Germination of Three Native Lupinus Species in Response to Temperature

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Germination of Three Native Lupinus Species in Response to Temperature

Abstract
Understanding germination requirements of native species is an important component of restoration in south Puget Lowland prairies (Washington, USA). We conducted an experiment to determine the effects of pre-germination treatment and germination temperature conditions on the proportional germination of three species of Lupinus. For one species, Lupinus lepidus, germination was highest following heat shock treatments to the dormant seed, suggesting adaptation to the high temperatures associated with wildfire. For a more widespread species, Lupinus polyphyllus, high heat treatments had no effect, and germination was mildly higher in a temperature sequence simulating winter chilling followed by cool, diurnally alternating temperatures. For a final intermediate species, Lupinus albicaulis, responses to germination temperatures were dependent on high-heat treatments. These data suggest interspecific variation in germination cues where these Lupinus species are influenced differentially by environmental conditions in both breaking of physical dormancy and the promotion of germination. Our results have the potential to improve the establishment of these species from seed in restoration. Specifically, there may be implications for the timing of manual seed sowing and the efficacy of seed establishment following exposure to fire in south Puget Lowland prairies.

Introduction
Studies that identify environmental limitations to seed germination in ecologically important native plants have the potential to significantly improve efficacy of ecological restoration in disturbed ecosystems (Primack 1996, Baskin and Baskin 1998, Seabloom et al. 2003, Corbin and D’Antonio 2004, MacDougall and Turkington 2006). In grassland ecosystems in particular, seed limitations may be important in controlling native species abundance (Turnbull et al. 2000, Foster and Tilman 2003, Zeiter et al. 2006, Clark et al. 2007, Stanley et al. 2011). Therefore, determining germination response cues for native species may be especially important in developing holistic restoration strategies in prairie ecosystems.

Restoration efforts in south Puget Lowland prairies (WA, USA) have taken a number of steps to re-establish native flora in a landscape heavily invaded by non-native plants (Stanley et al. 2008). However, plans to significantly expand the scale of restoration efforts highlight the need to test seed germination requirements of native plants under simulated natural conditions (Thomas and Carey 1996, Dunwiddie et al. 2006). Useful germination protocols have already been established for some important taxa native to these prairie ecosystems (Drake et al. 1998, Kaye and Kuykendall 2001). However, species in the genus Lupinus (Fabaceae) remain poorly understood in terms of germination cues.

Members of the genus Lupinus are especially important to restoration efforts because they have symbiotic associations with nitrogen-fixing bacteria. This allows them to play unique roles as arbiters of soil nitrogen dynamics in native plant communities following disturbance. For example, Lupinus lepidus Dougl. ex Lindl. represents the most abundant herbaceous plant on new volcanic substrates following the eruption of Mount St. Helens where it plays a major role in shaping plant community structure (del Moral and Rozzell 2005). Species in the genus Lupinus are also known for unique pollinator relationships with arthropods (especially Hymenoptera and Lepidoptera) and they have been vital in the conservation of endangered species in western Oregon and Washington prairies (e.g., Wilson et al. 2003, Schultz et al. 2011).

The conditions inducing germination in hard-seeded native plants in the genus Lupinus can be complex. Germination in the family Fabaceae is often a two-stage process starting with the breaking of physical dormancy followed by actual germination. Physical dormancy is dormancy provided by hard seed coats that do not allow the seed to absorb water and is especially common in Fabaceae. Van Assche et al. (2003) found that temperature fluctuations involving a chilling to 5 to 10 °C for six weeks followed by alternating day/night temperatures of 15 °C and 7 °C increased germination...
rates for Fabaceae species exhibiting physical dormancy. Other studies have demonstrated high germination for Fabaceae species with exposure to fluctuating spring temperatures (Roberts and Boddrell 1984, Baskin and Baskin 1998). However, other cues might also be involved. The germination of *Lupinus sulphureus* Douglas ex Hook. ssp. *kincaidi* (C.P. Sm.) L. Phillips was significantly increased by the interaction of manual scarification and cold stratification (Kaye and Kuykendall 2001). Heat shock by fire and extreme temperatures (> 80 °C) have also been identified as environmental cues that may break physical dormancy (Probert 2000, Maret and Wilson 2005). Extreme temperature fluctuations of 30 - 40 °C have been found to be sufficient to promote the germination of *Lupinus* spp. in Australia where summer temperature extremes are typical prior to fall rains (Quinlivan 1961, 1968, 1971). Seeds of native Californian Fabaceae species were made permeable by a heat shock treatment of 80 - 100 °C, simulating surface-soil temperatures of wildfires (Brochert 1989, Keeley 1991). This variation in germination cues highlights that species from the same genus and family can display a range of germination responses to a variety of treatments (Martin et al. 1975, Herranz et al. 1998, Pérez-Garcia and González-Benito 2006, Bossuyt and Honnay 2008). Such variation may be related to the evolutionary history of species and especially the fire history of ecosystems where they are most prevalent.

