Population dynamics and conservation biology of *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae)

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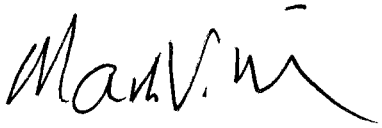

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Abstract: Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii* [Smith] Phillips) is a federally listed Threatened plant of Willamette Valley prairies. Both low reproduction, due to seed coat dormancy, and competition from invasive plants may threaten the long-term viability of this species. In separate studies, I investigated seed germination and an invasive species control technique. My objectives in the seed germination study were to test for a seed scarification requirement for germination and to identify an effective scarification treatment. I tested the effects of various durations of sulfuric acid immersion on rates of seed water imbibition, germination and emergence in three experiments, and found that sulfuric acid immersion increased germination and emergence rates and 20 min of immersion yielded optimal results. This indicated that Kincaid's lupine seeds have seed coat dormancy effectively broken by a short duration of acid scarification. My objective for the invasive plant control study was to evaluate the effects of growing season mowing on Kincaid's lupine growth and reproduction. This technique was shown effective in reducing cover of the dominant invasive plant in the studied population, tall oatgrass, but its effects on Kincaid's lupine were unknown. I tested the effects of mid-June mowing on the change in numbers of Kincaid's lupine racemes and leaves in permanent plots over one year following treatment. I found that mowing reduced the number of leaves and racemes produced the year following mowing, indicating that growing season mowing may not be a safe control for invasive species in Kincaid's lupine populations.

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Chapter 1

General Introduction

Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii*) is a rare endemic plant of Willamette Valley prairies. Along with many other native prairie plants, it is now quite rare and occupies few scattered populations throughout its formerly broad distribution. It provides an important ecological function in prairie communities by hosting most of the remaining populations of the extremely rare Fender's blue butterfly. The long-term persistence in remnant prairies of Kincaid's lupine and the associated butterfly is threatened by numerous factors, detailed below.

I have undertaken several studies focused on conservation and restoration concerns in Kincaid's lupine populations. The common theme in these studies has been the effort to expand knowledge of Kincaid's lupine biology, and provide applications to conservation issues. Efforts to maintain viability of this taxon must be guided by more complete knowledge of the underlying dynamics within individual populations.

In the introduction below, I provide some background on Kincaid's lupine taxonomic status, biology, range of distribution, and conservation concerns. I then describe the individual studies I have conducted.

Taxonomic Status

Lupinus sulphureus Hooker ssp. *kincaidii* [Smith] Phillips (Kincaid's lupine) was initially named early in the 20th century. However, its taxonomic position within the *Lupinus* genus is still unclear. The *Lupinus* genus is currently placed in the monotypic subtribe Lupininae, of tribe Genisteae, of Fabaceae. Palmately compound leaves and

terminal inflorescences (racemes) (Hitchcock, et al., 1955) distinguish members of Lupinus. *Lupinus sulphureus* is characterized by small flowers with slightly pubescent to glabrous and slightly reflexed banners, slightly gibbous calyx and narrow leaflets (Hitchcock et al, 1955) and was originally described by Hooker in 1833. The first description of Kincaid's lupine may have been what Heller named *L. oreganus*, collected from Eugene in 1911 (Heller, 1911). Later, Smith (1924) described a specimen collected from Corvallis in 1898 as *L. oreganus Kincaidii* var. *nov.*. His distinction of this specimen as a variety was based on short upper calyx lips in the flowers. No taxonomists since have been able to distinguish *oreganus* and *Kincaidii* varieties, so they may be the same. However, Hitchcock et al. (1955) later recognized the plant as a variety of *L. sulphureus*, and identified three geographic races of the species in our area: var. *sulphureus*, var. *subsaccatus* and var. *kincaidii*. Variety *kincaidii* is described as having blue to purplish flowers, leaflets glabrous on upper surface, and racemes 10-18 cm. Phillips renamed it as a subspecies of *L. sulphureus* in 1955, to comply with botanical nomenclature rules, so it is currently known as *L. sulphureus* ssp. *kincaidii*. Phylogenetic analysis using molecular data may allow finer resolution of relationships in regional *Lupinus* flora.

Biology

Kincaid's lupine appears to have very long-lived genets; one excavated specimen showed over 25 annual growth rings on a central caudex (M.V. Wilson, pers. comm.). Aboveground stems arise from the caudical branches at short (~2 cm) to long (>2 m) distances from connected stems (pers. obs.). A molecular genetics study of Kincaid's

lupine identified two apparent subpopulations of separate stems as merely two large genets, using isozyme markers (Liston et al., 1995). However, this plant is not thought to propagate clonally. If perennating organs are completely caudical, with no root development at distal stem bases, separate stems would all be physiologically dependent on the central plant, and unable to persist individually.

Few seedlings have been found in natural populations, indicating strong limitations on sexual reproduction. Very low seed set has been reported from many populations, so seed supply may be limited. However, dense cover of invasive species now crowding many populations may also inhibit germination and establishment of seeds successfully matured.

With low reproduction and potentially limited clonal propagation, populations may consist of spreading genets persisting through perennial vegetative growth. Physiological interdependence would increase the likelihood that eventual tissue senescence and stochastic events could damage entire genets. If populations contain few such genets, loss of a single individual could have a large impact on population persistence.

Inflorescences are indeterminate, maturing from the oldest flowers at the base. Floral development occurs over a broad range of time (ca. 14 days) within an inflorescence. On many racemes, upper flowers do not mature by the end of the growing season (pers. obs.).

