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# Scarification and Cultural Practice of Four Lupine Species Native to the Great Basin

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# Cultural Practices and Seed Scarification of Four Native

# *Lupinus* Species (Fabaceae) From the Great Basin

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ABSTRACT

Cultural Practices and Seed Scarification of Four Native *Lupinus* Species (Fabaceae) From the Great Basin

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The Great Basin is North America’s largest desert, encompassing 135 million acres. Grazing and other anthropogenic activities in the Great Basin have put heavy demands on the landscape over the last 150 years. Heavily grazed areas lack diversity which allows the spread of exotic weed species. Cheatgrass (*Bromus tectorum* L [Poaceae]) has invaded and shortened fire frequency intervals from historic 30—100 years to as few as three to five years. Post-fire reseeding of native species is requisite for restoration of highly invaded ecosystems thus, preventing complete conversion to exotic weeds. Most native shrubs and grasses are available for restoration projects, but native forbs are largely unavailable or expensive. This situation led to the creation of The Great Basin Native Plant Selection and Increase Project (GBNPSIP). In 2000 this project was initiated as a joint effort between the Bureau of Land Management, Forest Service Research, and the Utah Division of Wildlife Resources in an effort to make native seed more available and less expensive for landscape scale restoration projects. To meet restoration goals the GBNPSIP project promotes cultivation of native species to increase seed supplies. This research focuses on overcoming seed dormancy issues that have hindered cultivation through scarification and evaluating germination, establishment, and seed production in a cultural setting of four lupine species: hairy big leaf lupine, (*Lupinus prunophilus* M.E. Jones [Fabaceae]); silky lupine, (*L. sericeus* Pursh); silvery lupine, (*L. argenteus* Pursh); and longspur lupine, (*L. arbustus* Dougl. ex Lind) five scarification treatments were evaluated sulphuric acid and mechanical treatments significantly improved germination on three of the four species tested. All other treatments were unpredictable and not significant. No treatments significantly improved germination of *L.arbustus* and three of the five treatments significantly decreased seed germination from the control. Results demonstrate that scarification method, and exposure interval, differ in effectively increasing % germination among species. Germination, establishment, and seed production were evaluated using two planting methods for each species. Broadcast plots (covered) were covered with N-Sulate fabric™ and 5 cm (2 in) of sawdust. Control plots (uncovered) were drilled and left untreated. Germination was significantly improved for all four lupine species under treatment conditions. *Lupinus prunophilus* and *L. sericeus* exhibited the greatest improvement in germination when covered. Germination of *L. argenteus* and *L. arbustus* were also significantly improved ($p<0.0001$ and $p=0.004$, respectively) by the covered treatment. Higher germination in the covered treatment was mirrored in establishment for every species except *L. arbustus.* There is an advantage of using the covered treatment, but low yields make cultivation unprofitable.

Keywords: lupine, Great Basin, native forb cultivation, legume scarification

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CONTENTS

CHAPTER 1

# Optimizing Seed Scarification Methods for *Lupinus sericeus*, *L. argenteus*, *L. prunophilus* and *L. arbustus*

## ABSTRACT

Most lupine species in the Great Basin are hard seeded and thus hard to establish from seed in a cultural setting. This study was conducted to reduce seed dormancy through scarification. Five scarification treatments were evaluated on four Great Basin lupine species: hairy big leaf lupine, (*Lupinus prunophilus* M.E. Jones [Fabaceae]); silky lupine, (*L. sericeus* Pursh); silvery lupine, (*L. argenteus* Pursh); and longspur lupine, (*L. arbustus* Dougl. ex Lind). Two thermal treatments; first, a boiling water bath varying exposure time second, holding time constant at 60-sec while varying exposure temperature. Two chemical treatments, one using concentrated sulphuric acid, (18 M $H_2$) and the other using bleach ($SO_4$2.7% sodium hypochlorite) and one mechanical treatment using a Forsberg mechanical scarifier. Controls for each species were left untreated. Sulphuric acid and Mechanical treatments significantly improved germination on three of the four species tested. All other treatments were unpredictable and not significant. No treatments significantly improved germination of *L.arbustus* and three of the five treatments significantly decreased from the control. Results demonstrate that scarification method, and exposure interval, differ in effectively increasing % germination among species.

## INTRODUCTION

The Great Basin is North America's largest desert, encompassing 135 million acres of land spanning from the Rocky to the Sierra Nevada mountain ranges and covering much of Utah and Nevada with portions extending into Idaho, Oregon, and California. This vast expanse of territory provides an opportunity for multiple land uses. Early settlers of the Great Basin mined precious metals and ranched. A census conducted in 1890 recorded 3.8 million sheep and half a million cattle in Utah and most of these animals grazed in the Great Basin during at least some parts of the year (Harrison et. al. 2003). Heavy demands placed on the Great Basin by early settlers did not come without consequence. Heavy grazing areas reduced diversity of many native plant communities and caused the spread of exotic weed species.

Presence of cheatgrass (*Bromus tectorum* L [Poaceae]), an invasive annual, was identified as early as 1916 in the west and is currently prolific in the Great and Colombia Basin regions (Morrow and Stahlman 1984). This weedy annual shortens fire frequency intervals from historic 30—100 years to as few as three to five years (Whisenant 1990; Peters and Bunting 1994). The native ecosystems of the Great Basin are not adapted to such abbreviated fire intervals.

Post-fire reseeding of native species is requisite to restoring highly invaded ecosystems and preventing complete conversion to exotics. Most native shrub and grass seed for large-scale restoration projects are being sold at a reasonable price, but native forbs are largely unavailable or expensive. This situation led to the creation of the Great Basin Native Plant Selection and Increase Project (GBNPSIP) in 2000. The GBNPSIP project is a joint effort between the Bureau of Land Management and Forest Service Research in an effort to make native seed more available and less expensive for the landscape scale restoration projects needed (USDA Forest Service). To accomplish their goal the GBNPSIP intends to collect wildland seed from desired native species and increase seed supplies, through private production, to quantities necessary to meet restoration needs.

To help GBNPSIP reach its goals this study focuses on overcoming seed dormancy issues that have hindered cultivation of four native lupine species (*Lupinus* Tournefort [Fabaceae]). Seed dormancy is a survival strategy that better ensures the persistence of wildland species and “results from some characteristic of the seed” not the environment (Baskin and Baskin 1998). Dormant seed can pass through periods that are environmentally favorable for germination and remain in the soil profile as viable ungerminated seed. For wildland species, dormancy helps insure species survival as ungerminated seed can pass through periods of disease, drought, fire, or flood that decimates the plant population; dormant viable seed still persists in the seed bank and could germinate in subsequent growing seasons thus assuring survival of a species. Seed dormancy is characterized as exogenous if caused by factors outside the embryo, or endogenous if caused by factors within the embryo (Nikolaeva 1969). Exogenous dormancy is further characterized as physical, mechanical or chemical, while endogenous dormancy may be physiological or morphological. Combinations of these factors may be responsible for dormancy in some species (Jones and Nielson 1992). In the Great Basin, some lupine species exhibit physical, or exogenous, dormancy. These lupine species have a seed coat that is impermeable to water; another term for this type of dormancy is “hard seeded". The degree of dormancy for Great Basin lupine species is variable and differs both by species and within species. Quinlivan (1970) demonstrated that dormancy in lupine seed is correlated to relative humidity at the time the seed reaches maturity, with a higher percent of dormant seed as humidity decreases. In nature, soil bacteria or insects that penetrate the impermeable seed coat can overcome physical dormancy. Also, humidity and sharp daily temperature variation can increase permeability.

To efficiently cultivate a species, it is important that seed is viable and non-dormant. Using non-dormant seed or seed that has had dormancy broken allows uniform germination and good stand establishment. Scarification of hard seed can break the impermeable seed coat and reduce the percentage of dormant seed. However, overexposure to scarification treatments can be deleterious to the seed embryo and result in a non-viable seed. This study was undertaken to determine how different scarification treatments and exposure intervals to those treatments effect hard seededness and germination of four lupine species.

## MATERIALS AND METHODS

### Germplasm

In May and June of 2007 we identified four sites that would provide the lupine seed necessary to conduct scarification trials (*Fig 1*) and seed was collected from a single species and biotype from each of those four locations in June and July of 2007. Hairy big leaf lupine (*Lupinus prunophilus* M.E. Jones) and silky lupine (*L. sericeus* Pursh) were collected in central Utah at the eastern edge of the Great Basin; silvery lupine (*L.*

*argenteus* Pursh) and longspur lupine (*L. arbustus* Dougl. ex Lind) were collected in north central Nevada.

**Treatments**

Twenty-five good seeds (full seed with an intact seed coat) were selected for each treatment interval and replicated twice. The controls for each species were not treated. The experimental design employed tested ten intervals for each species with five scarification methods.

The 25 seeds for each species and subsequent treatment were placed on moist blotters in petri dishes. Petri dishes were randomly stacked and placed in clear plastic bags to prevent blotters from drying and replicated twice. Bags were left in ambient conditions (19-21º C) and placed in cardboard box on a laboratory bench for the duration of the study.