Our study examines species-specific variation in germination cues for three native plants in the genus *Lupinus*: *L. polyphyllus* Lindl, *L. lepidus*, and *L. albicaulis* Doug. var. *albicaulis*. These three species are all native to south Puget Lowland prairies. We specifically chose these species to represent a range of species distributions and life histories. We chose one relatively small species that occurs primarily in frequently-burned and open habitats (*L. lepidus*), one larger resprouting species common in moist sites with a wide geographic distribution (*L. polyphyllus*), and another species with distribution and life history traits intermediate to the other two (*L. albicaulis*). Since germination is a two stage process where the breaking of physical dormancy and the actual sprouting of the seed may be triggered by different mechanisms, we developed hypotheses regarding both processes, and that the three species would have responses corresponding to their life history traits. We hypothesized: 1) high temperature pre-treatments that simulated high summer temperatures or fire events would enhance germination of *L. lepidus* the most, followed by *L. albicaulis*, with *L. polyphyllus* being unaffected by these treatments; and 2) germination of all species would be enhanced by treatments that simulated winter chilling followed by fluctuating spring temperatures.

**Methods**

**Study Species**

*Lupinus lepidus* is a small (10 - 35 cm) perennial that only reproduces by seed. It is only found in the Pacific Northwest, especially in prairies and volcanic plains. The plant generally blooms from May - June, although we have seen this species blooming as late as October. It produces numerous small seeds (mean seed weight 4 mg ± 0.01 mg SD), with set and dispersal in June - August. *Lupinus polyphyllus* is a large perennial (up to 150 cm tall) that typically occurs in moist habitats. It is much more widespread than *L. lepidus*, occurring from California to Alaska and east to Newfoundland. It blooms in May, produces moderately sized seed (mean seed weight 18 mg ± 1.3 mg), with dispersal occurring in late June - July. The species readily resprouts from a rhizomatous base. Finally, *L. albicaulis* is a branching perennial (up to 120 cm tall) found throughout the Northwest primarily in mountain and prairie habitat. This species is fully herbaceous and can resprout from a subterranean crown and a moderately spreading rhizome. The plants bloom in May - June, and the large seed (mean seed weight 31 mg ± 1.9 mg) is dispersed between June - August with full senescence by September.

All *Lupinus* seeds were collected during the summer of 2007 from The Nature Conservancy’s Shotwell’s Landing Nursery (Littlerock, WA). All seeds were collected from native plants propagated from south Puget Lowland prairies. For *L. lepidus*, plants can be sourced to two populations from prairies on the Joint Base Lewis-McChord military base (Fort Lewis, WA). For *L. polyphyllus*, plants can be sourced to two prairie populations at Lewis and Clark State Park (Lewis County, WA), and one population at West Rocky Prairie Wildlife Area (Thurston County, WA). For *L. albicaulis*, plants can be sourced to two populations on the Joint Base Lewis-McChord military base, one population at Scatter Creek Wildlife area, one population at West Rocky Prairie Wildlife Area, and one population at Glacial Heritage Prairie Preserve (Thurston County, WA). The entire seed crop was collected weekly over a 4 week period to ensure a representative sample of the seed population for the year. The collected seeds were air-dried and stored in paper bags for three weeks at approximately 15 °C and 45% relative humidity. These
seeds were examined under a dissecting microscope and any seeds showing signs of herbivory by weevils (*Thychius* sp.) or other damage were removed prior to the study. The seed with the greatest predation by weevils was *L. albicaulis* with 22% of the seed damaged prior to the study. Random samples from the remaining seeds were used for the experiment.

Because proportions of seed with physical dormancy can vary, we performed a preliminary test for physical dormancy. Three replicates of 50 seeds of each species were manually scarified by nicking the seed coat at the distal end. Another three replicates of 50 seeds were untreated. All seeds were placed on moistened filter paper at 20 °C under grow lights (40 μmol photons m⁻² s⁻¹ PPFD) with a 14-hr photoperiod. The individual seeds were weighed (mg) at 0, 12 and 24 hrs. *Lupinus lepidus* and *L. albicaulis* had the highest percentages of seed exhibiting physical dormancy with 70 ± 6% SD and 71 ± 9% SD, respectively, while *L. polyphyllus* had only 19 ± 7% SD (data not shown). Each species exhibited some amount of physical dormancy, indicating pre-treatments designed to break physical dormancy could be effective.