Kaye & Kuykendall (1993) postulated obligate outcrossing in Kincaid's lupine, due to mechanical barriers to self-pollination. Some aspects of floral morphology support this hypothesis. The anthers are located above the stigma, and pollen collects in the tip of

the keel. Pressure on the keel causes anthers to force pollen through an opening in the keel tip. A system has been described in *Lupinus* where peristigmatic hairs prevent stigma contact with self-pollen during this process, unless the pollen is forced against the stigma (Juncosa and Webster, 1989). Protandry may also promote outcrossing in this plant, if the pollen is removed (by insect pollinators) or becomes inviable before the stigma is receptive. The dominant insect pollinators, *Bombus* spp., commonly forage from bottom to top in the inflorescence (pers. obs.; P. Severns, pers. comm.). This pattern should promote deposition of outcross pollen on lower flowers with receptive stigmata, and removal of viable pollen from upper flowers. A hand pollination study indicated obligate insect pollination for fruit and seed set (Kaye, 1999). However in the pollination study described below, fruit and seed were set in undisturbed, closed flowers, indicating a capacity for autonomous self-fertilization. Other lupine species have exhibited wide variation in selfing rate over time, and this has been anecdotally correlated to abundance of insect pollinators (K. Karoly, pers. comm.).

Known Range

Kincaid's lupine is considered an Oregon endemic, and confined primarily to the Willamette Valley. There are 50 known populations in the Willamette Valley, and three in the Umpqua Valley of Oregon. Additionally, one population is located in Lewis County, Washington, just north of the Columbia River.

Kincaid's lupine populations at Fern Ridge Lake, west of Eugene, Oregon are arranged along the shore, in upland prairie remnants. These populations are small, 30 meters diameter or less, and isolated from each other by woodland strips of ash and oak

and by open water. Scattered plants are found in areas where prairie fragments are connected, suggesting a more continuous distribution in the past. Some populations exist as discrete areas densely stocked with plants, while others are sparsely distributed among other vegetation. Community associates include both native prairie species, such as *Festuca roemeri* Wilson, *Danthonia californica* and *Sidalcea virgata*, and invasive exotics, such as *Arrhenatherum elatius, Cytisus scoparius* and *Rubus discolor* (nomenclature after Hitchcock and Cronquist, 1973).

Conservation Issues

Kincaid's lupine was listed by the US Fish & Wildlife Service as a Threatened species, in a package listing Fender's blue butterfly (*Icaricia icarioides fenderi* Macey) and Willamette daisy (*Erigeron decumbens* var. *decumbens* Nuttal) as Endangered species (USFWS, 2000).

Fender's blue butterfly primarily utilizes Kincaid's lupine as its larval food plant, and is considered obligate to it for long-term population survival (Hammond & Wilson, 1993). The fender's blue butterfly was presumed extinct since 1937, until rediscovered at a Kincaid's lupine population near Corvallis, Oregon in 1989 (Chambers, 1990; Hammond 1991). It is currently extant in only 10 populations of Kincaid's lupine (M.V. Wilson, pers. comm.). Efforts to preserve and increase populations of Fender's blue butterfly have focused on conservation of its plant host and on improving habitat quality. However, the absence of Fender's blue butterfly from many populations of Kincaid's lupine may be due to low abundance of native nectar plants for adult butterflies. Only the larvae feed on Kincaid's lupine foliage, while the adults require nectar from such plants

as *Sidalcea* spp.. Conservation of Fender's blue butterfly may require restoration of native prairie communities.

Threats to Kincaid's lupine populations are diverse, and indirectly threaten the associated Fender's blue butterfly. The largest threat is habitat loss. Nearly all (>99%) of native Willamette Valley prairie has been lost to development, agriculture and natural succession since the mid-19th century. Most extant remnants are degraded, and dominated by exotic invasive plants. Competition from these species may limit growth and reproduction of Kincaid's lupine and other native vegetation. Active vegetation management may be needed to restore remaining prairie habitat.

Due to the absence of the pre-European settlement (pre-1850) burning regime in Willamette Valley prairies, reproduction may currently be limited in Kincaid's lupine populations. Kincaid's lupine seeds may require scarification to germinate. This could have resulted from the rapid, low intensity prairie burns. Burning may have also cleared competing vegetation and humus from the soil, allowing seedling establishment.

Kincaid's lupine populations may suffer genetic limitations associated with low population sizes and spatial isolation. Isolation may limit gene flow between populations, due to limited pollen exchange. Small populations are subject to deleterious effects of genetic drift and bottlenecks. If this species is normally outcrossing, inbreeding depression may occur in populations with few genets.

Experiments

In partial fulfillment of my Bachelor of Science degree in the BioResource Research interdisciplinary program, I have undertaken several studies of population

dynamics associated with conservation of Kincaid's lupine. Competition from invasive species may threaten the viability of some Kincaid's lupine populations, leading to efforts to control pest plants (Wilson and Clark, in prep.; Wilson and Clark, in prep.) This has prompted me to examine the effects of one control technique, growing season mowing, on Kincaid's lupine growth and reproduction, in a population dominated by invasive species. Kincaid's lupine seeds have been thought to require scarification for germination. In order to develop an effective scarification technique for restoration efforts, I undertook a series of experiments testing acid immersion treatments. In a separate project, I am also investigating the spatial extent of vegetative spread in two Kincaid's lupine populations, by using molecular genetic markers to identify genets.

The mowing and acid-scarification studies are described in Chapters 2 and 3.