**Data Collection**

Association Official Seed Analysts (AOSA) germination rules have not been developed for the lupine species used in this experiment. AOSA rules suggest monitoring general lupine germination for 18 days; however in this study, germination was monitored for 22 days. Germinated seed were counted and removed three times during the 22-day period then petri dishes were re-randomized and returned to the plastic sack. *L. argenteus* and *L. prunophilus* are known to have hard seed coats and *L. arbustus* and *L. sericeus* are not considered to be hard seeded; however, these were treated to test the hypothesis that scarification would benefit germination.

**Analysis**

Germination data was analyzed using a GLIMMIX model in SAS (SAS Institute Inc. 2008) to determine statistical differences. Loess smoothing functions with +/- 2 standard error bands were used to generate figures in R statistical package 2.13.1 to demonstrate the relationship of various treatments with germination fraction.

## RESULTS

Of the five scarification treatments, only sulphuric acid and mechanical treatments impacted germination of *L. argenteus* significantly (*Fig 2*). It appears that 6-min of sulphuric acid scarification and 4 to 6-sec of mechanical scarification produce 82% and 84% germination, respectively. These are 24 and 26% better than the untreated control (58% germination). Other treatments produced erratic and unpredictable germination that was not significant according to confidence intervals.

Sulphuric acid was the only treatment that significantly improved germination of *L. prunophilus* (*Fig 3*). Initial germination of the untreated control for this treatment was 40%, after 5-min of treatment exposure to the sulphuric acid germination increased to 80%, a 40% increase above the control. The other scarification treatments on this species did not overcome the confidence intervals and were therefore not significant.

The response of the scarification treatments on *L. sericeus* were similar to that of *L. argenteus*, only sulphuric acid and mechanical treatments significantly improved germination (*Fig 4*). 3-min of exposure to the sulfuric acid treatment and 4-sec of exposure to the mechanical treatment produced 85 and 75% germination, respectively.

These are 45 and 35% better than the untreated control (40% germination). None of the other treatments significantly improved germination of this species.

None of the scarification treatments significantly improved germination of *L. arbustus* (*Fig 5*). However the constant temperature, sulphuric acid, and mechanical treatments all significantly decreased germination compared with the untreated control.

Overall the most predictable and most effective scarification treatments among the four species tested were either sulphuric acid or mechanical. With sulphuric acid scarification demonstrating significant improvement on germination with three of the four species tested and mechanical showing significant improvement in germination on two of the four species tested.

## DISCUSSION AND CONCLUSIONS

Mechanical scarification was the most efficient way to process large amounts of seed. In addition to efficiency, mechanical scarification was an effective treatment on *L. argenteus*, and on *L. sericeus.* The reason that mechanical scarification so effectively on *L. argenteus* are smaller seeded size, and has a noticeably thicker seed coat. Travlos et. al. (2006) found mechanical scarification to be more effective than sulphuric acid scarification on the Marama bean (*Tylosema esculentum* (Burch) L. Schreib[Fabaceae]), a hard seeded African legume. With mechanical scarification there is no added time for rinsing and drying like there is with chemical or thermal treatments. The other two species we tested did not respond favorably to mechanical scarification. The Forsberg mechanical scarifier used is very abrasive, even the shortest exposure times damaged the seed of *L. prunophilus* and *L. arbustus.*

Even though more time consuming, sulphuric acid was the only treatment that yielded a significant improvement in germination for *Lupinus prunophilus*. *Lupinus prunophilus* seed is much larger than seed of *L. argenteus* and was visibly damaged from the rotating action of the mechanical scarifier. Although mechanical scarification was not significant for this species it does have a more normal distribution and appear to be more predictable than the other treatments tested. Another option for *L. prunophilus* might be to use a less aggressive mechanical scarifier. Dreesen, (2004) used a rock tumbler with pea gravel and water to clean and scarify New Mexico olive (*Forestiera pubescens* Nutt. var. *pubescens* [Oleaceae]). A less abrasive mechanical treatment will take more time, but would be less likely to damage the seed embryo and the equipment needed for this treatment is easier to procure than sulfuric acid.

This was the first attempt of using sodium hypochlorite to scarify lupine seed. Although it is much easier to acquire than sulphuric acid it did not show any significant increase in germination for any of the species tested at any exposure interval. However, this does not mean that a longer exposure interval would not increase germination. Further research using sodium hypochlorite to scarify lupine seed with a longer exposure needs to be done.

Thermal scarification methods were unpredictable and did not significantly improve germination over the control for any of the four lupine species tested. Thus despite the long term use of thermal scarification with blue lupine (*L. pilosus* L.) (Hootman, 1941) thermal scarification cannot be recommended for these four lupine species.

In the past good seedling emergence has been observed without scarification treatments with *L. arbustus* and *L. sericeus*. This held true in the scarification treatments of *L. arbustus.* In fact, seed germination immediately declined with all scarification treatments was observed on this species. This is likely a result of a noticeably thinner seed coat with *L. arbustus* than the other species. The thin seed coat provides less protection for the seed embryo and any abrasive treatment meant to scarify the seed coat damages the embryo and lowers germination percent in the process. Unlike seed of *L. arbustus* germination of *L. sericeus* seed increased 45 and 35% with sulphuric acid and mechanical treatments respectively, over the control. The low germination percentage of the control (40%) may reflect that 22 days is not an adequate duration for germination without scarification. Even if additional seeds will eventually germinate without scarification, the short sulphuric acid or mechanical treatment will increase uniformity of germination and likely lead to quicker germination and better establishment in a field setting.

Our results are similar to Dittus and Muir (2010) who showed different scarification treatments had varying effects on similar legume species. Scarification can be a very effective tool to reduce dormancy and thus allow more efficient use of the limited seed. Both mechanical and sulphuric acid produced seed with better germination and were more predictable than other scarification treatments in three of the four species studied. Thus, when used appropriately, scarification should reduce the quantity of expensive seed needed to establish increase fields for these species. Our data clearly suggest a need to establish appropriate scarification methods for each species desired by growers. Additional information on less aggressive mechanical treatment than the Forsberg machine used also would be valuable.

| Site Name | Species | Zone | E | N | Elevation, m |
|---|---|---|---|---|---|
| Tintic | *L. prunophilus* | 12S | 406466 | 4424249 | 1950 |
| Buckskin Flat | *L. sericeus* | 12S | 441948 | 4392939 | 1920 |
| Soldier Canyon | *L. argenteus* | 11T | 638637 | 4517999 | 1768 |
| Bear Creek | *L. arbustus* | 11T | 628154 | 4632914 | 2469 |

*Fig 1*. Germplasm collection locations in Universal Transverse Mercator (UTM).

