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Factors Influencing Seed Germination of *Lupinus perennis*[1]

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Abstract

Seeds of *Lupinus perennis* Wats. (perennial lupine) were subjected to a variety of scarification and temperature treatments. Without scarification, <15% germination was observed within 1 week. Germination increased quadratically as the duration of acid scarification time increased. After 15 min scarification, nearly 90% of the seeds germinated and nearly 100 percent germination was obtained after 45 min scarification. Nicking the seed coats with a razor blade resulted in 100% germination. In contrast, soaking seeds in 22C (72F) water for 24 hr failed to enhance germination. Total germination of scarified seeds was >80% between 21 and 29C (70 and 85F) within 54 hr. The most rapid germination occurred within 24 to 29C (75 to 85F). At 32C (90F), total germination was reduced to approximately 60%. At temperatures ≥ 35C (95F), total germination was reduced to less than 4%. Germination occurred equally well in the light or dark. Our data indicate that seeds of *L. perennis* must be scarified to germinate and the most rapid germination occurs between 24–29C (75–85F).

Index words: sexual propagation, perennial lupine, temperature, scarification.

Significance to the Nursery Industry

Lupinus perennis is an obligate host for the endangered Karner Blue butterfly. However, lack of detailed information on seed propagation of this species currently limits the ability to propagate plants for research (e.g. study of plant/butterfly interactions) and for possible ecosystem restoration efforts. Our results indicate that scarification is needed to optimize seed germination. Placing seed in concentrated sulfuric acid for 15 min improves germination dramatically to >90%. Furthermore, results indicate the most rapid germination occurs between 24 and 29C (75 and 85F).

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Introduction

Lupinus perennis (perennial lupine) is the obligate host for the endangered Karner Blue butterfly (*Lycaeides melissa sanuelis* Nabokov). The plant grows only in dry, open edges or sandy savannas and scrubby woodlands. Unfortunately, urbanization, agriculture, and forestation of savannas with xeric conifers has destroyed much of the habitat of *L. perennis* within the rather restricted range of the butterfly (2). The butterfly feeds on several plant species, but oviposition and larval feeding are restricted to *L. perennis*. Even though *L. perennis* is distributed widely throughout the eastern United States, the butterfly is now restricted to very small populations from New Hampshire to Minnesota with the primary

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range in Wisconsin and eastern Michigan. Lack of detailed information on propagation of *L. perennis* by seed currently limits the ability to grow plants for further study of plant/butterfly interactions and efficient production of plants for ecosystem restoration efforts.

Like many legumes, seeds of *L. perennis* have seed coats impervious to water. This results in erratic germination and poor seedling stands. Studies with related species of *Lupinus* indicate that sulfuric acid scarification is needed to promote uniform and rapid germination (1, 3, 4, 6). Mechanical scarification treatments are also effective but generally have not been practical for large quantities of seed. Therefore, the objectives of the current investigation were to determine a) the germination response of *L. perennis* to sulfuric acid scarification of varying durations; b) the efficacy of other scarification treatments in promoting germination; c) the optimum temperature range for germination and d) the effect of light on germination.

Materials and Methods

Seeds were collected from five native populations of *L. perennis* in central Wisconsin (44°00' N to 44°23' N latitude and 89°57' to 90°45' W longitude) and pooled for the following experiments.

Acid scarification (Experiment 1). Seeds were placed in concentrated (36 N) sulfuric acid (60 seeds per 50 ml or 1.7 oz) for 0, 15, 30, 45, or 60 min at 22C (72F). Following scarification seeds were rinsed with distilled water several times before sowing.

Seed treatments (Experiment 2). Seeds were either soaked in water or mechanically scarified. In the water soak treatments, 25 seed each were (a) left nontreated (control), (b) soaked in room temperature (22C or 72F) tap water for 24 hr, (c) placed in 60, 80, or 100C (140, 176, or 212F) tap water which was allowed to cool for 24 hr, and for the mechanical scarification treatment a razor blade was used to nick the seed coat. After treatment seeds were placed in plastic petri dishes. Petri dishes were placed in a growth chamber with a 12 hr photoperiod provided by cool-white fluorescent lamps and a 12 hr day/12 hr night temperature regime of approximately 25/20C (77/68F). Maximal germination occurred within 3 days with germination (radicle emergence) monitored daily for 7 days. Experiments 1 and 2 were conducted three times.

