HRI's Mission:

To direct, fund, promote and communicate horticultural research, which increases the quality and value of ornamental plants, improves the productivity and profitability of the nursery and landscape industry, and protects and enhances the environment.

Effect of Seed Scarification and Gibberellic Acid Treatment on Seedling Emergence of Sky-Blue Lupine (*Lupinus diffusus*)[1]

Bijan Dehgan[2], Jeffrey G. Norcini[3], Steven M. Kabat[4], and Hector E. Pérez[5]
University of Florida, Institute of Food and Agricultural Sciences
Department of Environmental Horticulture, P.O. Box 110670, Gainesville, FL 32611-0670

Abstract

The efficacy of various scarification treatments and gibberellic acid (GA_3) treatment on seedling emergence of *Lupinus diffusus* Nutt. (sky-blue lupine) was evaluated. Seed scarified in concentrated sulfuric acid for 90 min followed by immersion in water for 24 hr resulted in the best emergence of viable seed (≈ 41%). Mechanical scarification with sandpaper for up to 30 min did not improve seedling emergence. Immersing seed in 90C (194F) water which was then allowed to cool for 24 hr apparently killed or severely injured the embryos as no seedlings emerged from seeds treated with hot water. There was only 5% seedling emergence for nonscarified seed that were soaked in water at room temperature for 24 hr. Emergence was not improved by soaking scarified seed in 1000 mg/liter (ppm) GA_3 for 24 hr prior to sowing the seeds.

Index words: seed germination, native wildflower, gibberellic acid, sulfuric acid, propagation.

Significance to the Nursery Industry

Sky-blue lupine is a short-lived perennial that produces showy racemes of light, true blue flowers in late winter and early spring. It is thought to have potential for roadside plantings, use in restoring of disturbed or burned sites, and other low maintenance areas because it occurs naturally on poor, sandy soils. Since lupines transplant poorly, it will be necessary to produce seeds of this species and then directly seed into desirable locations. Hence, it is essential that seed dormancy mechanisms of sky-blue lupine be understood and overcome. This study determined that scarifying the seed in concentrated sulfuric acid for 90 min followed by soaking in water for 24 hr resulted in the best seedling emergence (≈ 41% of viable seed). Since the percentage of viable seed appeared to be high on the basis of TZ testing, it was thought that in addition to hard seed coat dormancy, sky-blue lupine seed may also possesses physiological dormancy. Studies are underway to determine methods of improving the percentage emergence of viable seeds.

Introduction

Lupinus diffusus Nutt. (sky-blue lupine) is an attractive, widespread but infrequent perennial that is native to sandhills, sand pine or oak scrub, coastal strands, and pine flatwoods from North Carolina southward through Florida and Mississippi (14, 16). It has silky-pubescent, unifoliolate (appearing simple), oval to elliptic basal leaflets and numerous terminal racemes of light blue flowers. Because of its attractive floral and foliar features, perennial habit, and adaptation to dry sites, it is considered to be a suitable candidate for roadside plantings.

Similar to many other leguminous taxa, seeds of *Lupinus* L. spp. have proven to have low, erratic germination that is primarily attributed to seed coat water impermeability. There has been much work dealing with methods to overcome physical dormancy of *Lupinus* seeds, including research on native species. Seed of *L. texensis* Hook. (Texas bluebonnet) scarified with sulfuric acid for 30 to 60 min improved seedling emergence as did cutting, filing, and soaking in water at 85C (185F) water; freezing and thawing had no effect (2). Acid scarification of *L. havardii* S. Wats. (Big Bend bluebonnet) seed for 120 min resulted in 100% germination (7). Nicking the seed with a razor blade had the same effect but soaking in water for 24 hr had no effect. *Lupinus perennis* Wats. (perennial lupine) seed also had 100% germination when mechanically scarified by nicking the seed with a razor blade, with acid scarification for 45 min being nearly as effective (9). However, the best-known scarification methods for a particular *Lupinus* species do not always result in high rates of germination. Davis et al. (2) noted that one seed collection of *L. texensis* had only 80% germination whereas other lots of the same species exhibited 87% to 95% germination. According to propagation protocols for *L. versicolor* Lindl. (many colored lupine) (17) and *L. sericeus* Pursh. (silky lupine) (5) observed germination was 50% and 51 to 82%, respectively.