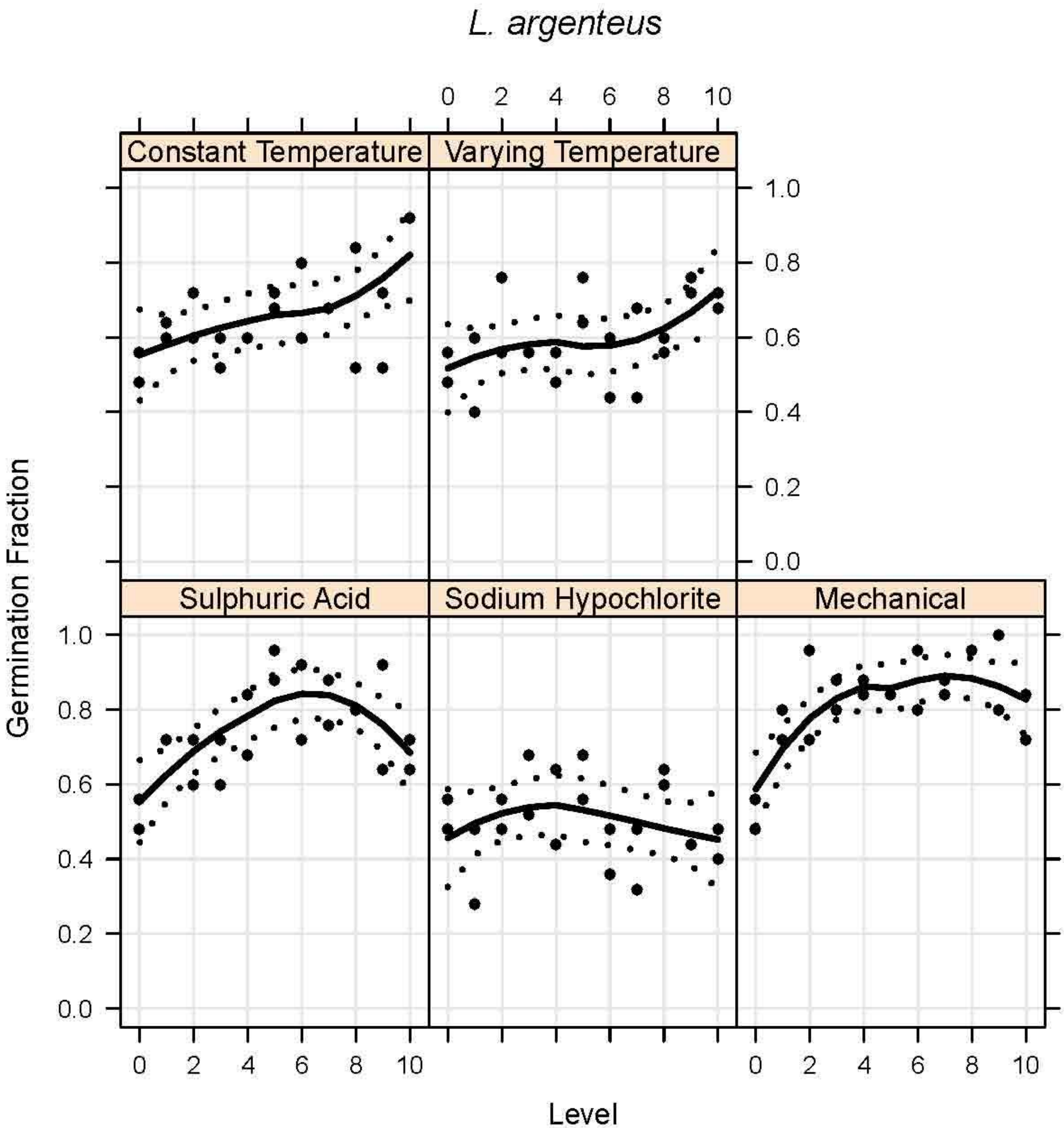


*Fig 2*. Loess smoothing functions with +/- 2 standard error bands. Constant Temperature = boiling water bath varying seed exposure in 5-sec intervals from 15 to 60-sec; Varying Temperature = time constant at 60-sec while increasing temperature 5º C from 50 to 95º C; Sulphuric Acid = 18 M $H_2SO_4$ increasing at 1-min intervals from 1 to 10-min; Sodium Hypochlorite (2.7%) = 1X concentration household bleach increasing 15-min intervals from 15 to 150-min; Mechanical = Forsberg batch scarifier one-sec exposure intervals from 1 to 10-sec.

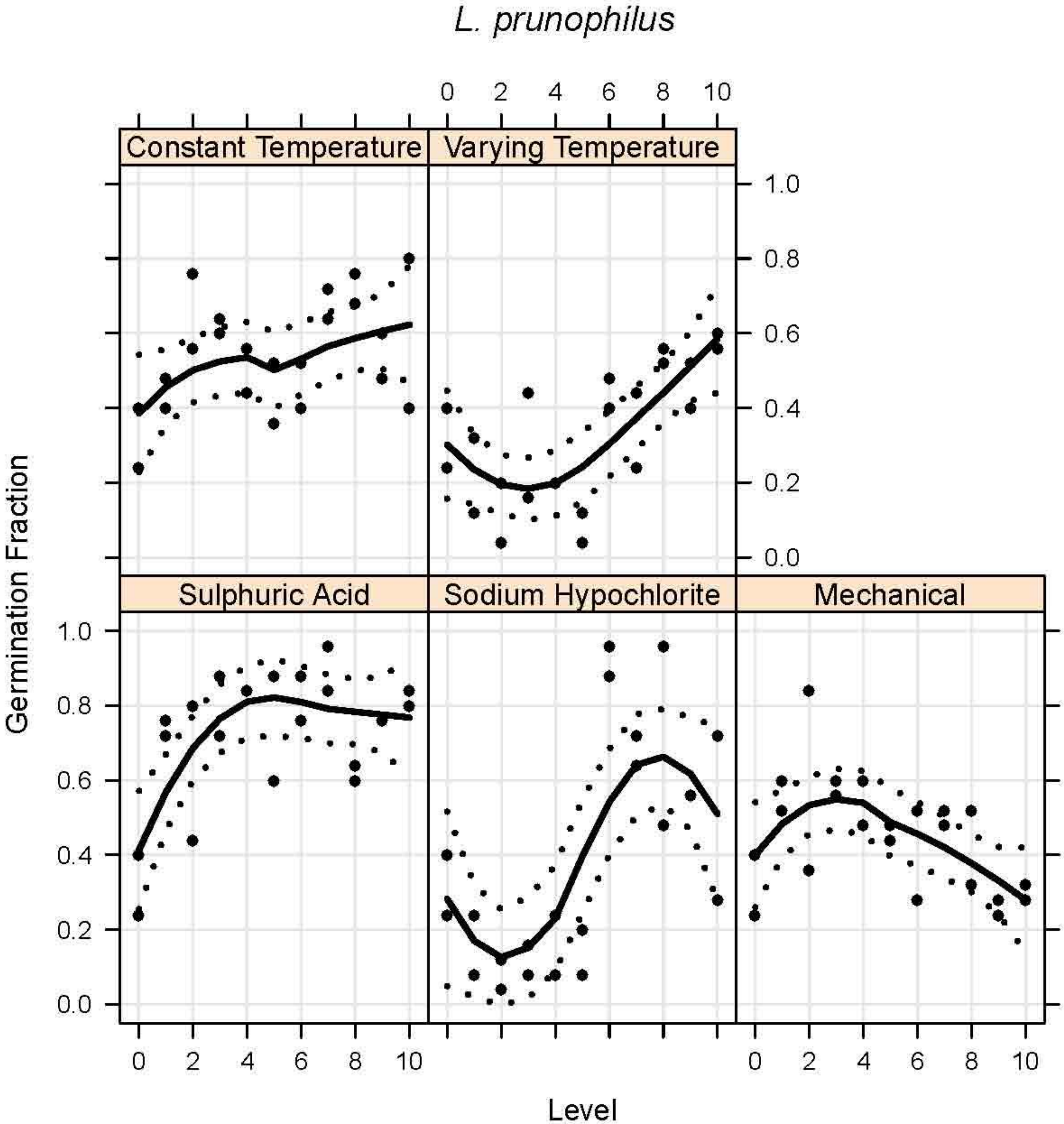


*Fig 3*. Loess smoothing functions with +/- 2 standard error bands. Constant Temperature = boiling water bath varying seed exposure in 5-sec intervals from 15 to 60-sec; Varying Temperature = time constant at 60-sec while increasing temperature 5º C from 50 to 95º C; Sulphuric Acid = 18 M $H_2SO_4$ increasing at 1-min intervals from 1 to 10-min; Sodium Hypochlorite (2.7%) = 1X concentration household bleach increasing 15-min intervals from 15 to 150-min;  Mechanical = Forsberg batch scarifier one-sec exposure intervals from 1 to 10-sec.

