

Germination of Seed of Three Varieties of Spotted Locoweed

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Abstract

Spotted locoweed (*Astragalus lentiginosus* Dougl.), one of the principal locoweeds of western rangelands, consists of 36 varieties. The objectives of this study included the definition of some of the factors influencing seed germination of the species. Five seed collections were studied; they represented five different geographical and edaphic locations. Three varieties of spotted locoweed were represented by these collections. The seed coat of the varieties studied was impermeable to water. Seed also contained a water-soluble germination inhibitor. Germination was not affected by light. Optimal germination occurred at the temperature regime 7/13° C. However, at -1/4 and 21/27° C, though it was slower, high percentage germination was ultimately achieved. Although salinity inhibited germination, seed from the different sources showed different tolerances to salinity.

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Spotted locoweed (*Astragalus lentiginosus* Dougl.), a polymorphic complex of 36 varieties, is distributed over western rangelands in the Columbia Basin, the Colorado Plateau, deserts of southeastern California, and Arizona and extends a short distance into northern Mexico (Barneby 1964). It is one of the important locoweeds of the West. Both livestock and game animals are subject to adverse effects of locoweed ingestion.¹

Populations of spotted locoweed fluctuate markedly from year to year and from area to area. Locoism losses of livestock tend to increase as the locoweed population increases. Since the plant, including the dried stems, is toxic in all stages of growth, livestockmen too often become aware of the danger only when they observe signs of locoism in their animals.

Environmental factors influencing germination and survival of juvenile plants largely determine the population densities. We collected seed of three varieties. Collections from two different sites for two of the varieties were from widely spaced geographical locations and divergent habitats, the objective being to identify major factors affecting germination. Hopefully this information will eventually be incorporated into a predictive model suitable for forecasting dense populations that may be dangerous to grazing animals.

Previous studies of other *Astragalus* have demonstrated a seed

¹Personal communication with Lynn F. James, Poisonous Plant Research Laboratory, Logan, UT 84321.

coat impermeable to water and a leachable inhibitory substance in a seed coat or embryo (Baskin and Quarterman 1969; Green 1973). Optimum germination required both scarification and leaching. Without these treatments, only a small percentage of the seed germinated. Seed of the genus *Astragalus* and the related locoism-producing genus *Oxytropis* DC remain viable in the soil for many years where a small percentage germinate annually (James 1972; Payne 1957).

Materials and Methods

Seed from five field collection sites were used in this study and included var. *albiflorus* (two collections), var. *araneosus* (two collections), and var. *salinus* (one collection). Pods from each site were collected from a small uniform population of plants. The pods were threshed and seeds were stored in a refrigerator at 4° C. Insect predation had reduced the number of seed obtained in some collections below a level that would permit their inclusion in all the experiments. Plants grown in the greenhouse from seed from each collection were identified and voucher specimens were deposited in the Intermountain Herbarium, Utah State University, Logan, Utah.

One collection of var. *albiflorus* (Gray) Schoener [identified here as var. *albiflorus* (m)] was collected 4.8 km south of Holbrook, Arizona, at an elevation of 923 m growing among creosote bushes [*Larrea tridentata* (DC) Cov.] and galleta grass [*Hilaria jamesii* (Torr.) Benth.]. A second collection, identified here as var. *albiflorus* (u), was made on a chained pinion-juniper site which had been seeded to crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.] at 1,940 m in the Henry Mountains in Garfield County, Utah. Variety *albiflorus* is reported to grow at elevations of 1,525 to 2,225 m and is widespread and locally abundant across northern Arizona and New Mexico, extending into southeastern Utah and southwestern Colorado (Barneby 1964).

One collection of var. *araneosus* (Sheld.) Barneby seed was obtained from a site near Callao, Juab County, Utah, at 1,323 m in an area dominated by shadscale [*Atriplex confertifolia* (Torr. & Frem.) S. Wats.]. This collection is designated as var. *araneosus* (c). A second collection, designated as var. *araneosus* (m), was obtained east of the Minersville Reservoir in Beaver County, Utah. The seed-producing plants on this site were growing in a stand of big sagebrush (*Artemisia tridentata* Nutt.) at 1,692 m. This variety grows over most of western Utah at elevations from 1,433 to 2,195 m (Barneby 1964).

Variety *salinus* (Howell) Barneby was collected from a big sagebrush community at 1,969 m on the east slope of the Muddy Range in western Box Elder County, Utah. This variety grows at elevations of 700 to 1,905 m from Oregon to southern Idaho and northern Utah (Barneby 1964).