Physiological dormancy mechanisms of *Lupinus* seed have also been investigated. Kaye and Kuykendall (6) found a significant difference in maximum germination of *L. sulphureus* ssp. *kincaidii* (Smith) Hitchc. (Kincaid's lupine), with 95% in one population and 55% in another, when scarification and cold stratification [4C (39.2F)] for 4 and 8 weeks were combined. Scarification plus cold stratification [3C (37F) for 30 days] is also recommended for *L. sericeus* Pursh. (5). In contrast, Nichols (12) reported that cold stratification [5C (41F)] for 71 days of *L. perennis* did not improve germination.

Therefore, the objective of this research was to determine the efficacy of various scarification treatments on seedling

[1]Received for publication October 17, 2002; in revised form January 30, 2003. Florida Agricultural Expt. Station Journal Series No. R-09110. We thank Fe Almira for technical assistance and Drs. Dennis McConnell and Rick Schoellhorn for careful review of the manuscript.

[2]Professor.

[3]Associate Professor, North Florida Research & Education Center, 155 Research Road, Quincy, FL 32351.

[4]Graduate Student.

[5]Graduate Student. Current address: Department of Tropical Plant & Soil Science Ecology, Evolution and Conservation, University of Hawaii at Manoa, 3190 Maile Way, Room 102, Honolulu, HI 96822.

emergence of *L. diffusus*. The effect of gibberellic acid (GA) on emergence was also evaluated to determine if *L. diffusus* seeds had some type of physiological dormancy in addition to physical dormancy. Some physiological dormancy mechanisms can be overcome by GA (15). As noted above, scarification can improve seed germination of various *Lupinus* spp. (5, 6).

Materials and Methods

Seed source. Seeds of *L. diffusus* were harvested in May 2001 from a native population growing in dry, deep yellow sand in Osceola County, FL (USDA Cold Hardiness Zone 9b; AHS Heat Zone 10). Seeds were cleaned and then stored in a sealed container in the dark at 5C (41F).

Seed viability. Seed viability was determined by tetrazolium (TZ) testing using AOSA guidelines of Grabe (4). Four 50-seed replications were placed into each of four tubes containing freshly prepared 1% TZ and incubated at 25C in the dark. Imbibed seeds were checked for viability 24 hr later. Hard, nonimbibed seed were nicked with a scalpel, reincubated in fresh 1% TZ, placed in the dark, and examined for viability 24 hr later.

Experiment 1. On April 3, 2002, seeds were scarified using acid, hot water, or mechanical abrasion. Seeds were acid scarified using 18M sulfuric acid for 20, 40, or 60 min. To assure uniform coverage, the acid was stirred occasionally with a glass rod. After acid scarification, the acid was decanted and seeds were rinsed under running tap water for 2 hr. Acid-scarified seeds were then divided into two groups and immersed for 24 hr in a 1000 mg/liter (ppm) solution of gibberellic acid (GA_3) or tap water. Hot water scarification was accomplished by immersing seed in 90C (194F) tap water and then allowed to cool for 24 hr. Half of the hot-water treated seeds were then immersed in a 1000 mg/liter (ppm) solution of GA_3 for an additional 24 hr. Mechanical scarification was done for 10, 20, or 30 min using 60 grit, D-weight, aluminum oxide sandpaper (Abrasive Leaders & Innovators, Fairborn, OH). Lining a tube with the sandpaper and moving back and forth in a horizontal motion until the scarified surface of the seeds were clearly visible accomplished this. The two control treatments were seed soaked in tap water for 24 hr with or without 1000 mg/liter (ppm) GA_3.

Seed of all treatments were sown 1 cm (0.4 inches) deep on April 4 (April 5 for the hot water/GA_3 treatment) in plug trays (120 cells per tray) containing MetroMix 300 (The Scotts Company, Marysville, OH). Seedling emergence (defined as emergence of the hypocotyl hook) was recorded every 3 days after sowing for 33 days. There were four 100-seed replications for each of the 16 treatments, with one plug tray per replication. Trays were arranged in a completely randomized design (CRD) on a mist bench (mist interval of 5 sec every 30 min from 8 am to 6 pm). The greenhouse temperature ranged from 26.7 to 29.4C (80 to 85F) during the day and 18.3 to 21.1C (65 to 70F) at night. The experiment was conducted under natural photoperiod and irradiance.