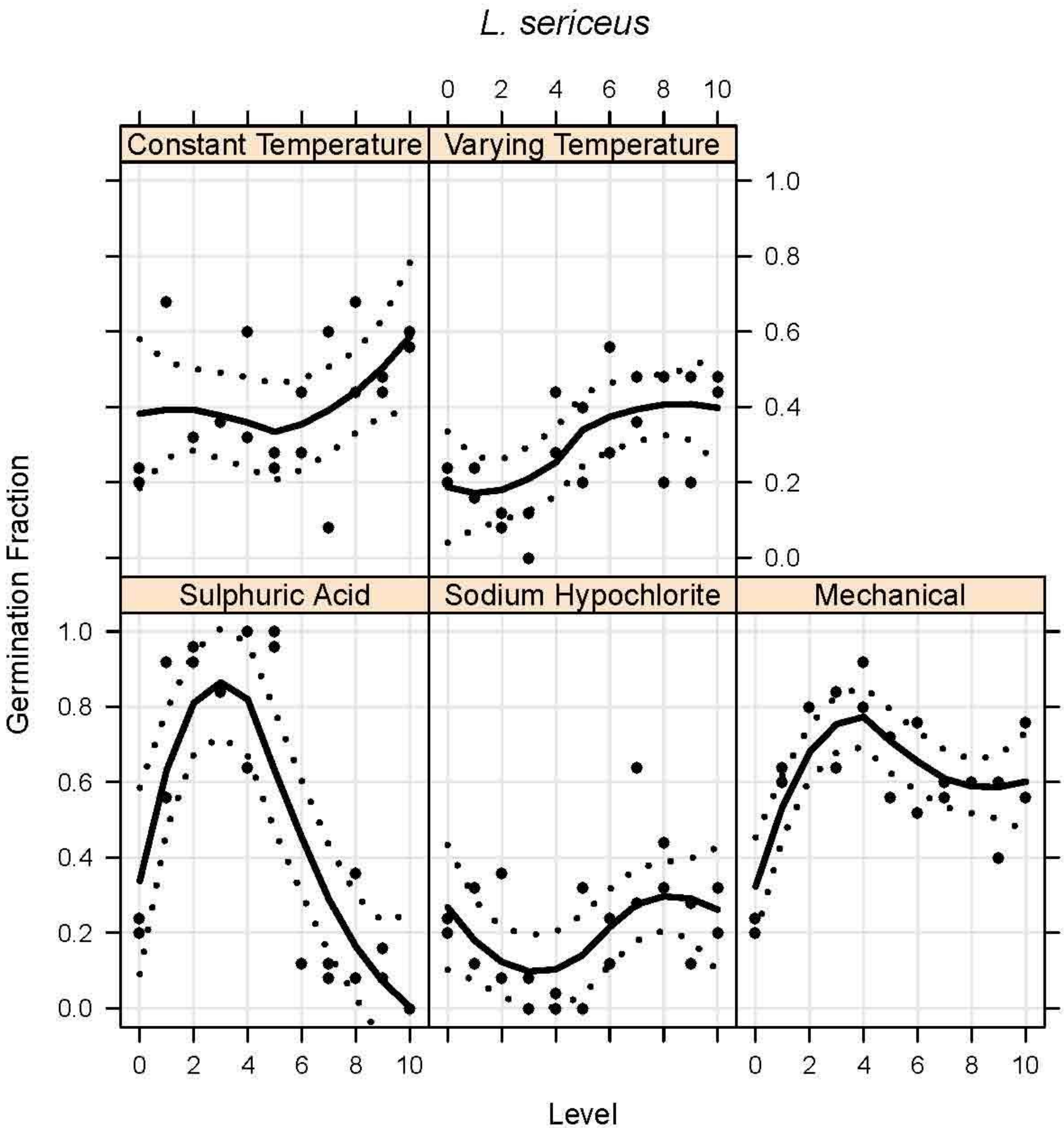


*Fig 4*. Loess smoothing functions with +/- 2 standard error bands. Constant Temperature = boiling water bath varying seed exposure in 5-sec intervals from 15 to 60-sec; Varying Temperature = time constant at 60-sec while increasing temperature 5º C from 50 to 95º C; Sulphuric Acid = 18 M $H_2SO_4$ increasing at 1-min intervals from 1 to 10-min; Sodium Hypochlorite (2.7%) = 1X concentration household bleach increasing 15-min intervals from 15 to 150-min; Mechanical = Forsberg batch scarifier one-sec exposure intervals from 1 to 10-sec.

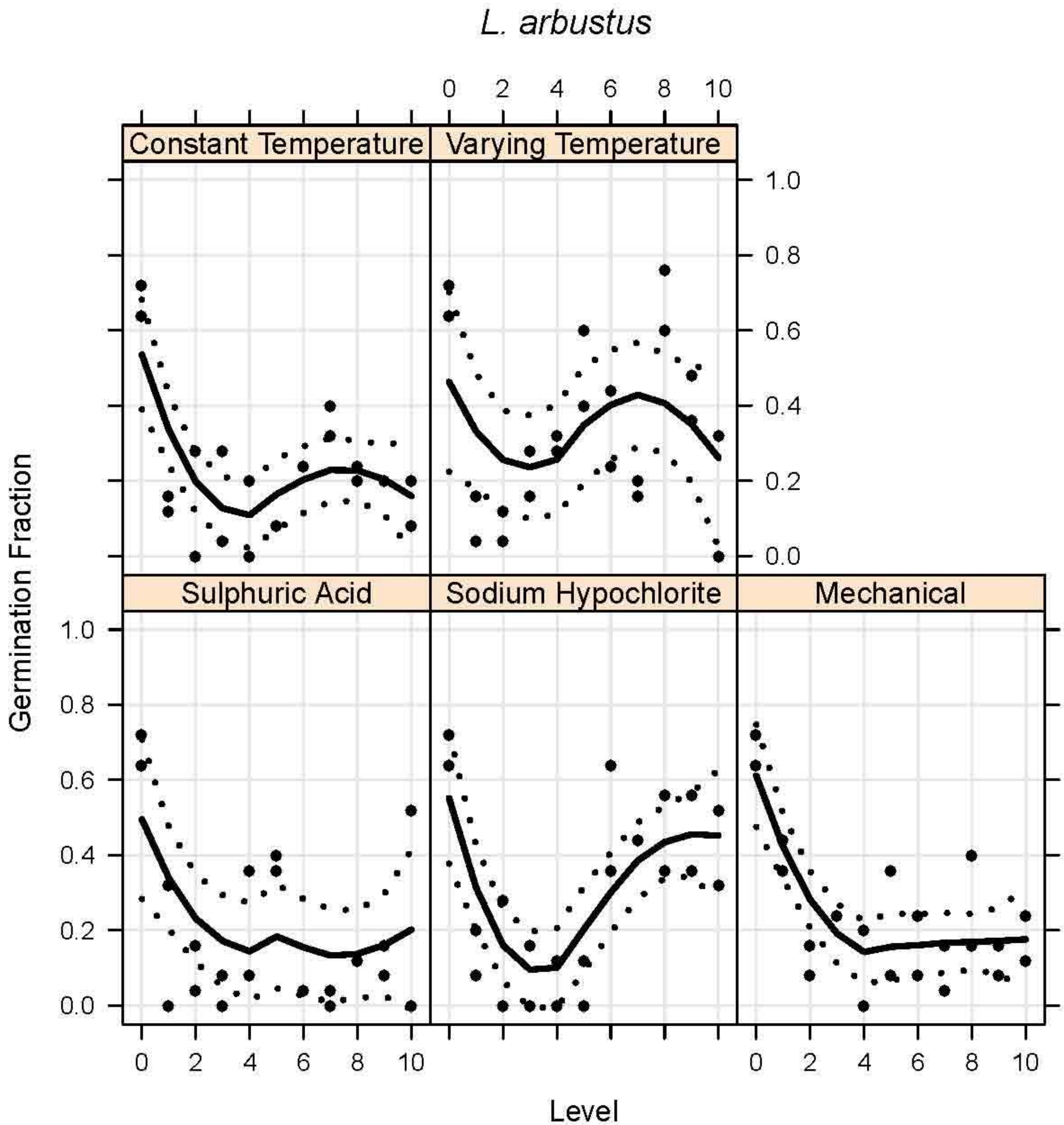


*Fig 5*. Loess smoothing functions with +/- 2 standard error bands. Constant Temperature = boiling water bath varying seed exposure in 5-sec intervals from 15 to 60-sec; Varying Temperature = time constant at 60-sec while increasing temperature 5º C from 50 to 95º C; Sulphuric Acid = 18 M $H_2SO_4$ increasing at 1-min intervals from 1 to 10-min; Sodium Hypochlorite (2.7%) = 1X concentration household bleach increasing 15-min intervals from 15 to 150-min; Mechanical = Forsberg batch scarifier one-sec exposure intervals from 1 to 10-sec.

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CHAPTER 2

# Identifying Cultural Practice Methods for *Lupinus sericeus*, *L. argenteus, L. prunophilus* and *L. arbustus*


## ABSTRACT

Fire and invasive weeds have increased the demand of native seed for restoration across the Great Basin. Cultivation of native forbs could provide less expensive seed in necessary quantities to meet restoration needs. This study evaluated the cultivation of four lupine species: hairy big leaf lupine (*Lupinus prunophilus* M.E. Jones [Fabaceae]), silky lupine (*L. sericeus* Pursh), silvery lupine (*L. argenteus* Pursh), and longspur lupine (*L. arbustus* Dougl. ex Lindl). Direct seeded plots were used to evaluate germination, establishment, and seed production of two planting methods for each species. A hand-held fertilizer spreader or a cone seeder was used to either broadcast or drill the seed on the surface. Post seeding, two 1 cm steel chains were pulled over the top to incorporate seed into the soil. Broadcast plots (covered) were covered with N-Sulate fabric™ and 5 cm (2 in) of sawdust. Control plots (uncovered) were drilled and left untreated. Germination was significantly improved for all four lupine species under treatment conditions. *Lupinus prunophilus* and *L. sericeus* exhibited the greatest improvement in germination when covered. Germination of *L. argenteus* and *L. arbustus* were also significantly improved ($p<0.0001$ and $p=0.004$, respectively) by the covered treatment. Higher germination in the covered treatment was mirrored in establishment for every species except *L. arbustus*. Only *L. argenteus* and *L. sericeus* produced seed by the second and third growing seasons. Both species demonstrated decreased per plant seed yields with increased plant density, but the higher densities still produced more seeds per hectare. There is an advantage of using the covered treatment, but low yields make cultivation unprofitable.

**INTRODUCTION**

The Great Basin is the largest desert in North America covering over 492,000 square kilometers across Utah and Nevada as well as portions of Idaho, Oregon, and California. In the late 1800's and early 1900's early settlers of the Great Basin mined precious metals and ranched. An 1890 census recorded 3.8 million sheep and 0.5 million cattle in Utah and most of these animals grazed in the Great Basin during at least some part of the year (Harrison et. al. 2003).

Many native plant communities experienced a "detrimental change in composition structure" due to heavy grazing (Vavra 2007). These impacted areas were then invaded with cheatgrass (*Bromus tectorum* L [Poaceae]), and this non-native weedy species is among the most detrimental invasive weeds found in the Great Basin. Cheatgrass was noticed as early as 1916 in the west and is currently the most prolific plant in the Great Basin (Morrow and Stahlman 1984). This weedy annual grass invades weakened ecosystems and shortens fire frequency intervals from historic 30 to 100 years to as few as three to five years (Whisenant 1990; Peters and Bunting 1994). The native ecosystems in the lower elevations of the Great Basin are not adapted to such abbreviated fire intervals and are quickly disappearing from the landscape. Functioning lower elevation native ecosystems in approximately 20 million hectares have been lost to cheatgrass; thus leaving only 54 million hectares of its original potential of 73 million hectares (Connelly et al. 2004).

