Presowing Seed Treatment and Temperature Effects on Germination of *Engelmannia pinnatifida* and *Indigofera miniata* var. *leptosepala*

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Abstract

Research was conducted to evaluate the effects of presowing seed treatments and temperature on germination of seeds of Engelmann daisy *(Engelmannia pinnatifida)* and western indigo *(Indigofera miniata* var. *leptosepala).* The seeds were imbibed in controlled environmental conditions for 14 days with night/day temperatures of 5/15, 10/20, 15/25, or 20/30°C and a 12-hour photoperiod corresponding with the high temperature. Seed treatments prior to imbibition included: mechanical scarification, immersion in hot water (80°C) for 3 min., acid scarification by immersion in concentrated sulfuric acid for 17 min., and an untreated control. Percent germination was affected by both temperature and presowing seed treatments. Maximum germination (43%) of Engelmann daisy was attained by untreated seeds at 20/30°C. Presowing seed treatments did not significantly increase germination of Engelmann daisy at any temperature. Western indigo seeds which were mechanically or acid scarified germinated over 90% in the 10/20, 15/25 and 20/30°C temperature regimes. Seeds immersed in hot water germinated from 59–68%, and untreated seeds germinated from 17–42% in the same environmental conditions.

Selection of adapted species for revegetation of deteriorated rangeland and surface mined lands is a very important consideration in a revegetation project. The revegetation specialist must often choose between introduced or native species of grasses, forbs, or woody plants to be planted in monocultures or mixtures. Presently, perennial warm-season grasses are used most frequently in revegetation of rangelands or surface mines in central Texas.

Planting mixtures of grasses and forbs in the western United States has been found to expedite the natural sequence of plant succession. Mixtures in contrast to a monoculture provide a greater variety for animals to graze, utilize the total soil profile more efficiently, and are generally better adapted to variable canopy, soil, terrain, and climatic conditions (Vallentine 1971).

Lack of available seed has been a deterrent to use of native mixtures in range seedings or reclamation projects in many areas (Eddlemann 1979, Sutton 1975). The USDA Soil Conservation Service in cooperation with the Texas Agricultural Experiment Station and the Texas Parks and Wildlife Department are continually seeking new plant varieties. Two of the native forb species being evaluated are Engelmann daisy *(Engelmannia pinnatifida)* and western indigo *(Indigofera miniata* var. *leptosepala)* (USDA Soil Conservation Service 1978).

Engelmann daisy, a perennial cool-season forb, is native to all of Texas except the Piney Woods (Gould 1975). This species produces high quality, palatable forage. Western indigo is a perennial warm-season legume that has the potential to improve soil fertility through nitrogen fixation. This species grows in the eastern two-thirds of Texas. Kneebone (1959) reported western indigo plants in Oklahoma were utilized by grazing animals and that nodule production was good. The species also rated fair in seeding habits, establishment potential, and first year's growth.

Both species have the potential to be used in seeding mixtures for revegetation of deteriorated rangelands or surface mines in central Texas. However, as with many forb species, we are lacking detailed information relative to the environmental requirements for seed germination and seedling growth and the potential for improving seed germination with presowing seed treatments. This information is vital if managers are to successfully establish these species in plant mixtures for revegetation projects.

Preliminary trials in our laboratory have indicated germination (radical longer than 5 mm) percentages of 49 and 37 after 14 days in an 18/24°C temperature regime for Engelmann daisy and western indigo seeds, respectively. Our hypothesis is that Engelmann daisy and western indigo seeds germinate well over a narrow temperature range and their seed germination may be improved with presowing seed treatments. The study objective was to evaluate the effect of temperature and presowing seed treatments on seed germination.

Materials and Methods

Seed of PMT-874 Engelmann daisy and PMT-1051 western indigo were obtained from the USDA Plant Materials Center, SCS Knox City, Texas. Germination trials were conducted with 3-year old seeds which were uniformly large-sized, fully developed, and undamaged. Western indigo seeds were sorted by air; Engelmann daisy seeds (achenes) were removed from their bracts.

Presowing seed treatments included mechanical scarification by cutting the seed coat with a razor blade (Latting 1961, Martin et al. 1975), immersion of seeds in hot water (80°C) for 3 minutes (Halls et al. 1970, Latting 1961), and acid scarification by immersion of seeds in concentrated sulfuric acid (H_2SO_4) for 17 minutes (Latting 1961).

Samples of 50 seeds previously dusted with a fungicide (Captan)[1], were imbibed on 2 pieces of Whatman[1] No. 1 filter paper in a 100-mm diameter petri dish. The paper was supported by a 5-mm thick piece of polyurethane foam with a cotton wick in the center

[1]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the Texas Agricultural Experiment Station and does not imply approval to the exclusion of other products that may also be suitable.