Experiment 2. In experiment 1, 60 min acid scarification was clearly the best treatment; however, emergence was still between 30% and 40% for the four replications. In this experiment, acid scarification (May 30) was conducted using the same methods as described above (including the GA_3 and water only treatments) except that the duration of acid scarification was increased to 90 or 120 min. For each individual treatment in this 2 × 2 factorial arrangement of treatments, CRD experiment there were four 100-seed replications, except five 100-seed replications for the 90 min acid treatment. Seeds were sown on May 31 as before. Seedling emergence was recorded every 3 days until emergence peaked at 21 days, at which time the experiment was terminated. Greenhouse conditions and misting frequencies were the same as in Experiment 1.

Data analysis. Observed emergence values were converted to percent emergence of viable seed (based on TZ viability test) prior to analysis of variance. Percentage data were transformed (arcsine of square root) prior to analysis but actual means are presented. Means were separated using Tukey's Studentized range test at $P < 0.05$.

Table 1. Effect of scarification and gibberellic acid treatments on seedling emergence of viable *Lupinus diffusus* seed 33 days after scarification. Each value represents the mean of four 100-seed replications.

Scarification treatment	Seedling emergence (%)[z,y]
None + soak in water 24 hr	5.4b
Acid for 20 min + soak in water 24 hr	11.1bcd
Acid for 40 min + soak in water 24 hr	19.4d
Acid for 60 min + soak in water 24 hr	37.4e
Acid for 20 min + soak in 1000 mg/liter GA_3 24 hr	8.9bc
Acid for 40 min + soak in 1000 mg/liter GA_3 24 hr	18.9cd
Acid for 60 min + soak in 1000 mg/liter GA_3 24 hr	37.7e
None + soak in 1000 mg/liter GA_3 24 hr	5.2b
Abrasion for 10 min + soak in water 24 hr	4.0b
Abrasion for 20 min + soak in water 24 hr	7.1b
Abrasion for 30 min + soak in water 24 hr	5.4b
Abrasion for 10 min + soak in 1000 mg/liter GA_3 24 hr	6.6b
Abrasion for 20 min + soak in 1000 mg/liter GA_3 24 hr	4.0b
Abrasion for 30 min + soak in 1000 mg/liter GA_3 24 hr	9.1bcd
Hot water + soak in water for 24 hr	0.0a
Hot water + soak in 1000 mg/liter GA_3 24 hr	0.0a

[z]Percentage emergence of viable seeds; seed viability determined by tetrazolium testing.

[y]Mean separation by Tukey's Studentized range test at $P < 0.05$.

Results and Discussion

Hard seeds comprised 91.0 ± 4.8% of the lot, about the same percentage as observed 10 months earlier (93.5 ± 4.4%). Although this percentage of hard seeds was similar to that of one collection of *L. texensis* (92% hard seeds) (2), the reported percentage of hard seeds for native lupines ranges from 20% to 80% (1, 2, 13). Percentage of viable seeds as determined by TZ testing was 87.5 ± 6.4%, with hard seeds 82.5 ± 7.6% viable, and non-hard seeds were 5.0 ± 1.2% viable. Although seed was nearly 1 year old, there was no significant loss of viability since August 2001 when 91.4 ± 3.4% of the seeds were viable (data not presented).

In Expt. 1, acid scarification was the only treatment that significantly increased seedling emergence (Table 1). Based on the follow-up Expt. 2, the optimal scarification treatment was 90 min followed by a 24-hr soak in water. However, while percentage seedling emergence for a 90-min exposure (40.9 ± 5.2%) was not significantly different than that of 60 min (Table 1), emergence rate was considerably higher: 92%