Chapter 2

Effects of Growing-Season Mowing on Kincaid's Lupine Foliar and Floral Production

Introduction

Competition from invasive plants may be limiting Kincaid's lupine growth and reproduction in degraded prairie remnants. Most known populations have infestations of such exotics as *Rubus discolor* [Himalayan blackberry], *Cytisus scoparius* [scots broom] and *Arrhenatherum elatius* [tall oatgrass] (Hammond and Wilson, 1993). The tall stature and rapid growth of these species, once they are established, tends to shade and inhibit native vegetation. Eradication techniques for these species have included burning and mowing treatments.

In this study a prairie containing a Kincaid's lupine population was mowed in late spring to remove an invasive exotic, *Arrhenatherum elatius.* Aboveground resource allocation of *Arrhenatherum elatius* var. *bulbosum* peaks in late May in the Willamette Valley (Tanphiphat and Appleby, 1990), although weather conditions may delay this peak. Tall oatgrass seeds mature by early July, and high aboveground reserves should persist from late May until seed maturation. Therefore, mowing during June should both remove a high proportion of biomass in aboveground parts and prevent seed from maturing. Repeated mowing treatments in late spring or early summer significantly reduce tall oatgrass abundance, allowing native grasses to increase (Wilson and Clark, in prep.)

The Kincaid's lupine population manipulated in this study is known as North Eaton. It is located on the northeast shore of Fern Ridge Lake (south of Eugene, Oregon), and is managed by the US Army Corps of Engineers (ACOE). The population is roughly circular and approximately 21 m in diameter, situated in the middle of an upland prairie remnant. From 1982 until the mid 1990's, this area has been mowed annually in late September to control invasive woody species (J. Beal, pers. comm.). As a result, there were many scattered small (< ½ m tall) plants of *Rubus discolor* and *Cytisus scoparius* at the beginning of the study, but no large plants. There was also a dense cover of tall oatgrass across the entire population, up to two m in height. Kincaid's lupine plants attained a maximum height of ~1m, and appeared strongly suppressed by the dense grass and woody cover.

If Kincaid's lupine growth and reproduction is limited by the presence of tall oatgrass, a mowing treatment reducing tall oatgrass cover should result in increased foliar and floral growth. There is concern that mowing causes a net negative impact to Kincaid's lupine, as it destroys any tall aboveground portions. Any direct harm to the plant could outweigh the benefits of decreased competition.

Materials and Methods

The population was divided across the diameter into two even halves. Twenty permanent 0.5 m^2 plots were established in each side. Plot locations were determined by randomly selecting a radius ($r < 10.5$ m), and an orientation from the marked center of the population to a plot corner. All four corners of the plot were staked and flagged. There were 40 plots established in the population, for a total of 20 m^2 in a 346 m^2 area, so six

percent of the area was sampled. One side of the population was randomly selected to be mowed, while the other side served as the control. Treatments were unreplicated because of constraints in mowing by ACOE staff.

Plots were allowed to overlap, so were independently arranged within the treatment blocks. Use of randomly selected polar coordinates did result in plots being clustered near the population center. However, this population displayed a greater stem density near the center, so this sampling design more nearly matched the plant distribution than a uniform sampling design would.

In each plot, the number of leaves and racemes were counted prior to treatment. The initial measurements were made on June 13, 1998. The treatment side was mowed on June 14, 1998, at 12-15 cm above ground. Measurements in the plots were repeated on June 11, 1999, to assess effects of the first year treatment. The treatment side of the population was mowed again on June 14, 1999. Measurements will be repeated in June 2000, to assess effects of the second year of treatment.

The entire population was mowed again in late September 1999 to reduce woody cover. However, aboveground portions of Kincaid's lupine had died back by this time, and should not have been affected.

Differences between treated and untreated areas in changes in leaf number and raceme number from 1998 to 1999 were tested with unpaired t-tests. Untransformed data met statistical assumptions of normality for raceme number, but leaf data required a square-root transformation: $(\text{leaves } 99)^{1/2} - (\text{leaves } 98)^{1/2}$.

Results and Discussion

The control was estimated to have a mean of 2.7 racemes per plot in 1998 and a mean of 3.7 racemes per plot in 1999, constituting a mean increase from 1998 to 1999 of 1 raceme per plot. The treatment was estimated to have a mean of 5.4 racemes per plot in 1998, and a mean of 4.5 racemes per plot in 1999, constituting a decrease of 0.85 racemes per plot. There was a significant decrease in raceme number change with treatment (t=2.35, p=0.024) (Table 1).

The control was estimated to have a mean of 45.9 leaves per plot in 1998, and a mean of 76.3 leaves per plot in 1999, constituting an increase of 30.4 leaves per plot. The treatment was estimated to have a mean of 72.5 leaves per plot in 1998, and a mean of 88.9 leaves per plot in 1999, constituting an increase of 16.4 leaves per plot. There was a significant decrease in square-root transformed leaf number change with treatment (t=1.90, p=0.064) (Table 1).

Table 1. Mean number of racemes and leaves in control and treatment plots in 1998 and 1999, changes in leaf and raceme number between 1998 and 1999, and results of two-sided t-tests for differences in leaf and raceme number changes between control and treatment plots.