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This report is approved for publication by the Director, Texas Agricultural Experiment Station, as Tech. Article No.

The authors greatfully acknowledge the Center for Energy and Mineral Resources at Texas A&M University for funding the project, the USDA-SCS, Plant Materials Center, Knox City, Texas, for providing seeds used in the study, and Mr. Don Shelton for assistance in the statistical analysis.

Manuscript received April 13, 1981.

(Haferkamp et al. 1977). The filter paper was kept moist with sterile distilled water (pH 6). One piece of moistened filter paper was placed beneath the dish cover to improve moisture availability.

Germination trials were conducted in a controlled environmental chamber. Night/day temperatures were set at 5/15, 10/20, 15/25, or 20/30° C with 12-hour photoperiods during the high temperature periods. These temperature ranges were selected to coincide with normal monthly mean temperatures in central Texas (December, January, and February—5/15° C; March and November—10/20° C; April and October—15/25° C; and May and September—20/30° C). All experiments were conducted in a light regime of 237 $\mu\epsilon$ m^{-2} sec^{-1} photosynthetic active solar radiation, during the high temperature periods. One trial was conducted at each temperature, and 3 replications per seed treatment were arranged in a randomized complete block design.

Germinated seeds were counted daily for 14 days. The germination criteria were based on the Association of Official Seed Analysts (Crosier 1970) definition of germination, i.e. that the seed embryo must develop the essential structures to produce a normal plant in favorable conditions. The two major criteria for germination were:

1) Normal—at least one cotyledon exposed and a radical greater than or equal to 5 mm in length.
2) Abnormal—neither cotyledon exposed or a radical less than 5 mm in length.

Statistical analyses were conducted with the ANOVA procedure of the Statistical Analysis System (Barr and Goodnight 1972), to determine if significant differences ($p \geq 0.05$) in germination existed between presowing treatments within temperatures. Differences among means were compared using Duncan's multiple range test. Counts were transformed prior to analysis using the following relationship:

$$x^1 = \sqrt{(n+H)}\, arc\ sin \sqrt{\frac{f+3/8}{n+3/4}}$$

where the proportion (germinated seeds/total number of seeds) is f/n.

Presowing Seed Treatments

Engelmann Daisy

Untreated Engelmann daisy seeds germinated better than those subjected to presowing seed treatments. Mechanical scarification, acid scarification, and soaking in hot water did not enhance the germination of Engelmann daisy seeds (Table 1). The water and acid scarification treatments inhibited germination in the 20/30° C and 18/24° C regimes (data not reported). Seed germination after mechanical scarification sometimes equaled germination of untreated seeds, but germination of mechanically scarified seeds in the 20/30° C regime was 40% less than germination of untreated seeds. Maximum germination was 43% for untreated seeds in the 20/30° C regime, which was significantly ($p \geq 0.05$) higher than the 14 day germination for any of the treated seeds.

Table 1. Percent germination of untreated Engelmann daisy seeds and seeds treated with presowing seed treatments of acid scarification (acid), soaking in hot water (water), or mechanical scarification (mechanical), and imbibed in 4 alternating temperature regimes.

Temperature (C°)	Treatment	Days 2	4	6	8	10	12	14
5/15	untreated	0a[1]	0a	0a	0a	0a	0a	0a
	mechanical	0a	0a	0a	0a	0a	0a	0a
10/20	untreated	0a	0a	1a	5a	11a	17a	23a
	mechanical	0a	0a	7a	15a	19a	20a	25a
15/25	untreated	0a	0a	14a	24a	31a	34a	35a
	mechanical	0a	0a	8b	15a	20a	23a	23a
20/30	untreated	0a	16a	29a	33a	38a	41a	43a
	mechanical	1a	6b	16b	20b	20b	23b	25b
	water	0a	0c	0c	0c	0c	0c	0c
	acid	0a	0c	2c	4c	5c	7c	7c

[1]Means within a column for each temperature regime followed by the same letter are not significantly different at $P<0.05$ level of significance using Duncan's multiple range test.

Western Indigo

Mechanical scarification enhanced normal germination of western indigo seeds at all temperatures studied (Table 2). Enhancement of germination also occurred with acid scarification. Water soaked seeds germinated at an intermediate level and untreated seeds germinated the poorest.

Mechanically scarified seeds began germination by day 8, and 31% of the seeds had germinated by day 14 in the 5/15° C regime (Table 2). This was significantly better than germination by all other seeds during the same time period. Germination was apparent by day 10 in the acid-scarified seeds, but only 9% of the seeds had germinated in 14 days. None of the untreated or water soaked seeds germinated normally in these cool temperatures.