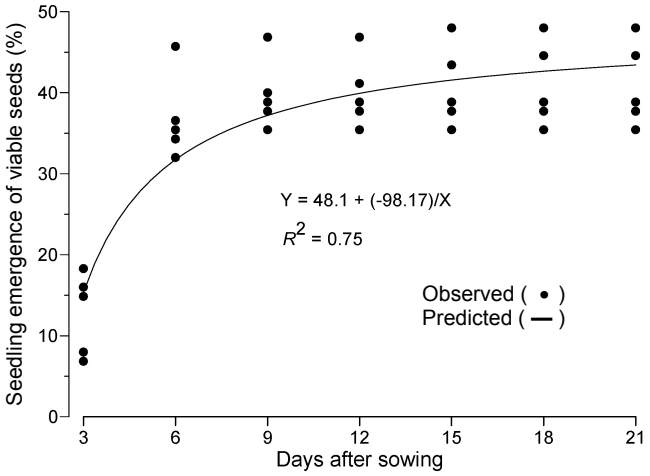

Fig. 1. Seedling emergence of viable *Lupinus diffusus* seeds after 90 min scarification in 18M sulfuric acid. Scarified seed were rinsed in tap water, then soaked in tap water for 24 hr prior to sowing. There were five 100-seed replications. Seed viability was determined by tetrazolium testing.

emerged 6 days after sowing as compared to 15 to 21 days for 60 min (Fig. 1). It was also estimated visually that there were fewer nonimbibed seed (24 hr after soaking in water) for the 90-min treatment compared to the 60-min treatment. Increasing the exposure time to 120 min did not improve seedling emergence (35.7 ± 12.4%). Compared to other native lupines, this is less time than the 120 min required for optimal germination of *L. havardii* (7) but more than that required for germination of *L. perennis* (30–45 min) (9) or seedling emergence of *L. texensis*. (30–60 min) (2). Seedling emergence of *L. diffusus* scarified in acid for 90 min peaked after 15 to 21 days (Fig. 1). In contrast, seedling emergence of *L. texensis* seeds peaked 1 month after sowing scarified seed (2). Germination of acid scarified *L. perennis* seeds peaked after 3 days (9) and after 1 week for *L. havardii* (7).

Mechanical scarification via abrasion for up to 30 min did not improve seedling emergence of *L. diffusus*. A longer period of abrasion would probably have made the seed coat permeable to water since nicking the seeds permitted imbibition. Manual mechanical scarification of individual seed of other native lupines significantly improved seedling emergence or germination compared to nontreated seeds (2, 7, 9). Hot water was ineffective and apparently killed or severely injured *L. diffusus* embryos as no seedlings emerged; seed soaked just in water resulted in about 5% seedling emergence. Seed of *L. texensis* placed in 85C (185F) water that was allowed to cool for 24 hr increased seedling emergence but was not as effective as acid or mechanical scarification (2). Hot water treatment slightly improved germination of *L. havardii* (7) and *L. arboreus* (8) but did not promote germination of *L. perennis* (9). However, hot water treatment is recommended for scarification of *L. sericeus* seed (5).

Gibberellic acid [1000 mg/liter (ppm)] had no effect on seedling emergence (Table 1), or reduced emergence. Emergence of seeds scarified in acid for 90 or 120 min and then immersed in GA_3 was 26.6. ± 3.0% and 10.2 ± 1.3%, respectively. No reports of GA enhancing germination of native lupines could be found in the literature, which is not surprising given that physical dormancy is the mechanism that usually restricts germination of these species (2, 7, 8, 9). However, seeds of *L. sulphureus* ssp. *kincaidii* (6) and *L. sericeus* (5) apparently possess some physiological dormancy based on the positive effects of stratification on germination. Seeds of *L. diffusus* might possess physiological dormancy as well given that only about 41% of viable seeds emerged for the best scarification treatment. Physiological dormancy is suspected based on observations of naturally occurring populations of *L. diffusus* in central Florida (J. Stout, personal communication). The lack of a positive response of scarified *L. diffusus* seeds to GA_3 is not contradictory to the notion that this seed may possess physiological dormancy as the GA_3 concentration may not have been optimal or another GA might substitute for cold stratification. For example, GA_7 was more effective than GA_3 in promoting germination of *Sanguinaria candensis* L. (bloodroot) (3).

There may be other reasons for the relatively low seedling emergence of viable *L. diffusus* seeds compared to other native lupines. It simply may have been that optimal scarification time was between 90 and 120 min. It is not likely that the greenhouse temperature played a significant role in reducing seedling emergence. Seedling emergence of *L. texensis* was 80% to 95% (depending on seed lot) under a greenhouse temperature regime of 27/20C (81/68F), which was similar to the temperature regime in our experiment (2). Also, Mackay et al. (9) observed high germination percentages of *L. perennis* at 21 to 29C (70 to 85F), with reduction in germination occurring at 32C (90F). One other possibility for the low percentage of seedling emergence was that *L. diffusus* might require smoke or a post-burn residue to stimulate germination. In Florida, *L. diffusus* occurs in fire-prone pinelands and could be a fire-adapted species. Seeds of *L. sericeus* and *L. caudatus* Kellogg (tailcup lupine) germinate after fire (10, 11). Studies are underway to determine if smoke affects germination of *L. diffusus*.