Variable	1998	1999	Change ('98-'99)	*t*-statistic	*p*-value
Racemes					
Control	2.7	3.7	+ 1.00	**2.35**	**0.024**
Treatment	5.4	4.5	- 0.85		
Leaves					
Control	45.9	76.3	+ 30.4	**1.90***	**0.064***
Treatment	72.5	88.9	+ 16.4		

* t-test conducted on transformed values: $(\text{lvs } 99)^{1/2} - (\text{lvs } 98)^{1/2}$

Thus, treated areas had significantly fewer racemes and leaves than untreated areas. Although the treated and untreated areas could have differed in ways other than mowing, the use of pre-mowing data suggests that this result can be safely attributed to the mowing treatment.

The decrease in raceme production the year following treatment likely resulted from direct damage to the plants. In this long-lived perennial, racemes have only been observed on stem bases greater than one year old (P. Severns, pers. comm.); individual stem bases may require a full growing season to accumulate enough carbohydrate reserves to produce a raceme. Since the mowing destroyed plant parts over 12-15 cm high; loss of photosynthetic tissue may have limited the amount of carbohydrates sequestered in the caudices that season. The following season, there may have been fewer stem bases with enough reserves to produce racemes.

The slight decrease in leaf production with treatment may also be attributed to decreased carbohydrate reserves. However, leaf production may be less dependent than raceme production on resources sequestered below ground.

Wilson and Clark (in prep.) found that repeated late spring mowing reduced cover of tall oatgrass, and increased cover of native species. However, significant decreases in tall oatgrass cover emerged only after two years of mowing. Kincaid's lupine may not respond positively to this treatment until the tall oatgrass cover, and its competitive pressure, is decreased. In contrast, negative responses to the mowing were expected to be visible after the first year, because these would result from direct damage to the plants. Data collected from these plots in late spring of 2000 will show if two years of treatment

decreases tall oatgrass cover, and the benefit of decreased competition outweighs the direct damage to Kincaid's lupine plants.

If the second-year mowing treatment increases leaf and raceme production, it may be informative to continue experimental mowing at this site. If the second-year mowing treatment results in further decreases, it may be prudent to discontinue the treatments. The potential value of mowing as a control for invasive species must be balanced with the danger of damaging an extant population of this rare and threatened plant.

Chapter 3

Effects of Acid Scarification Treatment on Germination Rates for Kincaid's Lupine Seeds

Introduction

If seeds of Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidi*i [Smith] Phillips), like many legumes, require scarification to germinate successfully, soaking in concentrated sulfuric acid should improve germination rates.

Germination of many types of seed is hastened by mechanical scarification (Hopkins, 1995), and previous studies with this species have indicated that razor nicking improves germination rate (P. Severns, pers. comm.). Ongoing studies of reproduction and future restoration efforts with Kincaid's lupine involve large quantities of seeds. Nicking individual seeds may be less efficient and accurate than acid-scarifying a group of seeds simultaneously. Soaking seeds in concentrated (36 N) sulfuric acid has significantly improved germination rates in two other *Lupinus* species, as a function of duration of immersion (Mackay et al. 1995; Mackay et al. 1996).

In three experiments, I examined the effect of sulfuric acid immersion time on germination of Kincaid's lupine seeds. In the first test, I evaluated germination rates on filter paper in petri dishes to allow close inspection of scarification effects. After effective treatments were identified in the first test, I evaluated total emergence rates and the number of days until emergence for individual seedlings in field soil. The ability of seeds to germinate on moist paper, in a controlled environment, may not be related to their ability to imbibe water from a soil matrix, and grow to emergence. The most effective

acid scarification treatments from these two tests were then compared to determine the optimum acid immersion duration. This third test was also used to evaluate the effectiveness of liquid nitrogen immersion.

If Kincaid's lupine seeds require seed coat disruption to germinate successfully, immersion in acid should result in a higher germination rate than the control. If sulfuric acid immersion damages embryos, immersion in acid should result in lower germination rates than the control. If duration of scarification affects the speed of germination, emergence times should have differed between acid immersion treatments.

I describe methods, predictions and summarized results for each experiment separately below.

Experiment 1

Materials and Methods

In the initial scarification test, I chose a broad range of scarification durations, based on results from experiments with *Lupinus havardii* and *L. perennis* (Mackay et al. 1995; Mackay et al. 1996). The ten treatments included a control, 7.5, 15, 22.5, 30, 40, 50, 60, 75 and 90 minutes of immersion in 36 N sulfuric acid. Only nine to ten seeds were used per treatment, due to limited supply. Despite low power to resolve individual treatments with so few replicates, it was more important to observe scarification effects over a broad range of immersion duration.

I randomly assigned nine to ten seeds to each treatment. Seeds were soaked for the specified duration in 36 N sulfuric acid, then rinsed thoroughly in distilled water. The

seeds were germinated on wet filter paper in petri dishes, with ambient temperatures between 18-24° C, under fluorescent lighting.