Some germination occurred in all treatments in the 10/20° C regime (Table 2). Presowing seed treatments caused seeds to germinate sooner. Germination was apparent on days 2, 4, 4 and 6, respectively, for the mechanically and acid scarified, water soaked and untreated seeds. Scarified seeds germinated more than 90% in 14 days. In contrast, water-soaked and untreated seeds germinated 59 and 17%, respectively. Mechanically and acid scarified seeds germinated significantly better than untreated or water soaked seeds during day 4 through 14 and day 6 through 14, respectively. Mechanical scarification appeared better than acid scarification during the interval day 4 to 8. Water soaked seeds germinated significantly better than untreated seeds from day 8 through 14.

A similar trend occurred in percent germination in the 2 warmest temperatures (Table 2). Germination was apparent on day 2 in mechanically scarified seed, and on day 4 in the acid scarified, water soaked, and untreated seeds in the 15/25° C regime. However, some acid scarified seeds in 20/30° C were germinated on day 2. In both regimes, more than 90% of the scarified seeds germinated, 65% of the water soaked seeds germinated, but only 40% of the untreated seeds germinated. Germination percentages of scarified seeds were significantly greater than percentages of water soaked and untreated seeds during day 4 through 14. Values were similar for the two scarification treatments in the 15/25° C regime. However, acid scarified seeds germinated better from day 4 through day 10 period in the 20/30° C regime. Germination of

Table 2. Percent germination of untreated western indigo seeds and seeds treated with presowing seed treatments of acid scarification (acid), soaking in hot water (water) or mechanical scarification (mechanical) and imbibed in 4 alternating temperature regimes.

Temperature C°	Treatment	Days 2	4	6	8	10	12	14
5/15	untreated	0a[1]	0a	0a	0b	0b	0b	0b
	mechanical	0a	0a	0a	0a	14a	20a	31a
	water	0a	0a	0a	0b	0b	0b	0b
	acid	0a	0a	0a	0b	1b	1b	9b
10/20	untreated	0a	0b	1c	2d	5c	10c	17c
	mechanical	1a	27a	69a	91a	93a	95a	95a
	water	0a	1b	5c	17c	39b	49b	59b
	acid	0a	7b	27b	72b	89a	92a	97a
15/25	untreated	0a	1b	3c	11c	25cd	33d	41d
	mechanical	8a	49a	85a	93a	94ab	94b	94b
	water	0a	2b	27b	41b	56c	65c	68c
	acid	0a	33a	78a	92a	98a	99a	99a
20/30	untreated	0b	4c	14d	28d	40d	41b	42b
	mechanical	29a	71b	82b	84b	84b	89a	91a
	water	0b	8c	33c	55c	59c	61b	64b
	acid	3b	91a	97a	97a	97a	97a	97a

[1]Means within a column for each temperature regime followed by the same letter are not significantly different at $P<0.05$ level of significance using Duncan's multiple range test.