Active habitat restoration using native plant materials, both pre and post fire, is critical to preserving biodiversity in the Great Basin. Native shrubs and grasses are currently being marketed in quantities and prices which allow for landscape scale restoration projects. However, native forbs are largely unavailable or expensive.

In 2000, The Great Basin Native Plant Selection and Increase Project (GBNPSIP) was initiated as a joint effort between the Bureau of Land Management and Forest Service Research to make native seed more available and less expensive for the landscape scale restoration projects necessary to restore the burned areas to their former state (USDA Forest Service 2009). One objective of the GBNPSIP is to collect wildland seed from desired native forb species and increase seed supplies through private production to quantities necessary to meet restoration needs. An initial list of desired species was generated when the GBNPSIP project was initiated. This list looked at restoration from the standpoint of whole plant communities. After working on the project for seven years, it was determined that some of the species on the initial GBNPSIP list are not suited to current agricultural technologies and equipment. GBNPSIP refocused the plant materials development program using species that had the following characteristics: tall 0.3-1.8 m (1-6 ft), upright growth habit, determinate flowering, good seed retention, abundant seed set, annually productive, easily established from seed, long lived, and disease resistant.

For our study we chose to evaluate germination, establishment, and seed production of four lupine species (*Lupinus* (Tournefort) [Fabaceae]). Lupines were chosen because they have some of the desired agricultural characteristics listed above and are on the GBNPSIP target forb list. These four lupine species have not been studied before and results will help provide useful information to commercial seed producers about what to expect when establishing these species in an agronomic setting.

We also evaluated seed production for each species as related to stand density. The evaluation of planting methods and seed production will provide important agronomic information for growers planning to incorporate these species into their seed production operations.

## MATERIALS AND METHODS

### Germplasm

In May and June of 2007 we identified four sites that would provide the lupine seed necessary to conduct our seeding trials. In June and July of 2007 we collected individual species from those four sites. Two species were located in central Utah at the eastern edge of the Great Basin: hairy big leaf lupine (*Lupinus prunophilus* M.E. Jones), silky lupine (*L. sericeus* Pursh), and two species in north central Nevada: silvery lupine (*L. argenteus* Pursh), and longspur lupine (*L. arbustus* Dougl. ex Lindl) (*Fig 1*).

### Study Area

The study was conducted at two locations, the Utah Division of Wildlife Resources (DWR) farm near Fountain Green, UT, and Snow Field station in Ephraim, UT (Table 1). The DWR farm is at 1836 m (6023 ft) elevation, receives 30 cm (12 in) of annual precipitation distributed evenly though out the year and located on loam soil. Snow Field Station is 30 km (18.6 miles) south east of Fountain Green and is 1690 m (5544 ft), receives at 27 cm (10.6 in) of precipitation mainly distributed among the fall, spring, and winter and is located on a clay loam soil.

**Seeding**

In October 2007 direct seeded plots were prepared using a double roller Brillion landscape seeder (Brillion Farm Equipment, Brillion, WI) to create an even surface with indentations for seed. Seeding rates were adjusted for each species to reflect 100% pure live seed (PLS). Seeding densities per 0.09 $m^2$ (1 $ft^2$) were; 19 seeds *L. argenteus*, 20 seeds *L. sericeus*, 25 seeds *L. arbustus*, and 17 seeds *L. prunophilus*. Using either a hand-held fertilizer spreader or a cone seeder; seed was either broadcast across the prepared surface or drilled through the custom precision cone seeder (Hege, Wichita, KS) on the surface. Immediately after seeding, two 1cm (3/8 in) steel chains were pulled over the top of the soil surface to help incorporate seed into the soil. At this point, drilled plots (uncovered) were left uncovered. Broadcast (covered) plots were covered with 5 cm (2 inches) of sawdust and N-Sulate fabric™ (DeWitt Company, Houston, TX). N-Sulate fabric is water permeable, 50g/$m^2$ (1.5 oz $yard^{-2}$) UV treated fabric designed to protect gardens, flowering annuals, bedding plants and vegetables from freezing temperatures. When placed directly on moist soil the fabric creates and prolongs a warm wet microenvironment beneficial for germination of some species. Thus we evaluated germination and establishment of these four lupine species when drill seeded and left uncovered or broadcast seeded and covered.

Sub-surface drip irrigation systems were installed using a single tooth ripper to put in drip tape 30 cm (12 inches) below the soil surface. Three rows of drip tubing were installed per treatment, one in the center and the other two equally spaced 46 cm (18 inches) on each side. This allowed equal distribution of additional water for all trials across the five blocks. This sub-surface drip system was used during the first two establishment seasons but was discontinued the third growing season.

**Germination and Establishment**

To estimate percent germination of the four species, five randomly selected areas were counted for the number of germinating seedlings and divided by the seeding density. To evaluate establishment, counts were repeated during the second growing season. Plants that overwintered were counted as established plants. Because establishment did not appear to be as evenly distributed across the plot as germination, we chose to count all established plants in each treatment instead of sub-sampling.

**Seed Collection**

Seed was mechanically collected a single time per growing season using a homemade pushcart forage harvester when the maximum amount of seed was ripe. Harvested material was dried on tarps and processed in a homemade debearder; then Carter-Day fractional aspirator (Carter Day International, Minneapolis, MN) was used to separate seed from chaff.

**Experimental Design and Analysis**

A completely randomized block design with five replications was used to compare a broadcast seeding, using N-Sulate fabric (covered treatment), and the traditional seeding method, of uncovered and drilled. Each treatment was 23 x 1.5 m (75 x 5 ft). Seed was harvested each year from mature plants at both locations using a forage type push harvester. Yields were averaged at both locations across all five blocks for each species.

Covered treatments were hypothesized to result in increased germination and consequently to have superior establishment and seed production. Germination and establishment data were analyzed by species, block, and treatment. We used a GLIMMIX model in SAS (SAS Institute Inc. 2008) to determine statistical differences.

## RESULTS AND DISCUSSION

### Germination

Germination was significantly improved for all four lupine species at both locations with the covered treatment ( *Fig 2*) averaging 26.6 and 7.3% for covered and not covered, respectivly. *Lupinus prunophilus* and *L. sericeus* seed had the greatest improvement in germination when covered: increasing 26 and 19 percentage points higher than the uncovered, respectively. There was small but significant improvement of germination with *L. argenteus* and *L. arbustus* ($p<0.0001$ and 0.004 respectively) by the covered treatment. Germination percentages were consistently low when not covered, with seed of *L. argenteus* being statistically lower (2.5%) and the other three similarly germinating species (7.2-13.1%). Schmal (2007) reported good success using N-sulate fabric and saw dust on tree and shrub plantings but varied success with forbs.

### Establishment

Higher percent germination in the covered treatment was mirrored in establishment rates for every species except *L. arbustus* (*Fig 3*). Shortly after germination most *L. arbustus* plants exhibited chlorosis, necrosis, and death. Establishment success with the covered treatment was statistically similar for the other three species averaging 10.1% (Table 1), which surpassed the goal of 7% establishment. Establishment for the uncovered seed was significantly lower than the covered seed and abysmally low (0-1.5%). This low percent establishment would require about six times more seed than the covered treatment and, because seed is limited for these species, this would translate to a six fold increase in the area that could be planted with the same amount of seed as uncovered. Germination and establishment data demonstrate the advantage of using N-Sulate fabric and saw dust to improve stand establishment when cultivating these lupine species. However, laying saw dust and fabric is time consuming and materials are expensive.

**Seed Production**

Only *L. argenteus* and *L. sericeus* produced seed by the second and third growing seasons. Yield estimates of 39.2 kg ha$^{-1}$ for *L. argenteus* (35.1 lbs ac$^{-1}$) and 28.2 kg ha$^{-1}$ (24.2 1 lbs ac$^{-1}$) for *L. sericeus* were obtained at the highest plant densities (*Table 1*). We anticipated seed yields would be higher for covered treatments due to higher plant densities. Uncovered treatments did not obtain desired densities with poor germination leading to subsequent poor establishment. Consequently, yield comparisons between treatments were not always comparable i.e. either on very dense or very lean stands.

## CONCLUSIONS

Most of the lupine species in the Great basin are perennials and will produce seed no earlier than the second growing season, and sometimes even later; of the four species included in study only two produced seed in either the second and third growing seasons. Despite essentiality for success, it is difficult to justify the expense of N-Sulate, at approximately $4077 dollars ha$^{-1}$ ($1650 ac$^{-1}$). If properly cared for, the fabric can be re-used at least once which lowers the cost to \$2038 dollars $ha^{-1}$ (\$825 $ac^{-1}$) over two uses, but that does not reduce the initial investment.