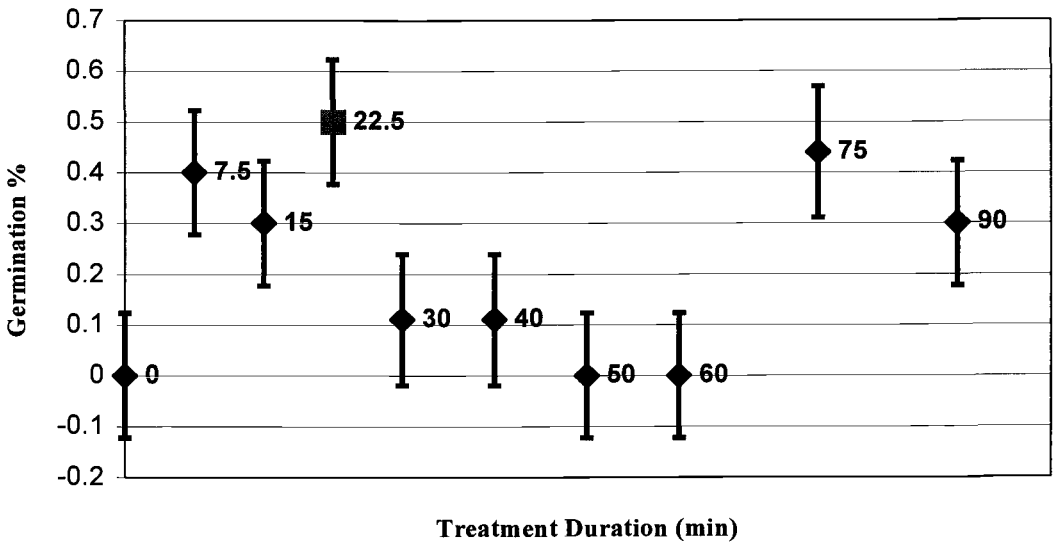
Seeds were checked daily for germination. Filter paper was re-moistened periodically for all treatments. Total germination rates were assessed at 7 days from scarification, by emergence of radicle from integument.

Results and Discussion

In this experiment, the control (0 minute) yielded no seed germination, while most treatments yielded some level of germination. The 7.5-minute treatment yielded 40% germination, the 15-minute treatment yielded 30% germination, and the 20-minute treatment yielded 50% germination. Both the 30 and 40-minute treatments yielded 11% germination, while 50 and 60-minute treatments yielded no germinated seeds. Treatments of 75 and 90 minutes yielded 44% and 60% germination, respectively.

Duration of immersion in sulfuric acid significantly affected mean germination rate (F=2.47, p=0.0145, from ANOVA F-test on 9 and 96 dF). One scarification treatment significantly increased germination rate over control: 22.5 minutes of immersion (Dunnett's test). Estimates for mean treatment effects over control are illustrated below (Figure 1):

Figure 1. Mean germination rates of Kincaid's lupine seed for ten acid scarification durations at 7 days following scarification. Standard error bars are shown. Squares represent values significantly different from control (0 min) (Dunnett's test).



While a low duration treatment and an intermediate duration treatment increased germination rates, the overall pattern was inconsistent. This could have been caused by seed heterogeneity. Since few seeds were available, some small and apparently immature seeds were used for this test, increasing the chance of variability among treatments. The 75-minute treatment increased mean germination rate, contrary to my predictions. However, cotyledons of the germinated seedlings displayed lesions consistent with acid burns. This indicated that under prolonged exposure, sulfuric acid penetrated the integument and damaged the endosperm, and such lengthy scarification treatments are unwise.

Mean germination rate peaked at treatments of 15 and 22.5-minute durations, but was lower at treatments of 30 and 40 minute duration. Both the 50 and 60 minute duration treatments resulted in no seed germination. All seeds in these two treatments

were infected with a *Pythium sp.*, while only immature-appearing seeds were infected in other treatments.

This infection pattern may have been resulted from a tradeoff between seed coat disruption permitting rapid germination, and allowing fungi to infect the endosperm. Seeds subjected to short scarification durations may resist penetration of fungal hyphae, but be capable of imbibing water to germinate. Seeds subjected to long scarification durations may imbibe water and germinate too rapidly for infection to take place. Seeds subjected to intermediate duration treatments may be vulnerable to fungal infection, yet not capable of imbibing water fast enough to germinate prior to rotting.

Experiment 2

Materials and Methods

In the second experiment, I narrowed the range of scarification duration, based on results of the first experiment, and increased replication. The nine treatments included a control, 5, 10, 15, 20, 25, 30, 35 and 40 minutes of immersion in 36 N sulfuric acid. Emergence in field soil was tested, to assess treatment effects under typical propagation conditions. I tested the effect of sulfuric acid scarification duration on total emergence after six weeks (42 days). I also tested the effect of scarification duration on number of days until emergence, because degree of scarification may affect the speed at which the seeds imbibe water and germinate. Both measures are important in determining the effectiveness of the treatments in enhancing germination.

In the initial study, some seeds germinated within one day, while several others germinated after more than one week. Emergence from soil takes longer than germination, so seedlings were expected to appear later.

Measuring emergence, rather than germination, is more germane to the application of this scarification technique to field studies. Most experiments involving establishment and growth of this species use a soil medium. Restoration efforts often involve direct field sowing. The ability of seeds to germinate on moist paper, in a controlled environment, may not be related to their ability to imbibe water from a soil matrix and to grow to emergence.

Twenty eight to thirty seeds were randomly assigned to each treatment. Seeds were immersed in sulfuric acid for the specified duration. Immediately following treatment, the seeds were rinsed thoroughly in distilled water, then in 10% chlorine bleach to disinfect.