Seed were germinated in petri dishes with 25 seeds per dish. Seeds were placed on Whatman filter papers discs, moistened, covered, and placed in the germination chamber under various temperature regimes. Five to ten replications were used for the various experiments. The first visual evidence of germination is the emergence of the radicle from the seed coat. Germination is defined as emergence and extension of the radicle 2 mm from the seed coat. Scarification was accomplished by rubbing seed between two sheets of medium grit sandpaper. Leachate from the scarified seed was obtained by agitating a known number of seed per ml of distilled water for 24 hr with a magnetic stirrer. When indicated, scarified seed were placed in a screened tube and leached with running tap water for 12 hr.

Light intensity was measured with a Lambda Instruments quantum sensor model LI-190Sr in microeinsteins/m² second ($\mu\text{E}/\text{m}^2/\text{sec}$).

The capacity of scarified seed to imbibe water was compared to that of untreated threshed seed. Varieties (*albiflorus* (h), *albiflorus*

(u) and *salinus*) were used. Seeds were moistened with 5 ml distilled water and placed in the germination chamber at 4° C for 45 days.

Leachate (four seeds/ml distilled water) of varieties *albiflorus* (h), *albiflorus* (u), and *salinus* were subjected to the lettuce hypocotyl test (Frankland and Wareing 1960).

Seed that had been scarified, scarified leached, or unleached scarified seed of var. *salinus* were moistened with 5 ml of leachate (4 seed/ml distilled water) or 5 ml distilled water. Seed were germinated in the dark at 10 hr low and 14-hr high temperature alternation of 0/15° C. Germinated seed were counted over 16 days to evaluate the effects of the leaching and leachate on germination.

Scarified seed of var. *salinus* were germinated in three concentrations of leachate: 3, 4, and 10 seed/ml distilled water. Seed were placed in a germination chamber with 10/14 hr alternating temperatures of 0/15° C.

Secondary dormancy is exhibited by some seed which have imbibed water and dried before germination can occur (Koller 1972). Scarified seed of var. *araneosus* (m) were subjected to three cycles of 12 hr moisture and 12 hr drying. Germination of these seed was then compared with germination of scarified seed in a 10/14 hr alternating 0/15° C temperature regime.

The influence of temperature and light were examined in a 3 × 2 factorial experiment. Seed were placed in a germination chamber with 10/14 hr temperature regimes of -1/4, 4/7, and 7/13° C. Half of the samples received 14 hr of 12 $\mu\text{E}/\text{m}^2/\text{sec}$ light from an incandescent light and half were germinated in the dark. Varieties *albiflorus* (h), *albiflorus* (u), and *salinus* were used for each treatment. Germination was determined after 70 days.

A 5 × 4 × 3 factorial experiment was conducted to evaluate the effects of salinity, temperature, and variety or seed collection. Five levels of salinity (NaCl) were studied. Salinity is expressed as bars of water potential. The water potentials used were: -2.18, -4.13, -6.41, and -8.52 and 25° C. Ten ml of one of the above solutions

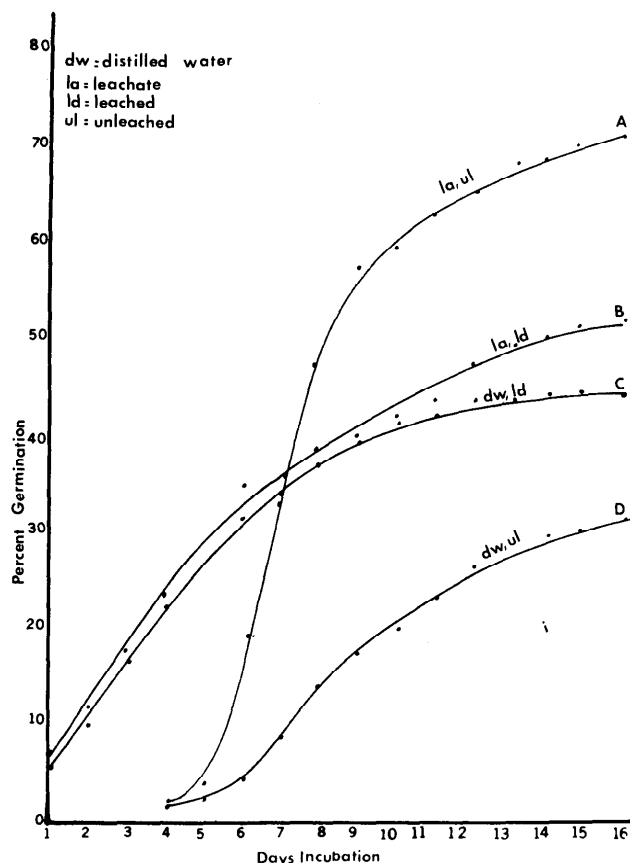


Fig. 1. Percent of scarified seed of *Astragalus lentiginosus* var. *salinus* germinating in the dark at 0/15° C when subjected to various treatments. Points with the same letters are not significantly different at the 95% confidence level.

²Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

were added to each petri dish. Collections of var. *albiflorus* (u), var. *araneosus* (c), var. *araneosus* (m), and var. *salinus* were used in this experiment. Seed were germinated in growth chambers with 10/14 hr regime of $-1/4$, 7/13, or 21/27° C and counted over a 49-day period.

Results and Discussion

All scarified seed imbibed water, swelling to about twice their dry volume. Only 20% of the unscarified seed of var. *albiflorus* (u), 25% of var. *salinus*, and 26% of var. *albiflorus* (h) imbibed water. Apparently the seed coat is responsible for at least a portion of the seed dormancy in this species. Results also indicate that more variation exists between collection sites of the same variety than between varieties.

The lettuce hypocotyl test with the leachate from seed of all three collections was positive, indicating the presence of a gibberellin-like substance in the seed which also contributes to the seed dormancy exhibited by this species.

Leached, scarified seed of var. *salinus* began germinating almost immediately. However, unleached seed to which leachate was added ultimately reached the highest level of germination, though delayed. Unleached seed in distilled water germinated least (Fig. 1). The germination of var. *salinus* increased as the concentration of leachate increased (Fig. 2). Knowledge of the interaction of promoter-inhibitor complexes in seed dormancy is limited and the analysis is beyond the scope of this study. However, the data indicate that such complexes may be operative in the seed of spotted locoweed.

Repeatedly allowing seed to imbibe water followed by a drying cycle did not impose secondary dormancy on seed of spotted locoweed.

Germination of seed collected 5 years earlier was 94%, whereas 98% of seed collected 6 months before the germination trials

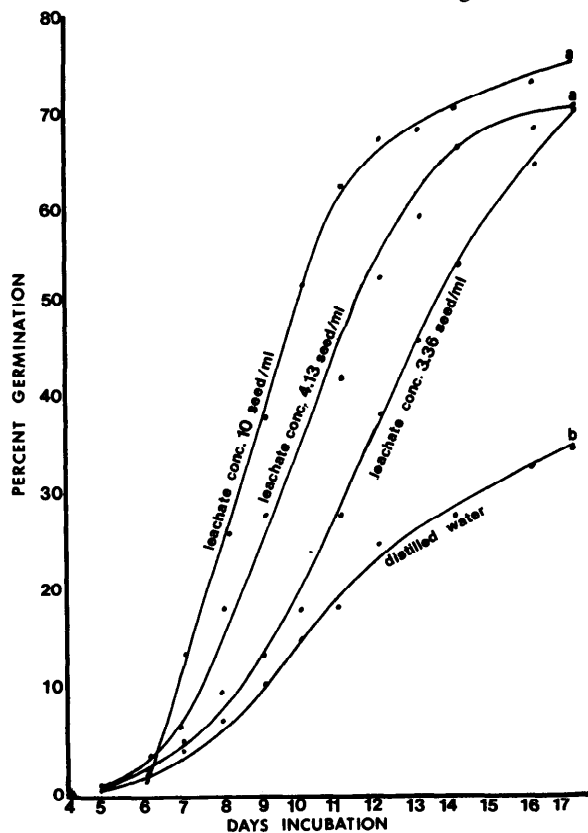


Fig. 2. Percent of *Astragalus lentiginosus* var. *salinus* seed germinating in the dark at 0/15° C when unleached, scarified seed were incubated in 5 ml leachate of 0, 3.36, 4.13, 10.0 seed per ml distilled water for 24 hr. Points indicated by the same letter are not significantly different at the 95% confidence level.

germinated. The results support the assumption that locoweed seed are long-lived and tend to persist for long periods in the soil (Payne 1957; James 1972).

Light supplied by an incandescent bulb did not affect germination, nor were there any significant interactions among light, temperature, and variety or collection of seed tested.