### Pre-Germination and Germination Temperature Regimes

A fully factorial experimental design was used to examine sequential temperature cues for germination among our species. The experiment included all three *Lupinus* species in 15 combinations of pre-treatments and germination temperatures with three replicates of 50 seeds in each combination (n = 3) and a total of 6750 seeds.

The first factor (hereafter pre-treatments) was designed to test responses to cues that might break physical dormancy. The five pre-treatments we tested included two dry high-heat treatments simulating hot summer conditions (37 °C and 47 °C), two heat-shock treatments simulating fire temperature (dry and wet 80 °C), and a constant temperature control treatment (15 °C). The pre-treatments were carried out by subjecting separate batches of seeds to one of the following five procedures: 1) seeds were placed in a drying oven (Yamato DKN600, Yamato Scientific America Inc., Santa Clara, CA) at 37 °C for 8 hr, then placed at 15 °C for 16 hr; 2) seeds were placed in a drying oven at 47 °C for 8 hr followed by exposure to 15 °C for 16 hr; 3) seeds were placed in a drying oven at 80 °C for seven min, then removed to 15 °C and allowed to cool; 4) 50 ml of 80 °C water was poured over the seeds, which were then allowed to soak for 4 hr; and 5) seeds were stored in paper envelopes at 15 °C and 35% relative humidity until the germination study commenced. For each species, the pre-treatments were conducted on the same day the seed was transferred to glass Petri dishes filled with a substrate of silica sand. The seeds were moistened by adding de-ionized water to the saturation point of the substrate.

The second factor (hereafter germination temperatures) was designed to test germination responses to fall and spring temperature variation. These treatments included three germination temperature regimes that would likely be found on typical Puget Lowland prairies consisting of: 1) alternating diurnal temperature of 15 °C/7 °C to simulate fall germination temperatures, 2) chilling at 5 °C for six weeks to simulate winter followed by alternating diurnal temperatures of 15 °C/7 °C approximating spring conditions, and 3) a constant ambient temperature of 20 °C.

All germination temperature regimes were conducted using a 14-hr photoperiod (about 40 μmol m⁻² s⁻¹, 400-700 nm of cool white fluorescent light). The germination temperature regimes were: 1) diurnally alternating temperatures at 15 °C for 14 hr and 7 °C for 10 hr in a growth chamber (Illuminated Incubator 818, GCA Precision Scientific, Chicago, IL); 2) six week cold treatment at 5 °C then alternating temperatures of 15 °C for 14 hr and 7 °C for 10 hr per day in a growth chamber; and 3) a constant temperature of 20 °C. All the replicates were monitored and enumerated every other day. Seeds were counted as germinated on radical elongation > 2 mm. All rotting or obviously fungal-infected seeds were removed and counted. The seeds were kept moist by adding deionized water to the saturation point of the substrate as needed (the water was kept at the same germination temperature as the germination series). At the end of 35 days, all remaining seeds were characterized as: 1) germinated, 2) imbibed/rotten, or 3) un-imbibed/hard-seeded. The seeds were considered hard-seeded if their final weight was within the range of seed weights for dry seed of that species.

### Statistical Analysis

All data are presented as a proportion of all seed that germinated. However, data were arcsine square-root transformed prior to analysis, as is standard with percentage or proportion data. For each species, a two-way ANOVA was used to compare the mean proportion germination among five pre-treatments, three germination temperature regimes, and all possible pre-treatment x germination temperature (PT x GT) interactions. To determine differences among treatment
levels in significant ANOVA models, post hoc Tukey’s honest significant difference (HSD) tests were used. For all tests, statistical significance was determined based on $P < 0.05$.

**Results**

Pre-Treatments and Germination Temperatures

Results of two-way ANOVA revealed that the three species were significantly affected by pre-treatment and germination temperature regimes, but the nature of the responses was different among species (Table 1). Moreover, the variation among species spanned the range of possible outcomes. One species (*L. lepidus*) demonstrated significant response to high pre-treatment temperatures, one species showed responsiveness to germination temperatures only (*L. polyphyllus*), and another species demonstrated responsiveness to an interaction between pre-treatments and germination temperatures (*L. albicaulis*).