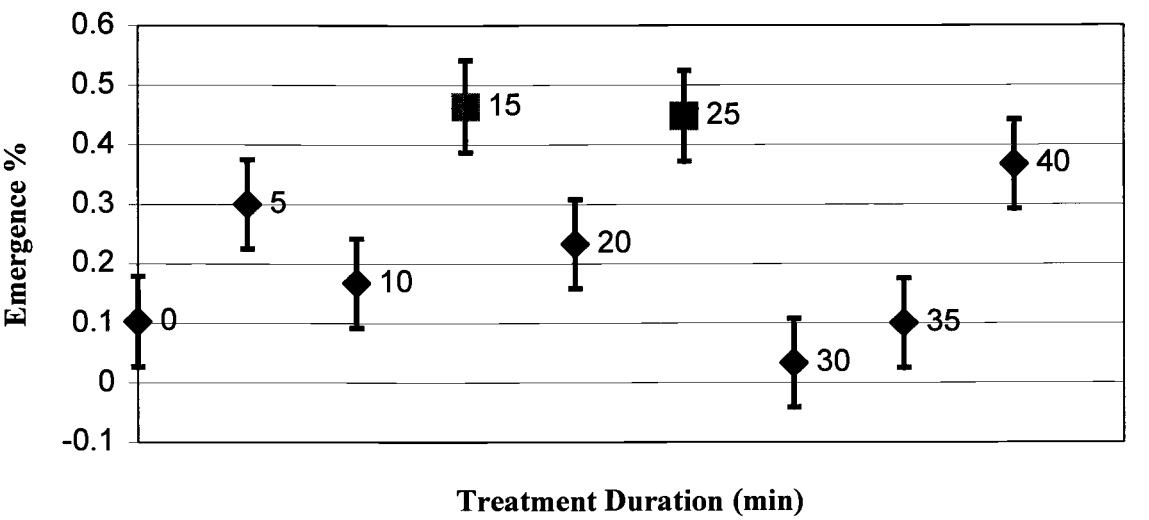
The scarified seeds were sown to a uniform depth (5 mm) in moist soil in individual soil cylinders. The medium consisted of A horizon soil collected near a Kincaid's lupine population, with perlite, vermiculite, coarse sand and worm castings added to improve drainage and aeration in the narrow containers. The seeds were germinated under natural light in a greenhouse, with temperatures ranging from 18-24° C. Every 24 hours, the containers were checked for emerging seedlings and watered. Containers with visible lupine cotyledons were scored as emerged. At 42 days from scarification, total emergence rates were assessed.

Results and Discussion

In this experiment, the control (0 min) treatment yielded 10% seed emergence after 42 days. The 5-minute treatment yielded 30 % emergence, and the 10-minute treatment yielded 17% emergence, while the 15-minute treatment yielded the highest mean emergence rate, 46%. The 20-minute treatment yielded only 23% emergence, but the 25-minute treatment yielded 45% emergence. The 30-minute treatment yielded only 3% emergence, and the 35-minute treatment yielded 10% emergence. However, the longest duration treatment, of 40 minutes, yielded 37% emergence.

Duration of immersion in sulfuric acid significantly affected mean germination rate (F=4.32, p=0.0001, from ANOVA F-test on 8 and 265 dF). Treatments of 15 and 25 minutes of immersion significantly increased germination rate over control (Dunnett's test). Estimates for mean emergence rates of treatments are illustrated in the figure below (Figure2):

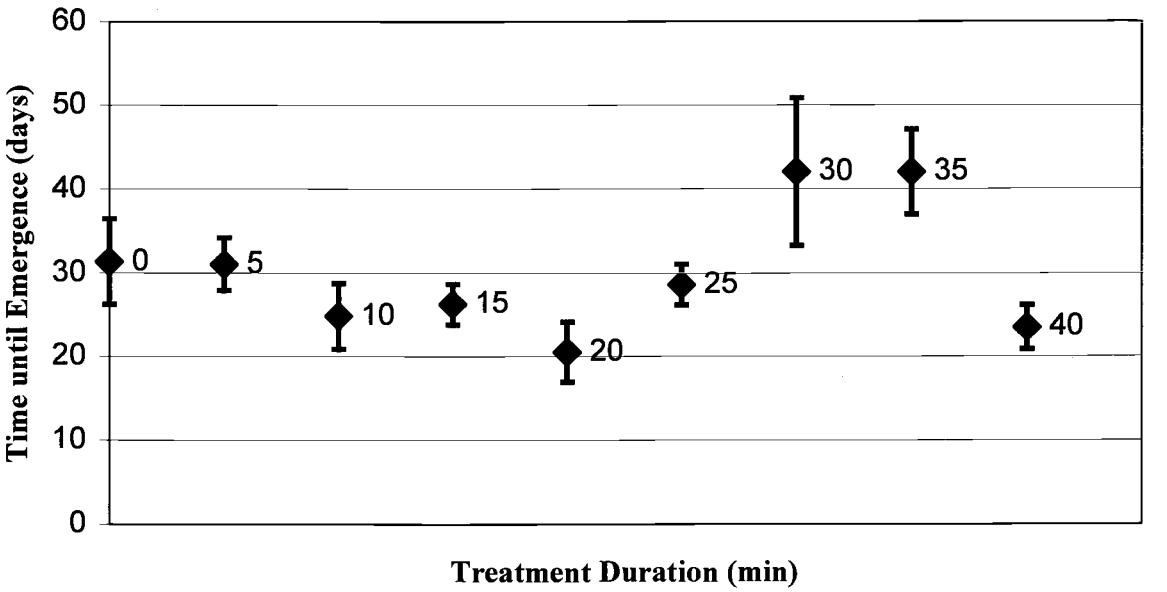
Figure 2. Mean emergence rates of Kincaid's lupine seed for nine acid scarification durations at 42 days following scarification. Standard error bars are shown. Squares represent values significantly different from control (0 min) (Dunnett's test).



Mean time until emergence from soil for seeds in each scarification treatment varied from 20 to 42 days, with an overall average of 30 days.