Temperature effects (Experiment 3). Temperature effects on seed germination were investigated using seeds that had been scarified in sulfuric acid for 30 min. Seeds were divided into five replications, 15 seeds (10 seeds in the third experiment) in each, and placed on a thermogradient plate. Seeds were placed on one sheet of Whatman 4 filter paper (Whatman Paper, London) in Pyrex petri dishes moistened with 2 ml (0.7 oz) deionized water and sealed with parafilm. Deionized water was added as needed to maintain constant moisture during the experiments. Petri dishes were arranged five per column (replications) perpendicular to the temperature gradient on a thermogradient plate. The aluminum thermogradient plate measured 61 × 122 × 2.5 cm (24 × 48 × 1 in: width/length/thickness) and was insulated on the bottom and sides. A 30 × 30 cm (11.8 × 11.8 in) heat plate in contact with the bottom surface at one end provided heat,

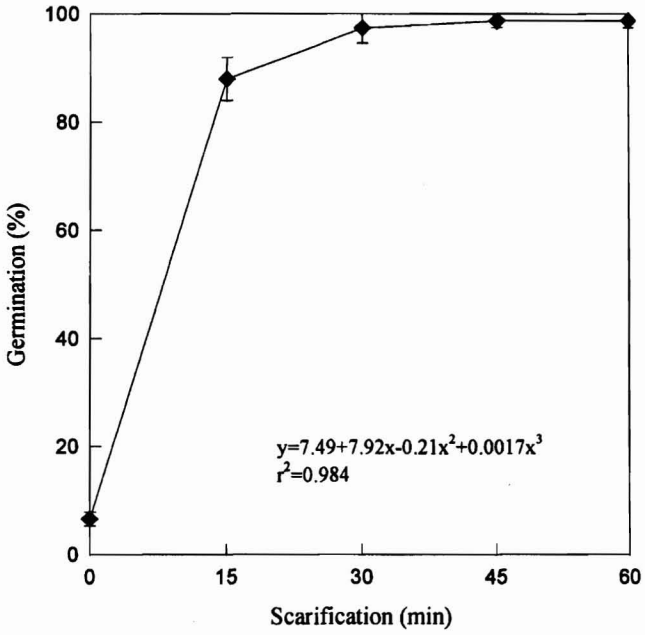

Fig. 1. Germination of *L. perennis* after scarification with concentrated sulfuric acid for varying lengths of time. Germination was determined after 7 days. Vertical bars indicate standard error of the mean (n = 75).

whereas compressed freon circulating through eight, 6.4-mm (0.25 in) diameter lateral holes in the opposite end cooled the plate. A photoperiod of 16 hr was provided by two cool-white fluorescent lamps positioned 40 cm or 15.8 in (~50 $molm^{-2}s^{-1}$ in the center of the plate) above the plate surface. Each temperature range experiment was conducted three times.

A thermocouple was placed in the bottom of an empty petri dish on each temperature column. Temperatures were recorded every 30 min by a datalogger for the duration of each test. Daily temperature variation occurred within the plate concurrently with changes in room temperature. However, temperatures varied <2.0C (3.8F) with no overlap between the columns. Germination, defined by radicle emergence ≥2 mm, was recorded at 2 hr intervals for 54 hr in the temperature experiment [21.1, 23.9, 26.7, 29.4, 32.2, 35, 37.8C (70, 75, 80, 85, 90, 95, 100F)] after which no further germination occurred.

Effect of light (Experiment 4). Twenty-five seeds were placed in each of two petri dishes containing moistened filter paper as described for the temperature experiments. The petri dishes were placed in a growth chamber with a 12 hr photoperiod and a 12 hr day/12 hr night temperature regime of approximately 27/23C (80/73F). The dark treatment was provided by wrapping one petri dish in aluminum foil. Germination was recorded after 72 hr. The experiment was conducted four times. Statistical analyses were conducted using the PC SAS General Linear Models package (5).