*Lupinus lepidus* germinated at significantly higher rates only when the pre-treatment included an 80 °C water scarification ($F_{4,30} = 93.76, P < 0.001$; Figure 1). In treatments other than the wet 80 °C water scarification, germination rates were generally less than 15%, and the wet 80 °C treatment only increased germination to near 50% (Figure 1). Neither the germination temperature nor the PT x GT interactions were significant factors affecting germination of *L. lepidus*.

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**Table 1.** Analysis of variance (ANOVA) of the effect of pre-germination treatments, germination temperature, and their interaction (PT x GT) on the proportional germination of three *Lupinus* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Squares</th>
<th>F-Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lupinus lepidus</em></td>
<td>Pre-Treatment</td>
<td>4</td>
<td>2.01</td>
<td>0.5025</td>
<td>93.77</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Germination Temp</td>
<td>2</td>
<td>0.03</td>
<td>0.015</td>
<td>2.96</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>PT x GT</td>
<td>8</td>
<td>0.07</td>
<td>0.00875</td>
<td>1.73</td>
<td>0.133</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>30</td>
<td>0.161</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>44</td>
<td>2.274</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lupinus polyphyllus</em></td>
<td>Pre-Treatment</td>
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<td>0.03</td>
<td>0.008</td>
<td>1.8</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td>Germination Temp</td>
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<td>0.045</td>
<td>12.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>PT x GT</td>
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<td>0.02</td>
<td>0.003</td>
<td>0.58</td>
<td>0.784</td>
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<tr>
<td></td>
<td>Error</td>
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<td>0.004</td>
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<tr>
<td></td>
<td>Total</td>
<td>44</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lupinus albicaulis var. albicaulis</em></td>
<td>Pre-Treatment</td>
<td>4</td>
<td>1.02</td>
<td>0.255</td>
<td>5.1</td>
<td>0.003</td>
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<tr>
<td></td>
<td>Germination Temp</td>
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<td>0.535</td>
<td>10.7</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>PT x GT</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Total</td>
<td>44</td>
<td>4.64</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Figure 1. Germination response to pre-treatments by *Lupinus lepidus*. Vertical bars represent means +/- one standard deviation. Different letters denote significant differences at $p < 0.05$. Figure 1. In treatments other than the wet 80 °C water scarification, germination rates were generally less than 15%, and the wet 80 °C treatment only increased germination to near 50% (Figure 1). Neither the germination temperature nor the PT x GT interactions were significant factors affecting germination of *L. lepidus*.
*Lupinus polyphyllus* demonstrated enhanced germination in response to the germination temperature regime of 5 °C followed by alternating temperatures of 15 °C/7 °C simulating spring conditions \(F(2,30) = 12.08, P < 0.001\); Table 1). Germination in this alternating temperature regime was mildly higher than either the static 20 °C or just the alternating 15 °C/7 °C treatment (Figure 2). However, neither the pre-treatments nor the PT x GT interaction significantly influenced mean germination of *L. polyphyllus*.

For *L. albicaulis*, a two-way ANOVA revealed a significant pre-treatment effect \(F_{(4,30)} = 5.10, P = 0.003\), a significant germination temperature effect \(F_{(2,30)} = 10.69, P < 0.001\), and a significant PT x GT interaction \(F_{(8,30)} = 2.57, P = 0.029\); Table 1; Figure 3). The highest germination rates were found for the pretreatment control subjected to the 5 °C to 15 °C/7 °C germination temperature regime (Figure 3), but this pre-treatment group did not exhibit high germination rates in any other germination temperature regime. In contrast, the combination of a wet 80 °C pre-treatment and high germination temperature of 20 °C resulted in an exceptionally low mean germination rate. However, the dry 80 °C pre-treatment temperature also generally resulted in high germination (Figure 3). In fact, across all germination regimes, the dry 80 °C pre-treatment group was statistically indistinguishable from the highest-germination groups.

**Discussion**

Our data suggest that *L. lepidus* may respond to heat during a fire with enhanced post-fire germination, a common response in species adapted to recruitment following fire (Gill 1981, Keeley 1991). This species had low germination rates overall (generally < 50%; Figure 1), and thus conditions that enhance germination are especially important. Interestingly, physical dormancy in *L. lepidus* was broken by high heat treatments of immersion in 80 °C water but not at 80 °C dry heat. This discrepancy in response to heat may be explained by differences in duration and possibly intensity of the heat treatments which interact to break the physical dormancy in seeds (Auld and O’Connell 1991). In our experiment the 80 °C water likely conducted more heat to the seed than oven-drying at 80 °C for seven minutes. Both methods have been used to break physical dormancy and identify seeds that germinate after the passage of fire (Martin et al.)
Our results suggest that the wet 80 °C treatment provided enough heat over enough time to break physical dormancy. This could indicate the species is adapted to seed germination following exposure to high temperatures associated with fire. In fact, *L. lepidus* is most common in either Pacific Northwest prairies with historic high frequency fire, or open montane habitat (USDA 2011). This obligate-seeder may display physical dormancy sensitivity to simulated fire temperatures since its persistence on the landscape depends on seed germination post-fire, rather than resprouting. The species also did not show sensitivity to germination temperature regimes. Although the current practice in Washington prairie restoration is to use a seed drill to sow native seed following controlled burns, our results suggest that greater germination of *L. lepidus* might be achieved by sowing this species at the soil surface prior to burning.