The current market value of wildland collected lupine seed purchased by the state of Utah for restoration is about \$135 $kg^{-1}$ (\$60 $lb^{-1}$). One of the goals of this study was to see if we could reduce that cost through cultivation. Yields obtained of 39.2 and 28 kg $ha^{-1}$, at current market value a hectare would gross \$5,189 ha on *L. argenteus* and \$3,706 $ha^{-1}$ on *L. sericeus*(\$2,100 and \$1,500 $ac^{-1}$, respectively). Thus the low per hectare yields of these species make cultivation difficult to justify. Even with good stand establishment, low seed production makes it less profitable than more proven crops. For example, sainfoin, a non-native legume that ecologically fills a similar niche, yields over 1,100 kg $ha^{-1}$ (1,000 lbs $ac^{-1}$) at the current market price of \$6.35 $kg^{-1}$ (\$2.88 $lb^{-1}$) would generate \$7,124 dollars $ha^{-1}$ (\$2,880 an acre). This is an additional \$2,000 $ha^{-1}$ (\$780 $ac^{-1}$) above the value of the highest yielding lupine, and with seed costs less than one tenth that of lupine seed for restoration. The lower cost/higher yield translates to a higher demand from those doing restoration because more area can be treated for the same cost.

The covered treatment is expensive but was beneficial for lupine and has been shown to be beneficial for several other native species that we are currently cultivating in the GBNPSIP project (Jensen, Unpublished data USFS 2011). N-Sulate fabric was beneficial to germination and establishment with 16 of 22 Great Basin native forb species recently evaluated by the Forest Service (Jensen, Unpublished data USFS 2011). For perennial species that are difficult to establish, N-Sulate fabric could be the key to good stand establishment. However establishment yields would need to be substantially better than observed with lupine.

An alternative to cultivating these species would be to locate dense, naturally occurring stands across the Great Basin where seed can be obtained without the costs of cultivation. It would be necessary to manage these stands to maintain a thriving lupine community while at the same time obtaining optimal seed production. Using wildland stands managed for optimal seed production could potentially meet restoration needs and lower the cost of lupine seed.

When cultivating these Great Basin lupine species N-Sulate fabric will improve germination and establishment. However, low yields from cultivation will not likely reduce the current market value of the seed. A better understanding of cultural practices such as fertilization and better timing of irrigation could improve yields. More research needs to be done to improve in this area for commercial seed production to be a viable option.

| Site Name | Species | Zone | E | N | Elevation, m |
|---|---|---|---|---|---|
| Tintic | *L. prunophilus* | 12S | 406466 | 4424249 | 1950 |
| Buckskin Flat | *L. sericeus* | 12S | 441948 | 4392939 | 1920 |
| Soldier Canyon | *L. argenteus* | 11T | 638637 | 4517999 | 1768 |
| Bear Creek | *L. arbustus* | 11T | 628154 | 4632914 | 2469 |
| Fountain Green | | 12S | 446980 | 4384666 | 1749 |
| Ephraim | | 12S | 450184 | 4357992 | 1686 |

*Fig 1*. Germplasm collection and research plot locations in Universal Transverse Mercator (UTM).

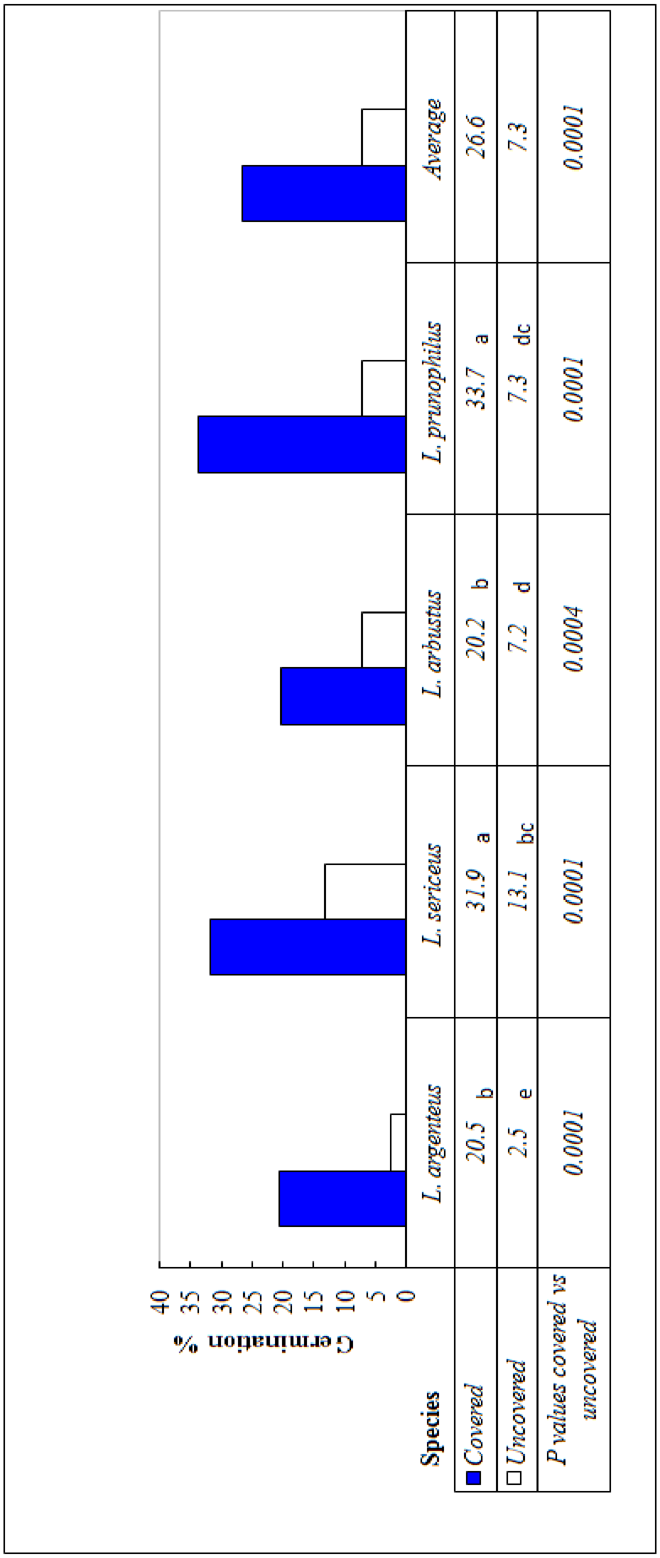


*Fig 2.* Average percent germination of four lupine species either covered or uncovered the average of two locations (Fountain Green and Ephraim). Values followed by the same letter are not significantly different at P<0.05.

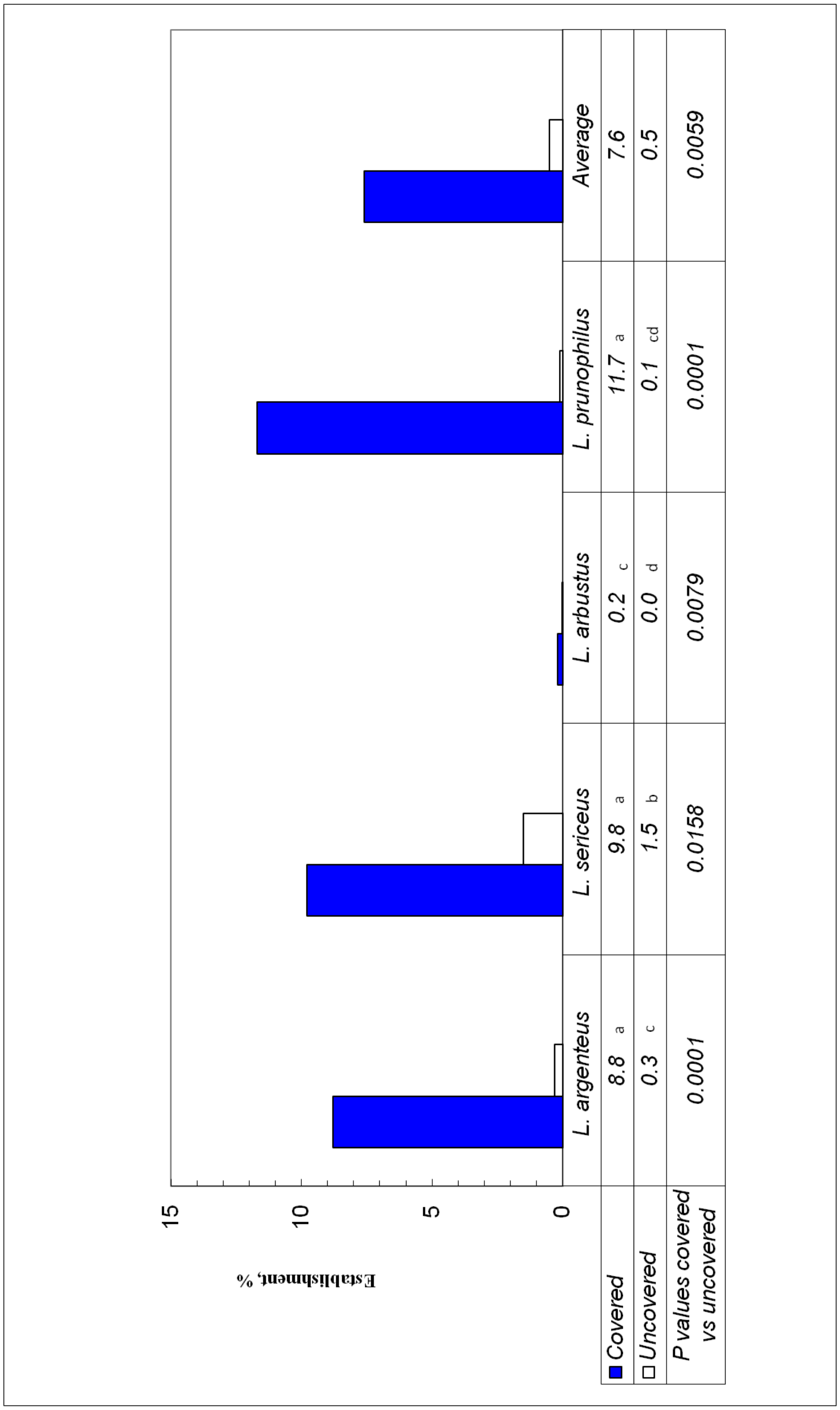


*Fig 3*. Average percent establishment of four lupine species either covered or uncovered the average of two locations (Fountain Green and Ephraim). Values followed by the same letter are not significantly different at P<0.05.

