this is likely due to the shorter exposure to cold, moist conditions and the inherently inconsistent

conditions of the field. Based on this field data, we would suggest that seeding of this species be done in the fall to allow for seed exposure to the cold conditions that lead to the embryo growth and seed germination that occur with the emergence of the seed from dormancy.

The *Lomatium dissectum* var. *multifidum* seeds from the highest elevation (LODI-86) exhibited relatively rapid embryo growth in response to the shorter treatment lengths when compared to the other sites. This seed lot reached peak embryo length after 14 weeks of cold, moist treatment. However, germination of this accession occurred more slowly and it may not have reached its full germination potential by the end of the 26 week incubation period, showing that the physiological dormancy requires additional time before it is broken. The *Lomatium dissectum* var. *dissectum* embryos from the lower sites (LODI-100 and LODI-102) also reached their greatest length after 14 weeks but with more even growth over all the treatment lengths. It was also at this treatment length when the percent germination also reached its higher range, showing a more synchronized release from the MPD dormancy.

The growth of the *L. dissectum* var. *dissectum* embryos from the middle elevation sites (LODI-18 and LODI-61) was somewhat unexpected. Embryos from both sites had increasing lengths for the first 10 weeks of cold, moist treatments, but then decreased. Recovery of embryo length was only slight by the end of the experiment. The germination of seed from these two sites was slow but steadily increasing through the entire experiment, reaching a low total germination, which, as in the case of the highest elevation site, was likely not its full germination potential. From these results, we can determine that these two varieties have different needs for breaking dormancy. For the

seeds collected in 2006, *L. dissectum* var. *dissectum* broke dormancy after shorter exposures to cold conditions and were able to germinate when embryo lengths were shorter than those of *L. dissectum* var. *multifidum*, suggesting that both the morphological and physiological dormancies are more quickly broken in the *dissectum* variety. This ability to germinate after less exposure to cold temperatures could be an adaptation of *L. dissectum* var. *dissectum* due to its environment, which has warmer, less extreme temperatures in the winter and spring. Within the *L. dissectum* var. *multifidum* seed lots, we observed variation between populations. The results revealed that embryo growth and to a smaller extent germination was stimulated faster in seeds from the higher elevation site compared to those from the lower elevations. This elevational response could be an adaptation of the seeds to a site that has a shorter growing season. However, all three sites may require more than 26 weeks of exposure to cold, moist conditions before reaching their full germination potential. It is also possible that the seeds of the *multifidum* variety naturally have much lower germination percentages than the seeds of the *dissectum* variety. These two possible hypotheses could be tested by extending this experiment beyond 26 weeks and adjusting the germination results with seed viability.

In the elevation experiment described above, there were complications that occurred due to the quality and quantity of the seed used. The poor quality and low quantity of seed lead to fewer replications and lower sample sizes. The poor seed quality also lead to more difficulties in determining whether germination had occurred or not. This experiment was repeated with seeds collected in 2007 when the plants showed higher seed yield. For the 2007 seeds, the final germination was about 80% for all populations except for *L. multifidum* seeds collected at Moore's Mountain (2200m). This

suggests that the seed from the other populations were of similar quality. With these seeds, the results differed from those obtained in 2006. In 2007, variety *dissectum* seeds required more hours of stratification than those of the variety *multfidium*. These contrasting results may reflect differences in seed quality among seeds collected in 2006. For example, if the *dissectum* variety seeds were of better quality than those of the *multifidum*, this could have lead to higher rates of embryo growth and germination in the former. An alternative possibility is that the contrasting results reflect differences in environmental conditions during seed development, which can alter the degree of seed dormancy (Fenner 1991; Benech-Arnold et al. 1992; Clua et al. 2006). Under this scenario, the environmental conditions, at the sites, during seed development, could have resulted in a higher degree of dormancy in *multifidum* in 2006. While in 2007, the environmental conditions may have resulted in a higher degree of dormancy in *dissectum*.

The notion that environmental conditions during seed development can affect seed dormancy in *Lomatium dissectum* is supported by some observations. For seeds collected at Harper, OR, differences in stratification requirements were observed between seeds harvested 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 (Scholten et al. 2009). 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 stored for approximately the same periods, suggesting that differences in the degree of dormancy cannot be attributed to disparity in the age of the seeds.

In addition to the environmental factors and hormones investigated in this study, other factors may affect embryo growth and germination. For example, an Australian study with the Apiaceae species *Actinotus leucocephalus* Benth. found a positive germination response to exposure to smoke and very high temperatures (Tieu et al. 2001). Another study, including *L. nevadense* (S. Watson) J.M. Coult & Rose, *L. canbyi* (J.M. Coult & Rose) J.M. Coult & Rose, *L. watsonii* (J.M. Coult & Rose) J.M. Coult & Rose, *L. donnellii* (J.M. Coult & Rose) J.M. Coult & Rose, *L. triternatum* (Pursh) J.M. Coult & Rose, *L. vaginatum* J.M. Coult & Rose, examining the response of forbs to fire, found that some *Lomatiums* had a positive responses to fire. Some of the favorable responses observed included increased reproduction, later senescence and greater vegetation production in plants that germinated following fire (Wrobleski and Kauffman 2003). This study did not explore the possibility of smoke playing a role in seed dormancy and germination but it offers an opportunity for future experiments. Perhaps smoke during the late summer may cause physiological changes that facilitate embryo growth under cold, moist conditions. A role for smoke during germination seems less likely since the seeds germinate during the early spring when fires are uncommon.

As with all experimental projects, there were complications that occurred during this study that created some difficulties. One complication in the study was the degradation of the seeds placed in the field. As previously mentioned, this experiment was set to run for a full two years but had to be ended before completing one year. This was due to seeds deteriorating to the point that signs of germination could no longer be confidently detected. A practical solution to this problem is not readily available as seed degradation is relatively inevitable and seeds with this type of dormancy and germination

strategy do not form long-surviving seedbanks (Thompson and Grime 1979; Forbis et al. 2002; Hawkins et al. 2007).

There were also some aspects of the experimental design that if changed could have led to a clearer picture of dormancy breakage. The majority of these alterations involve the experiments exploring the effect of temperature on embryo growth and germination. The first change would be to record the germination of each germination box right after treatment instead transferring each box to 15°C for 2 weeks before recording germination. The purpose of the exposure to the warmer temperatures was to simulate spring conditions. The results of the field study certainly suggest that the seed do not require the warmer temperatures to germinate. The experiments conducted in the lab with seeds collected in 2007 also clearly indicate that the seeds do not require warm temperatures to germinate. Dormancy break and high germination both occurred at temperatures between 3 and 5°C. I think that more informal exploratory experiments prior to this study would have allowed us to eliminate some of the treatments, such as short-term after-ripening, and concentrate on more promising treatments, such as cold exposure. Nevertheless, the after-ripening experiment suggests that dry, warm conditions similar to those that the seeds experience in the natural environment do not play a major role in the dormancy status of the seeds.

From this research, I was able to determine that *L. dissectum* seeds exhibit deep complex MPD. For seeds to be released from this dormancy, they must be subjected to cold, moist temperatures for more than 3 months. We know that there is likely an upper limit defining what temperatures are effective for cold stratification, but this is above 10°C. We also know that the seeds respond to constant cold temperature better than

fluctuating temperatures as was shown by the studies in the laboratory and in the field. Although there are variations between varieties and populations, the procedures for dormancy release differed only in the length of exposure required. This added knowledge about the germination of *L. dissectum* will allow for more efficient and effective use of this important plant in restoration efforts throughout the Great Basin, leading to more stable plant and animal communities.

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