Literature Cited

1. Davidson, E.D. and M.G. Barbour. 1977. Germination, establishment, and demography of coastal bush lupine (*Lupinus arboreus*) at Bodega Head, California. Ecol. 58:592–600.

2. Davis, T. D., S. W. George, A. Upadhaya, and J. M. Parsons. 1991. Improvement of seedling emergence of *Lupinus texensis* following seed scarification treatments. J. Environ. Hort. 9:17–21.

3. Deno, N.C. 1996. Seed Germination Theory and Practice. 2nd ed., 1st suppl. N. C. Deno, State College, PA.

4. Grabe, D.L. 1970. Tetrazolium Testing Handbook for Agricultural Seeds: Contribution no. 29 to the Handbook on Seed Testing. Assoc. of Offic. Seed Analysts, Springfield, IL.

5. Hosokawa, J., D. Wick, and T. Luna. 2001. Propagation protocol for production of container *Lupinus sericeus* Pursh. plants (172 ml containers); Glacier Natl. Park, West Glacier, MT. *In*: Native Plants Network. http://www.nativeplantnetwork.org (accessed Aug. 28, 2002). Moscow, ID: Univ. of Idaho, College of Natural Resources, For. Res. Nursery.

6. Kaye, T.N. and K. Kuykendall. 2001. Effects of scarification and cold stratification on seed germination of *Lupinus sulphureus* ssp. *kincaidii*. Seed Sci. Technol. 29:663–668.

7. Mackay, W.A., T.D. Davis, and D. Sankhla. 1995. Influence of scarification and temperature treatments on seed germination of *Lupinus havardii*. Seed Sci. Technol. 23:815–821.

8. Mackay, W.A., T.D. Davis, and D. Sankhla. 2001. Influence of scarification and temperature on seed germination of *Lupinus arboreus*. Seed Sci. Technol. 29:543–548.

9. Mackay, W.A., T.D. Davis, D. Sankhla, and D.E. Riemenschneider. 1996. Factors influencing seed germination of *Lupinus perennis*. J. Environ. Hort. 14:167–169.

10. Matthews, R.F. 1993a. *Lupinus caudatus*. *In*: U.S. Dept. Agr., For. Serv., Rocky Mountain Res. Sta., Fire Sciences Lab. (July 2002). Fire Effects Information System, [Online]. http://www.fs.fed.us/database/feis/ [accessed Aug. 28, 2002].

11. Matthews, R.F. 1993b. *Lupinus sericeus*. *In*: U.S. Dept. of Agr. For. Serv., Rocky Mountain Res. Sta., Fire Sci. Lab. (July 2002). Fire Effects Info. System, [Online]. http://www.fs.fed.us/database/feis/ [accessed Aug. 28, 2002].

12. Nichols, G.E. 1934. The influence of exposure to winter temperatures upon seed germination in various native American plants. Ecology 15:364–373.

13. Nixon, E.S. 1964. Edaphic responses of *Lupinus texensis* and *Lupinus subcarnosus*. Ecol. 45:459–469.

14. Taylor, W.K. 1998. Florida Wildflowers in their Natural Communities. Univ. Press of Florida, Gainesville.

15. Taylorson, R.B. and S.B Hendricks. 1977. Dormancy in seeds. Annu. Rev. Plant Physiol. 28:331–354.

16. U.S. Dept. Agric., Nat. Res. Cons. Serv. 2001. The Plants Database, Version 3.1. http://plants.usda.gov/plants. Natl. Plant Data Center, Baton Rouge, LA 70874-4490 USA (accessed Aug. 12, 2001).

17. Young, B. 2001. Propagation protocol for production of container *Lupinus versicolor* Lindl. plants (Deepot 16); Golden Gate National Parks, San Francisco, CA. *In*: Native Plants Network. http://www.nativeplantnetwork.org (accessed Aug. 28, 2002). Moscow, ID: Univ. of Idaho, College of Natural Resources, For. Res. Nursery.