Duration of immersion in sulfuric acid had a significant effect on mean time until emergence (F=2.46, p=0.0239 from ANOVA F-test on 8 and 62 dF). However, no scarification treatments significantly changed mean time until emergence (Dunnett's test). Estimates for mean days until emergence are illustrated below (Figure 3):

Figure 3. Mean time until emergence of Kincaid's lupine seed for nine acid scarification durations following acid scarification. Standard error bars are shown. No values significantly differed from control (0 min) (Dunnett's test).



While two intermediate duration treatments significantly increased germination rate, the overall pattern was inconsistent. This may have been due to heterogeneity of the soil. Due to the high clay content of the field soil, there may have been pockets of dry or over-saturated soil, which could have lowered emergence rate. The soil could have also harbored harmful microorganisms. Seeds not emerging by the end of the test were excavated to determine their fate. Many seeds had germinated, but rotted before

emergence. Very low emergence was noted in the 30 and 35-minute treatments, where most seeds had rotted.

Seeds subjected to intermediate scarification durations were associated with apparently higher fungal infection rates than other seeds in this test, as in the first scarification experiment. This may again be due to a tradeoff between seed coat infection resistance and a dormancy-breaking requirement for germination. The treatment durations at which severe infection occurred differed between experiments, but growth media also differed.

Experiment 3

Materials and Methods

In the third experiment, I tested the effects of acid immersion and liquid nitrogen ($N_{2(l)}$) immersion on germination rate. The four treatments included a control, 20 and 40-minutes of immersion in 36 N sulfuric acid, and liquid nitrogen immersion. The 20-minute treatment had not significantly increased emergence in the second experiment, but the 15 and 25-minute treatments had. The 40-minute treatment also significantly increased emergence rate in the second experiment. This experiment used more seeds per treatment than previous tests to identify the more effective of the two acid scarification treatments.

Fifty seeds were randomly assigned to the four treatments. Seeds in the acid scarification treatments were immersed in 36 N sulfuric acid for specified duration, then rinsed thoroughly in distilled water. Seeds in the liquid nitrogen treatment were placed in a 500 ml graduated cylinder, and covered with 100 ml of liquid nitrogen. The nitrogen

was allowed to boil until evaporated. Seeds were immediately rinsed in distilled water. Seeds in the control treatment were rinsed in distilled water. All seeds were then rinsed in 10 % chlorine bleach to disinfect.

In order to test the effect of scarification treatment on water imbibition, I soaked all treated seeds in distilled water at 26° C for 24 hours. Imbibition rate was calculated as the germinated fraction of total seed per treatment, assessed by visual inspection. Kincaid's lupine seeds are hard and often compressed when mature and dry. Upon initial imbibition of water, they usually expand markedly. Imbibition seems to be always accompanied by softening and at least some expansion (pers. obs.). Seeds in this test had clearly either imbibed water, or were hard and compressed after 24 hours. Visual inspection did not allow the extent of water imbibition to be assessed, but effectively distinguished seeds that had imbibed substantial water and those that had imbibed very little or no water.

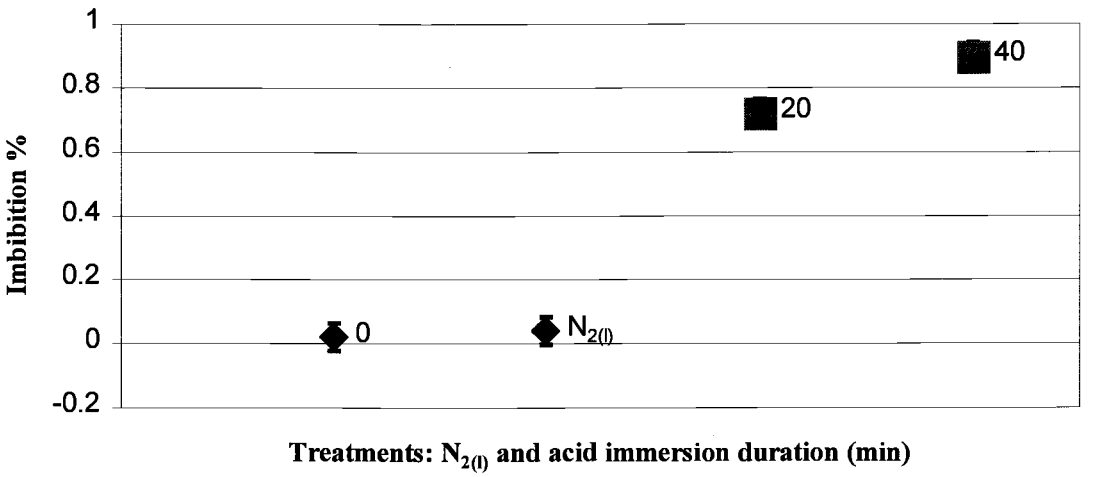
Filter paper was soaked in 100 % ethanol to disinfect, and allowed to dry thoroughly in closed petri dishes. The filter paper was moistened with distilled water. Soaked seeds were placed on filter in the petri dishes, and incubated at 26° C for one week. Germination rates were then assessed by emergence of radicle from integument.

Results and Discussion

In this experiment, the control treatment yielded a mean imbibition rate of 2%, and the liquid nitrogen soak yielded a mean imbibition rate of 4%. The 20-minute acid scarification treatment yielded a mean imbibition rate of 72%, and the 40-minute treatment yielded the highest mean imbibition rate, at 90%.