| | | No. Plants $m^{-2}$ | Yield $kgha^{-1}$ |
|---|---|---|---|
| Covered | *L. argenteus* | 19.01 | 39.2 |
| | *L. sericeus* | 22.4 | 28.3 |
| | *L. arbustus* | 0 | 0 |
| | *L.prunophilus* | 0 | 0 |
| Uncovered | *L. argenteus* | 0.3 | 3.7 |
| | *L. sericeus* | 2.2 | 7.2 |
| | *L. arbustus* | 0 | 0 |
| | *L.prunophilus* | 0 | 0 |

*Table 1.* Yields $ha^{-1}$ at specific plant density.

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# CHAPTER 3

## LITERATURE REVIEW: Overcoming Difficulties with Lupine Cultivation and Seed Increase

## INTRODUCTION

The Great Basin is truly one of the west's greatest treasures; this vast expanse of territory has provided an opportunity for multiple land uses. Starting in the mid 1800's until present, the Great Basin has provided the inhabitants with jobs mining gold, silver and other precious metals. The open range has provided opportunity for ranching, grazing thousands of sheep, cattle, horses and many species of native wildlife. By 1890 a census done on Utah's livestock recorded 3.8 million sheep and half a million cattle in the state, most of these animals grazed in the Great Basin during at least some parts of the year (Harrison et. al. 2003). These hefty demands placed on the Great Basin by the most recent inhabitants have not come without consequence. Heavily used and abused areas caused loss of diversity in native plant species and showed population decline. The majority of range degradation occurred below 7000 feet elevation. Native ecosystems in the lower elevation Great Basin are not adapted to such abbreviated fire intervals and are quickly disappearing from the landscape. Functioning native ecosystems in approximately 20 million lower elevation hectares have been lost to cheatgrass. Only 53.8 million hectares of its original potential, 73 million hectares are currently intact (Connelly et al. 2004).

These degraded ecosystems allowed- exotic weed species to be introduced, some of which have proven to be invasive changing historical habitat drastically. Cheat grass (*Bromus tectorum L.*) is among the worst of invasive weeds that have been introduced into the Great Basin, cheatgrass was noticed as early as 1916 in the west and is currently most prolific in the regions of the Great and Colombian Basins (Morrow and Stahlman 1984). This weedy annual grass not only invades weakened ecosystems but changes fire regimens in the areas it persists causing a shift in the fire frequency from a fire every 30 to 100 years to a possible fire every three years (Whisenant 1990; Peters and Bunting 1994). These more frequent fires make it impossible to sustain native habitat. However if there existed a seed source of native species to re-seed the landscape before exotics had a chance to invade there is a greater possibility of restoring degraded rangeland. Although there are some native species available in the seed market many native seed sources still not available in the necessary quantities needed for landscape scale restoration. This is one of the reasons that the Great Basin Native Plant Selection and Increase Project (GBNPSIP) was initiated (USDA Forest Service).

The GBNPSIP project started with a list of plants, mostly native forbs, which would be useful in large scale restoration projects. Goals of the project included harvesting seed from a number of sites for each desired species in the Great Basin and then establishing common gardens to determine which sources would work best to accomplish the goal of bringing the plant into large scale cultivation. The best sources for each species would then be selected from the common garden and agronomic production would begin to increase seed availability for these species thus lowering seed cost. More, less expensive seed would allow for large-scale restoration projects that are needed in the degraded and disturbed areas. These restoration projects will also hinder the spread of exotic species in the Great Basin and provide better range condition for wildlife and domesticated animals. Some plant species in the project have been more successful than others because they have lent themselves well to agronomic production displaying certain characteristics similar to domesticated plants, examples of which are determinate seed set and seed harvestability that allows the use of harvesting equipment that is readily available. Other species have preformed well in common gardens but modern agriculture equipment and techniques are not currently available that would facilitate harvesting for such species. Lupines (*Lupinus* spp.) are a wide spread range plant that persist across the Great Basin. They can be deleterious to cattle and are one example of a genus of plants that have had varied success in common gardens across Utah and Nevada.

Lupine was chosen for a number of reasons the upright habit of the plant along with the ability to produce prolific seed made it a real candidate. Also lupine was chosen because the role it plays in the historic plant communities of the Great Basin its wide distribution of lupine makes it a critical group of plants.

**SEED**

Some annual species of lupine are commonly used in Australia for grazing and also as a green manure crop, thus much of the literature published on lupine originates there. Quinlavian (1970) found that yellow lupine, (*L.luteus*(L.)) blue lupine (*L. angustifolius*(L.)) and sandplain lupine, (*L. varius*(L.)) were hard-seeded to some degree. Hard-seededness is of incredible value in the wild. It helps plants survive periods of disease, drought, fire, and flood but in agriculture these characteristics are counterproductive. A survival mechanism like hard-seededness would be essential to the persistence of a plant species in pastures of Australia's hot dry climate (Quinlivan 1970). In the case of European and North American pastures, perennial forb species are more common and hard-seededness would be less necessary. However, hard-seeded lupine species do persist in these types of environments (Quinlivan 1970). In nature there are a number of factors that contribute to the softening of hard seeds, some of which are; soil bacteria, insects, humidity change, and sharp daily temperature change.

Quinlivan (1970) found that hard-seededness was related to the seed moisture content, which was correlated to the relative humidity that it was exposed during the drying period. Sandplain lupine had the hardest seed and also persisted in the driest area. When seed of this species was allowed to dry to a moisture content below 8.5% it was extremely hard (over 90% of the seed remained unimbibed when exposed to water over a 12 week period). On the other hand, when sandplain lupine was allowed to dry in a more humid area of 13%, hard-seededness was not present (after 7 weeks exposed to water all seed had imbibed). Seed collected and dried in a humid area and then sown in a field would require as little as 10% the quantity of seed to establish the same number of plants as untreated seeds dried in a less humid area (Quinlivan 1970).

Another method of overcoming hard-seededness would be to plant early so the seed can go through a natural temperature cycle. Studies have revealed that temperature fluctuation of the soil surface in one site in Australia reaches an average high of 60º C (140º F) in the day and an average low of 15.5º C (60º F) at night over a five month period. Quinlivan (1970) found that fluctuating the temperatures, mimicking the natural environment temperature swings for five months, produced 35% germination verses holding the temperature constant at either 60º C or 15.5º C for five months producing only approximately 5% germination. Exactly why this occurred was not clear.

Scarification is another method that can break down a hard seed coat allowing the seed to imbibe and germinate. There are several methods of seed scarification which can be classified into three basic methods, chemical, thermal, and mechanical.

Burns (1958) evaluated the effect of chemical scarification on hard seeded strains of blue lupine using highly concentrated sulfuric acid. After two weeks 50-75% of seed were determined to be permeable, non-permiable seed was considered hard. Hard seed were placed in a sulfuric acid bath for three hours after the acid bath on it was determined that the water entered through either the hilar fissure or pits eroded through the testa. This same area is the permeable location in a soft seed (Burns 1958

Thermal scarification methods were being employed for lupine as early as 1941 (Hootman). Hootman (1941) collected seed from blue lupine in July then planted the seeds that fall but they failed to imbibe for germination. First he attempted to manually score the seed but found that tedious. Next he soaked seed in either cold or warm water, both methods failed to produce germination, furthermore, the seed soaked in warm water rotted when planted. Finally, he poured hot (almost boiling) water over the seeds and found that within hours some of the seeds had imbibed and later germinated after planting. He hypothesized from these experiments that the high temperature helped germination because these plants often come in after fire and were accustom too extreme environments (Hootman 1941). Cushwa et al. 1968 found that moistening and heating increased germination of (*Cassia nictitans* L.)*"* Segelquist (1970) conducted a similar study on (*Lespedeza cuneata* Dum. Cours). They found that exposing seed of this species to 100º C of moist heat increased germination steadily from 1-15 min. with a sharp drop in germination at 16 min and no germination when exposed to 100º C for 32 min. Lower temperatures (40-80º C) increased seed germination but required more time (Segelquist 1970).