In contrast, we found a small but significant increase in the germination of *L. polyphyllus* under the series-dependent germination temperature regime of 5 °C to 15 °C/7 °C (Figure 2), but the seed did not have any significant response to pre-treatment temperatures. This pattern could be reflective of a widespread species responsive to variable spring temperature regimes, but without a particular adaptation to high summer temperatures or fire. Unlike *L. lepidus*, *L. polyphyllus* occurs in moister habitats that may be less fire-prone. Thus, it appears to have developed a reproductive strategy—resprouting—that allows it to tolerate a variety of disturbance regimes, rather than relying on a mechanism solely adapted to fire. Restoration techniques in prairies may see highest success with this species when sowing in fall or winter, but without specific attention to fire or summer temperature effects on germination.

The interaction between pre-treatments and germination temperatures on the germination of *L. albicaulis* was more complex. Our data for *L. albicaulis* suggested the 80 °C pre-treatment generally increased germination in this species and the wet 80 °C treatment resulted in high germination rates when followed by alternating simulated spring temperature regimes (5 °C to 15 °C/7 °C regime). Similar high germination rates could also be achieved in *L. albicaulis* without high pre-treatment temperatures if the germination temperature regime included near freezing temperatures followed by alternating 15 °C/7 °C. The effect of the germination temperature regime of 5 °C to 15 °C/7 °C was reduced when the seed was exposed to the summer heat treatments of 37 °C and 47 °C. This species apparently has plasticity for responding to variable spring temperatures and may also have some plasticity in response to fire and high summer temperatures. This is consistent with other studies on germination cues for temperate Fabaceae species (Roberts and Boddrell 1984, Baskin and Baskin 1998, Van Assche et al. 2003).

Our results for *L. albicaulis* also suggest other interactions that reduce germination rates. The interaction of wet 80 °C pre-treatment and 20 °C germination temperature caused a significant number of seeds to rot and a significantly lower seed germination rate than the cooler germination temperature regime at 5 °C to 15 °C/7 °C. This interaction may be explained by how the rate of imbibition affects the germination of the seed. In seeds with physical dormancy, both the site of water entry and the rate of water entry are important for the emergence of the radical and cotyledon development. Manning and Van Staden (1987) found that mechanical methods of breaking physical dormancy allowed for water entry in areas other than the strophiole, leading to a decrease in germination and seedling survival (Baskin 2003). In our experiment, the warmer germination temperature of 20 °C may have similarly increased water imbibition leading to higher seed rotting rates than the lower temperatures of 15 °C/7 °C and 5 °C to 15 °C/7 °C. Similar patterns have been suggested for *L. sulphureus var. kincaidii* where high rot-associated seedling mortality was found in scarified seeds compared to un-scarified seeds (Severns 2003). For *L. albicaulis*, ecological restoration techniques that augment seed in fall after high summer temperatures have passed may have larger spring seedling recruitment dependent on cool and fluctuating spring conditions. Restoration techniques may also see similarly high germination rates following exposure to fire.

In combination, our data suggest interspecific variability in germination cues among these three *Lupinus* species. The three species demonstrated patterns that spanned the range of potential responses to high summer temperatures, simulated fire temperatures, and fluctuating cool germination temperature regimes. The species that are more restricted to frequently burned prairies (*L. lepidus* and *L. albicaulis*) demonstrated stronger responses to high pre-germination temperature regimes. In contrast, the species with a broader distribution (*L. polyphyllus*) showed less responsiveness to high pre-germination temperatures, but minor responsiveness to fluctuating spring temperature regimes. These findings highlight non-uniformity in germination response cues in the genus *Lupinus*. A more thorough examination of seed ecophysiology in the genus *Lupinus* may be useful since the range of germination cues for the genus is not
well understood (Keeley 1991). However, managers might successfully predict germination responses to fire concomitant with species life history. Specifically, species that are more prairie-restricted in range might also show stronger germination responses to fire.

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Literature Cited


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