Duration of immersion in sulfuric acid significantly affected mean imbibition rate over 24 hours (F= 113.27, p<0.0001, from ANOVA F-test on 3 and 194 dF). The 40 minute treatment significantly increased imbibition rate over the control (Dunnett's test). The 20 minute treatment also significantly increased imbibition rate over the control (Dunnett's test). The liquid nitrogen treatment did not significantly increase imbibition over the control (Dunnett's test). Mean imbibition rates are illustrated below (Figure 4):

Figure 4. Mean imbibition rates of Kincaid's lupine seed for acid and $N_{2(l)}$ scarification treatments at 24 hrs. following scarification. Standard error bars are shown. Squares represent values significantly different from control (0 min) (Dunnett's test).



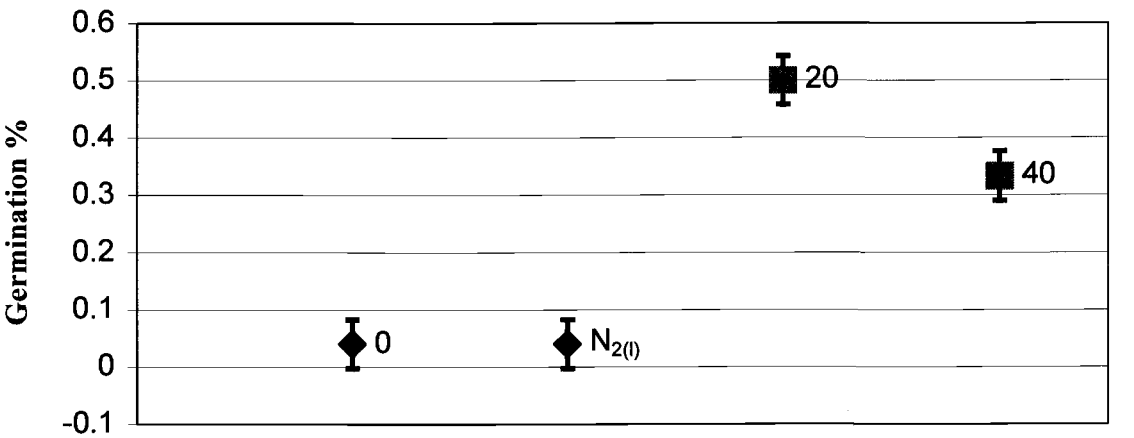
Imbibition rate was highest with acid scarification treatments, and the longer duration yielded the highest imbibition rate. Long durations of acid immersion have previously been found to cause severe seed coat degradation (see Experiment 1.) These results suggest that water imbibition is directly related to the extent to which the seed coat is disrupted by acid.

In this experiment, the control yielded a mean germination rate of 4%, and the liquid nitrogen treatment also yielded a mean germination rate of 4%. The 40 minute acid

scarification treatment yielded a mean germination rate of 33.3%, while the 20 minute acid scarification treatment yielded the highest mean germination rate: 50%.

Duration of immersion in sulfuric acid significantly affected mean germination rate (F=18.60, p<0.0001 from ANOVA F-test on 3 and 194 dF). The 20-minute treatment significantly increased germination rate over control (Dunnett's test). The 40 minute treatment also significantly increased germination rate over the control (Dunnett's test). Mean germination rates are illustrated below (Figure 5):

Figure 5. Mean germination rates of Kincaid's lupine seed for acid and $N_{2(l)}$ scarification treatments at 24 hrs following scarification. Standard error bars are shown. Squares represent values significantly different from control (0 min) (Dunnett's test).



Liquid nitrogen did not increase germination rate, contrary to my prediction. The extreme freezing and thawing of the seed was expected to disrupt the seed coat. However, the seeds may require several freeze-thaw cycles. The seeds may also not have a high enough water content to crack with freezing.

Conclusions

In all three experiments, intermediate acid immersion duration significantly increased seed germination rate, showing that Kincaid's lupine seeds have a scarification requirement. Prolonged immersion was observed to result in acid penetrating the integument and damaging endosperm. Although several treatments in the first two tests did not yield results consistent with these mechanisms, the overall pattern of scarification response was replicable.

The direct damage to seed tissue observed with long acid immersion durations in the first experiment was clearly due to acid penetrating the seedcoat. However, a study of acid scarification in Indian ricegrass (*Oryzopsis hymenoides* [Roem. and Schult.] Ricker) found that acid immersion delayed protein synthesis for 48 hours, reduced soluble protein and increased electrolyte leaching in the seeds (McDonald and Khan, 1983). Acid scarification may have these less obvious effects on Kincaid's lupine seed, which may explain the irregular pattern of fungal infection found in experiments one and two of this study.

The third test provided conclusive evidence that an intermediate immersion duration, 20 minutes, resulted in the highest germination rate. The 40-minute treatment yielded the highest imbibition rate, likely due to more severe seed coat disruption. However, the imbibition rates after 24 hours did not closely match germination rates after seven days. Overly severe scarification may allow rapid water imbibition in the seed, but damage the embryo.

Other lupines have demonstrated high germination rates at higher acid immersion durations. *Lupinus havardii* germination rate increased with increasing immersion

duration up to 120 minutes (Mackay et al., 1995). *Lupinus perennis* germination rate increased with increasing immersion duration up to 90 minutes (Mackay et al., 1996). Kincaid's lupine may have thinner seed coats than these agriculturally important species, and require less severe scarification to germinate successfully. Based on the irregular germination rate and tissue damage found with long acid immersion durations, Kincaid's lupine seeds should be treated for the minimum duration (20 min) yielding a high germination rate.

Application of this optimum 20 minute acid immersion treatment in large-scale propagation efforts should improve overall seed germination rate. This technique may also allow finer resolution of the effects of other factors on germination rate, in further studies.

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