Indian ricegrass (*Orzopsis humenoides* (Roem. and Schult)) is an important grass species in lower precipitation zones of the Great Basin and has mechanical and physiological methods to remain dormant through long droughts. Jones and Nielson (1992) evaluated mechanical scarification and pre-chill period requirements for their effect on germination of this species. When comparing scarified pre-chilled seed with the unscarified non pre-chilled control they found that older seed germinated best. Physical damage to the seed embryo occurred to approximately 7% of the seed but the remaining intact seed improved germination for 12 of the 13 lots by 9.5 to 29.7%. Pre-chilling also improved the germination for 10 of the 13 lots (Jones and Nielson, 1992).

## CULTIVATION

Despite difficulties, cultivation of lupine species is not new. The Egyptians cultivated lupine as far back as 2000 B.C. (Raza et.al. 2001). Lupine's symbiotic relationship with *Rhizobium sp.* (soil bacteria) results in the fixation of nitrogen allowing it to grow on nutrient poor, sandy soils and leave excess nitrogen in the soil for the following crop or the surrounding community. Lupine also has the ability to improve soil texture (Gonzalez et.al. 2004 and Raza et.al. 2001). The plant communities where lupines persist in the Great Basin tend to have low nutrient soils (Gonzalez et.al. 2004). This is one of the reasons that lupine are so important to restoring historical ecological communities. They directly benefit the lupine itself but but also indirectly provide nutrients for other plants in the community (Gonzalez et.al. 2004).

Although lupine has the ability to grow on nutrient deficient soils it is not the perfect plant for all poor soils, pH and iron respectively affect root and shoot growth. A number soil factors including but not limited to; soil bicarbonate concentration, lack of oxygen, extreme temperature, high salinity, and competition between soil microorganisms can effect nodulation (Raza et.al. 2001). Some micronutrients including cobalt are also necessary in very low concentrations for nodulation to occur in *L. angustifolius* (Riley and Dilworth, 1985).

Tang and Robinson (1993) compared the effects of high soil Ca and the effect of high pH on the growth of *L. angustifolius.* Both nitrogen-fixing and non-nitrogen-fixing fertilized plants were examined. Ca was applied as $CaCO_3$ and much more potent $CaSO_4$. Both applications of Ca stunted shoot and root growth. $CaSO_4$ is much more available source of Ca and Ca was found at higher ratios of in plant tissue the stunting effects were not more pronounced (Tang and Robinson, 1993). A second study examined the effects of higher pH but not higher Ca and compared *L. angustifolius* with an alkaline tolerant species *L. pilosus* when ionic solution increased as pH increased *L. angustifolius* preformed more poorly than *L. pilosus* (Tang and Robinson, 1993). This information coupled with the fact that the amount of Ca present had no effect on growth suggests that poor growth of *L. angustifolius* in alkaline soils might be more of a result of pH levels and not directly caused by a Ca uptake. There are at least two lines of evidence that *L. angustifolius* is sensitive to soil pH. First, this species of lupine has a tendency to have an iron deficiency in alkaline soils; and second, the R*hizobium sp.* that infects the roots causing nodulation and ultimately N fixation is present at much lower levels in alkaline soils (Tang and Robinson, 1995). Not all lupine species have the same tolerances to alkaline soils this can be respectively related to the iron need for each species. Species more tolerant to lack of availability of iron in high pH's are able to grow on more alkaline soils. Unavailable iron depresses nodule formation and nitrogen uptake for all species evaluated (Tang et. al. 1995). Iron is required for initiation of nodulation for *L. angustifolius*. Plants inoculated with R*hizobium sp.* that were iron deficient hardly formed any nodules while plants supplied with iron under the same conditions formed many nodules. Iron deficient plants that were supplied with N had much less of an effect on growth than did iron deficient plants that were inoculated to provide nitrogen suggesting that iron is critical in nodule formation and not as critical in other plant processes (Tang et. al. 1990).

Nitrogen deficiency because of lack of nodulation can effect plant growth. For example nitrogen deficiency *L. angustifolius* had little effect in leaf initiation but slowed leaf emergence along with that flowering was not affected but time to produce a flower was significantly increased (Ma et. al. 1997). Furthermore, forced nitrogen deficiency in nodulated plants and naturally occurring nitrogen deficiency in plants lacking nodules for *L. angustifolius* decreased stem growth, flower production, and pod and seed set (Ma et. al. 1998).

Other factors can effect lupine cultivation even when plants are being grown on the right soil type. As mentioned above, in Australia lupine is an important cultivated grain legume crop and because of its importance there have been a number of studies done on different aspects of lupine cultivation. Blue lupine (*L. angustifolius*) is one of the lupine species of interest for the Australians (Landers, 1995). When cultivating this species it is important to have the longest flower duration possible to produce the highest quantity of seed however if flowering occurs to early then repeated frosts can damage flower reproductive parts and therefore impact pod set and seed production (Landers, 1995). Time for flowering in this species is mainly controlled by a requirement for vernalization and response to long days (long days have less of an effect than does vernalization) (Landers, 1995). The wild type non-cultivated plants in this species have evolved to flower later in the year to over come the frost issue but that minimizes grain production (Landers, 1995). Most cultivated lupines have the K*u* gene, which removes the requirement for vernalization (Landers, 1995). Vernalization response has been studied more in white lupine (*L. albus*) and vernalization responses occur between 1 and 14 degrees C and possibly up to 17 degrees C but more rapid responses are found between 1 and 11 degrees C (Landers, 1995). Critical vernalization period differed between genotypes from two to four weeks. When critical vernalization time was not met abnormal inflorescence and flowering parts were a common problem for plants not containing K*u* gene (Landers, 1995).

When production plots are established then seed predators can pose a problem (Maron and Simms, 2001). In wildland settings research has shown that 50-80% of possible seed produced is lost due to predation pre or post dispersal (Maron and Simms, 2001). This study examined how rodent granivore predation on bush lupine (*L. arboreus*) seeds related to recruitment. Bush lupine is an important ground stabilizing shrub found on the costal dunes of California. Like other lupine species bush lupine is a nitrogen fixer that provides nutrients to the surrounding community in an otherwise low nutrient sandy soil. This study showed that 75% of dispersed bush lupine seed was consumed by rodents inside the study control plots but how and if this seed predation is really effecting establishment of bush lupine is the question (Maron and Simms, 2001). Data from the first year showed a significant difference in control and rodent excluded plots. From a pool of 476 seeds in year one of a three year experiment 43 seedlings emerged in the rodent excluded plots as compared to 7 seedlings that emerged from the control plots (Maron and Simms, 2001). Years two had 46 seeds emerge from the exclusion and 17 in the control plot and year three was the worst for the control plots with 2 seeds emerging and 17 emerged in rodent excluded plots (Maron and Simms, 2001). This study showed that rodents could in some situations severely limit lupine recruitment in wildland situations. Rodents and birds could also be a major factor regarding seed loss with the cultivation of lupine especially if animals are consuming the seed while it is still green on the plant.

**RESTORATION**

After successfully cultivating and harvesting seed putting it back on the ground in a wildland setting raises concern for some land managers this is because of induced defenses. When animals are attacked by a pathogen their immune system quickly attacks the disease (Johnson et. al. 1989). Although plants do not have immune systems they do have defense systems to discourage herbivore attacks. Plants under attack often have evolved induced defenses which allow them to become less palatable and some times toxic. Lupine is known to produce alkaloids as an induced response to herbivore attack. The health of the plant effects it's ability to create these induced responses. Johnson et. al. conducted this study on (*L. succulentus* (Douglas ex K. Koch)) an annual lupine known to produce alkaloids, he evaluated both healthy plants with the ability to fix nitrogen and less healthy nitrogen deficient plants that lacked the ability to fix nitrogen (Johnson et. al. 1989). Leaves were then injured and lupine alkaloid concentration was monitored as it increased in plant tissue. Induction increased alkaloid production by 55% over control plants in damaged areas for both N fixing and N deficient plants but in N fixing plants a 33% rise in undamaged tissue was also observed (Johnson et. al. 1989). N fixing plants increased alkaloid levels with in 4 hours where as N deficient plants were much slower taking from 4 to 24 hours to induce alkaloid production (Johnson et. al. 1989). This study showed a direct relationship between N and induced plant defense many that are necessary to more fully understand induced defenses (Johnson et. al. 1989). Alkaloids in lupine plants are one of the biggest issues that effect grazers and their herds in the Great Basin. A fuller understanding of plant-induced defenses could offer possible solutions to this obstacle and provide less toxic or non-toxic plants to overcome this issue.

**CONCLUSION**

In conclusion lupine is an important species world-wide. Cultivation of lupine is not new but there are problems which have not been over-come associated with cultivation. This multifaceted study will address several of those problems and provide useful information in regards to cultivation of these Great Basin species. The information provided will then be used to cultivate and increase seed availability and initiate large restoration projects incorporating native lupine seed.

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