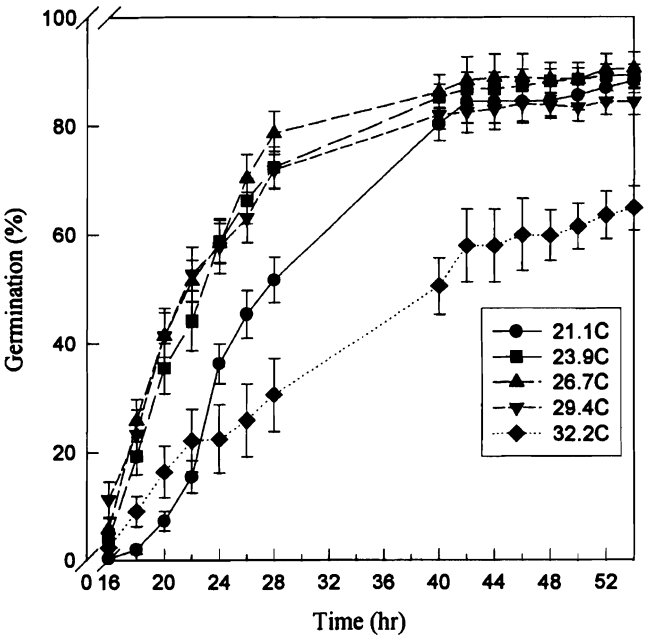

Fig. 2. Germination of acid-scarified seeds of *L. perennis* placed on a thermogradient plate at a range of temperatures. Vertical bars indicate standard error of the mean (n = 40). The regression equations and r^2 values are: 21.1C) $y = 8.23x - 0.0833x^2 - 115.8$, $r^2 = 0.88$; 23.9C) $y = 21.85x - 0.4959x^2 + 0.0038x^3 - 234.1$, $r^2 = 0.81$; 26.7C) $y = 24.75x - 0.58x^2 - 0.0045x^3 - 259.31$, $r^2 = 0.80$; 29.4C) $y = 20.49x - 0.4717x^2 + 0.0036x^3 - 210.87$, $r^2 = 0.75$; 32.2C) $y = 5.07x - 0.065x^2 + 0.0028x^3 - 63.22$, $r^2 = 0.55$.

Results and Discussion

Without scarification, <15% of the seeds germinated within 1 week (Fig. 1). Germination increased as the duration of acid scarification increased, with an asymptotic approach to 100% germination when treated with sulfuric acid for periods ≥30 min. Response of seeds of *L. perennis* is similar to that of *L. texensis* where 30 to 60 min scarification in sulfuric acid is necessary for maximum germination (1). This is less than *L.* Russell-Hybrid and *L. havardii* which require exposure to concentrated sulfuric acid for 45 to 60 and 90 to 120 min, respectively, for maximum germination (4, 6). Although *L. perennis* is similar to *L. texensis* in the duration of exposure necessary to achieve maximum germination, it differs from *L. texensis* and *L. havardii* in the amount of germination at shorter exposure times. A high percentage (90%) of seeds will germinate after only 15 min of exposure to concentrated acid whereas <25% of *L. texensis* and 40% of *L. havardii* will germinate when exposed to concentrated acid for 15 min (1, 4). The germination response of *L. perennis* is similar to *L.* Russell-Hybrid in which only 3% of the seeds germinate with no scarification but 54% will germinate with 15 min of acid scarification after 4 days (6).

Nicking the seed coat with a razor blade resulted in 100% germination after 1 week (data not presented). Soaking seeds in water for 24 hr failed to promote seed germination regardless of initial water temperature. This is similar to studies with *L. cosentinii*, *L. havardii* and *L. texensis* where hot water soaks failed to promote germination while scarification in concentrated sulfuric acid increased germination to >95% (3, 4).

Germination occurred rapidly at particular temperatures on the thermogradient plate (Fig. 2). Seed germination was >70% 28 hr after placement on the thermogradient plate for the 23.9, 26.7 and 29.4C (75, 80 and 85F) treatments and within 42 hr approximately 80% of the seeds germinated in the 21.1 to 29.4C (70 to 85F) treatments. At the end of the experiment (54 hr), seeds in all but the three highest temperature treatments [32.2, 35.0 and 37.8C (90, 95 and 100F)] had reached ≥80% germination. Seed placed in the lowest temperature treatment [21.1C (70F)] exhibited delayed germination with 51% of the seed germinated at 28 hr compared to the 23.9–29.4C (75–85F) treatments wherein approximately 70% of the seeds had germinated. After 40 hr, germination at 21.1C (70F) was approximately the same as the other treatments. Less than 4% of the seeds placed in the two highest temperature treatments [35 and 37.8C (95 and 100F)] germinated (data not presented).

Light did not affect seed germination. Ninety-seven ± 2% of the seeds germinated in the light whereas 93 ± 4% of the seeds germinated in the dark. This is similar to that of *L. havardii* where light is not required for germination (4).

Results suggest that optimum seed germination of *L. perennis* requires a minimum acid scarification treatment of 30 min. This method has resulted in near 100% germination and seedling emergence in our greenhouses with no apparent damage to seedlings or loss of vigor. The most rapid germination occurs at 24 to 29C (75–85F). The main factor which appears to limit germination is the impervious seed coat. Results suggest that seed scarification and monitoring seedbed temperatures prior to sowing could aid in the restoration of *L. perennis* to its native habitat.

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