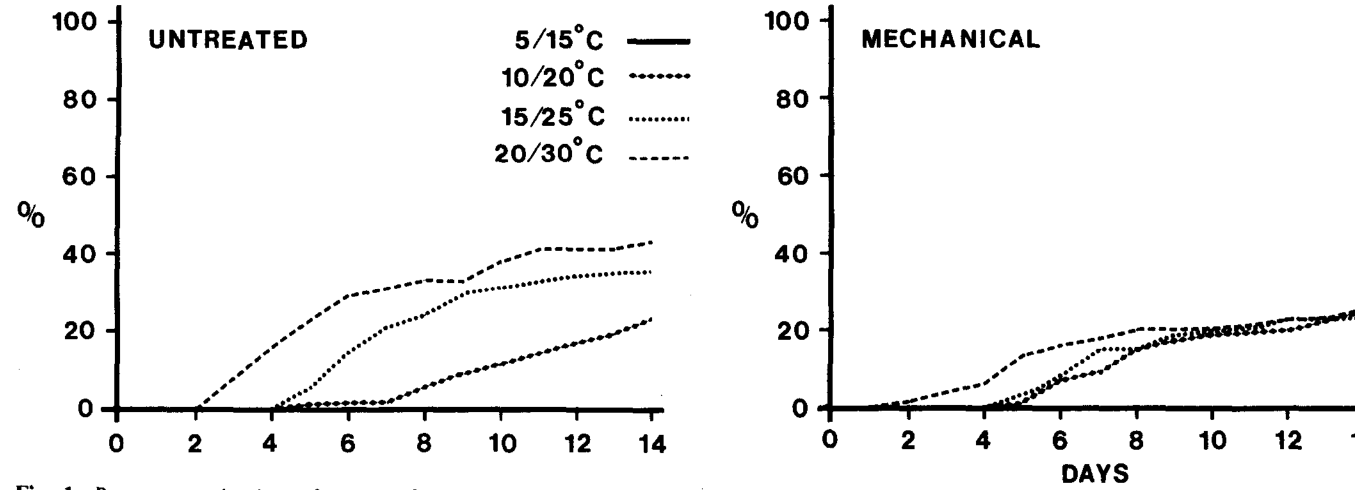

Fig. 1. *Percent germination of untreated and mechanically scarified Engelmann daisy seeds as affected by temperature during imbibition.*

water soaked seeds was significantly better than untreated seeds from day 6 through 14 in 15/25° C and from day 6 through 10 in 20/30° C.

Temperature effects

Engelmann Daisy

Germination of untreated Engelmann daisy seeds began sooner in the warmer temperatures (Fig. 1). Germinated seeds were apparent by day 3 in the 20/30° C regime and by day 5 in both the 10/20 and 15/25° C regimes. The highest ultimate level of germination occurred in the 20/30° C regime, and germination percentages after 14 days imbibition decreased with a decline in temperature.

No normal germination occurred in the 5/15° C regime, b some seeds were metabolically active in this low temperatu About 5% of the seeds had either a short radical or cotyled exposed after imbibing for 14 days. Abnormal germination was 10, and 2% in the 10/20, 15/25 and 20/30° C regimes, respectivel

Mechanically scarified seeds also germinated sooner and abo 25% better in the 3 warmest temperature regimes than in t 5/15° C regime (Fig. 1). In contrast to the untreated seeds, ultima germination levels were about equal in the 10/20, 15/25, a 20/30° C regimes. About 10, 5, 2, and 2% abnormal germinati occurred in the 5/15, 10/20, 15/25, and 20/30° C regime respectively.

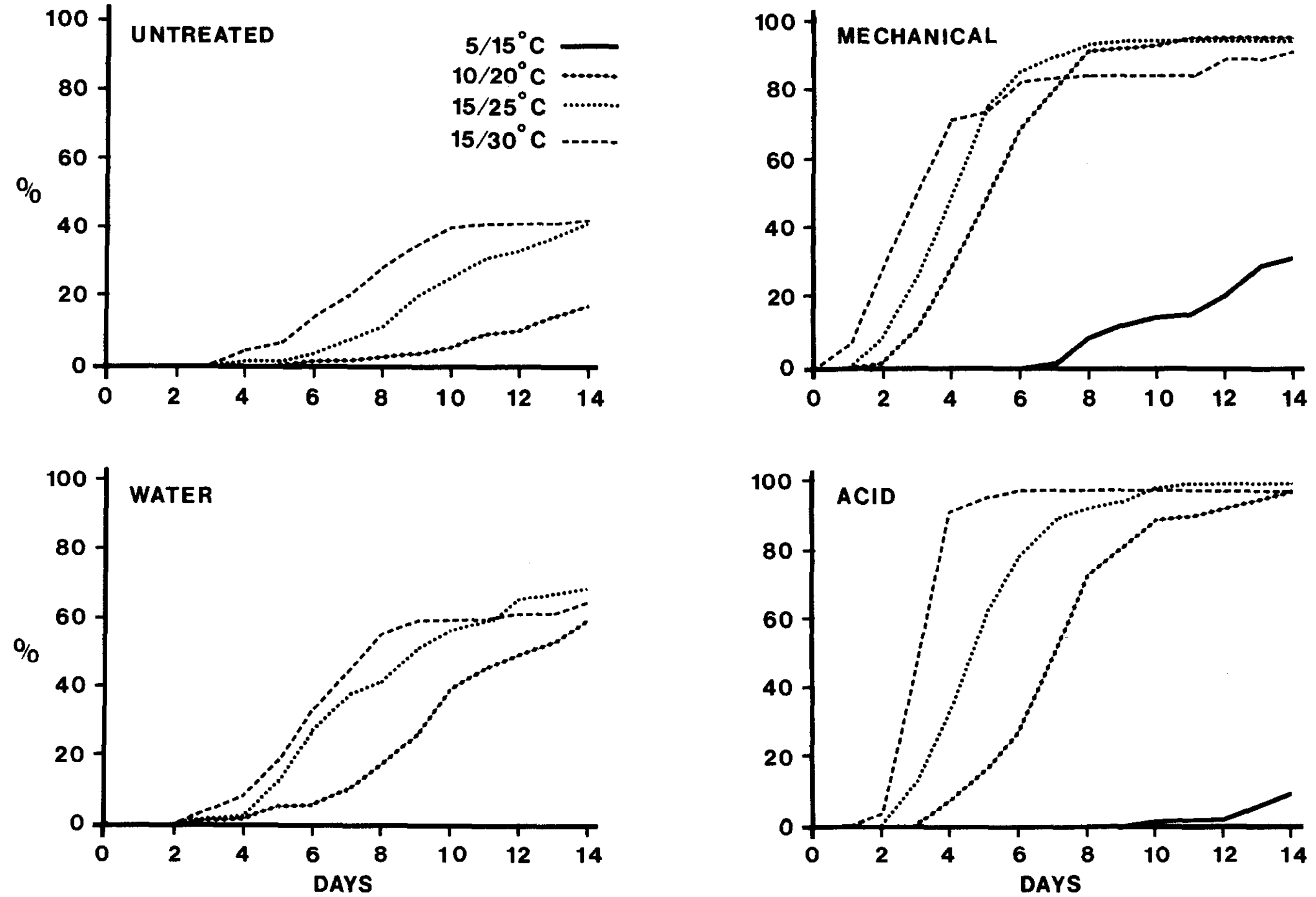

Fig. 2. *Percent germination of untreated and treated western indigo seeds as affected by temperature during imbibition.*

Western Indigo

Some untreated western indigo seeds had germinated in 4 to 6 days in the warmest temperatures (Fig. 2). Seeds in the 5/15° C regime were metabolically active, but no normal germination occurred in 14 days. About 5% produced cotyledons and short radicals.

Germination percentages of untreated seeds after day 4 were generally highest in the 20/30° C regime, intermediate in 15/25° C, low in 10/20° C, and lowest in 5/15° C (Fig. 2). The ultimate germination percentages were about equal in the 2 warmest regimes and twice as large as the percentage in the 10/20° C regime.

Normal germination of treated seeds imbibed in the 10/20, 15/25, or 20/30° C regimes was apparent on or before day 4 (Fig. 2). Some mechanically and acid scarified seeds had germinated by 7 and 10 days, respectively, in the 5/15° C regime.

Treated seeds appeared to germinate at a faster rate than untreated seeds (Fig. 2). Water soaked seeds germinated at an intermediate rate, and scarified seeds germinated at the most rapid rate. Germination of mechanically scarified seeds was very rapid during the initial 2 to 7 days of imbibition, and germination of acid scarified seeds was very rapid in the 2 to 8-day period. Following the rapid period of germination, an ultimate level was attained earliest in the warmest temperature regimes and later in the coolest regime.

Abnormal germination was more evident in the cooler temperature regimes. On day 14 in the 5/15° C regime about 5, 58, 40, and 83% of the untreated, mechanically scarified, water soaked and acid scarified seeds, respectively, showed signs of germination, but did not exhibit both an exposed cotyledon and radical longer than 5 mm. Only about 3 to 8% abnormal seed germination was recorded after 14 days in the 3 warmest temperature regimes. Interestingly, the mechanically scarified samples consistently had more seeds (2.5 to 8%) with a cotyledon emerged but with a radical shorter than 5 mm in length. This phenomena may have been due to the cotyledons simply being pushed through the cut in the seed coat upon imbibition of water.

Summary and Conclusion

Presowing seed treatments enhanced germination of western indigo seeds but did not improve germination of Engelmann daisy seeds. Mechanical and acid scarification and soaking in hot water all enhanced germination of western indigo seeds. Scarification was the superior treatment, and little difference was found between mechanical or acid scarification. Mechanically scarified seeds, however, appeared to germinate slightly faster during the initial 2-day period in the 2 higher temperature regimes.

Mechanical scarification would be the more practical treatment for field plantings if a method of treating bulk seed lots was available. One might plant a mixture of scarified and untreated seeds. The scarified seeds would germinate rapidly and improve the potential for successful stands, and the untreated seeds would provide potential for germination with subsequent moisture. However, additional research would be needed to evaluate the effect of scarification on the deterioration of seed viability and seedling damage.

The data suggest that germination of Engelmann daisy and western indigo seeds began sooner, proceeded at a more rapid rate, and the ultimate level was generally higher in the warmer temperature regimes. The 5/15, 10/20, 15/25 and 20/30° C regimes often occur during the December, January and February; March and November; April and October; and May and September periods in central Texas, respectively. The 15/25 and 20/30° C temperature regimes usually occur during the periods of greatest rainfall. Planting prior to these moist periods could provide successful stand establishment.

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