In the temperature salinity seed source factorial experiment, total germination for all seed sources at all levels of salinity was 59% at 7/13° C, 43% at 21/27° C, and 37% at $-1/4$ ° C. Germination at 7/13° C was significantly different from that observed at 21/27° C and $-1/4$ ° C. Differences in germination at the latter two temperature regimes were not significant.

Average germination for all varieties and seed sources at all three temperature regimes was 90% at 0 bars (distilled water) but declined to 2% in a NaCl solution of -8.52 bars. However, scarified seed imbibed water in solution more concentrated than -188 bars (Lang 1967).

Seed of var. *araneosus* (c) had the highest germination when the effects of salinity and temperature were pooled. The germination of var. *araneosus* was significantly higher than that of var. *salinus* (44%), var. *araneosus* (m).

Pooling the effects of salinity and temperature indicated that germination of var. *araneosus* (c) was 57%, which was significantly higher than for other seed sources. There were no significant differences in germination among the var. *albiflorus* (u) with 45%, var. *salinus* with 44%, and var. *araneosus* (m) with 41% germination.

Figure 3 portrays the interactions of temperature, salinity, and seed source. While analyses of variance of the data did not indicate a significant interaction, mean comparisons among treatment levels indicate that significant differences existed. Means for any two factors in the interactions are achieved by pooling the data for the third factor (Ostle 1963).

A significant increase in germination where data for the seed source were pooled was recorded under the temperature regime of

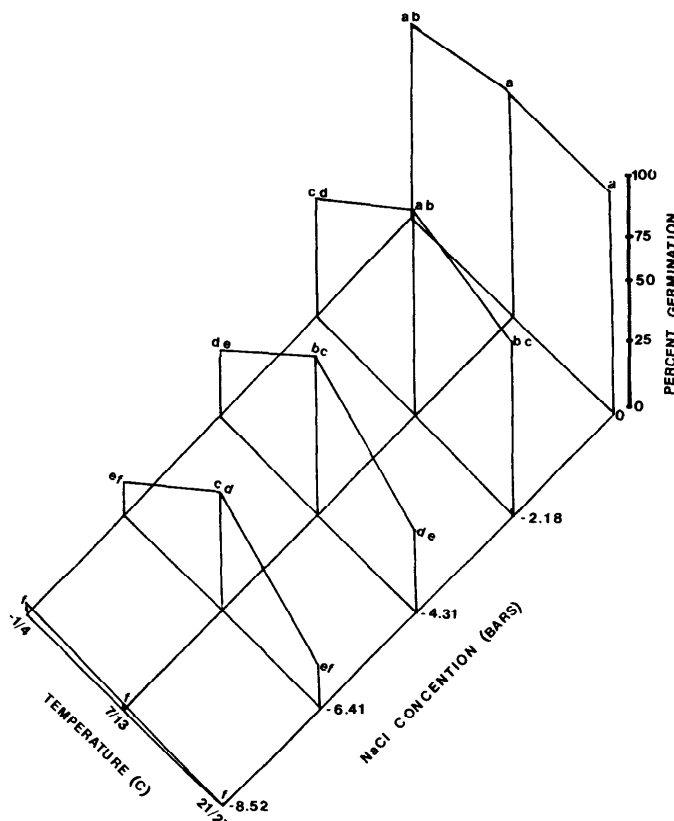


Fig. 3. Average percent germination for four seed collections (three varieties) of *Astragalus lentiginosus* after 49 days as influenced by temperature and salinity. Points indicated by the same letter are not significantly different at the 95% confidence level.

7/13 at NaCl concentrations of -4.31 and -6.41 bars, as compared to germination under temperature regimes of -1/4 and 21/27° C. There was also a significant increase in germination at -2.18 bars under the 7/13° C regime as compared to the 21/26° C regime. At 0 bars and at -8.52 bars, temperature did not significantly influence germination.

All seed sources produced their highest germination at 7/13° C, but var. *araneosus* (m)'s germination was significantly lower than germination of seed from other sources at that temperature. Germination of var. *araneosus* (c) was significantly higher than seed from other sources at -1/4° C and not significantly lower than germination at 7/13° C, indicating a broader tolerance limit to temperatures required for germination.

Variety *araneosus* (c) also consistently exhibited the highest germination at increasing salinity levels. However, only at -6.41 bars of NaCl concentration was germination significantly higher than germination of seed from other sources.

The most rapid germination of all varieties occurred at 7/13° C in distilled water (Fig. 4). Temperatures higher or lower than 7/13° C slowed germination, as did salinity levels. At -1/4° C in distilled water, no germination was observed for the first 8 days after initiation of the experiment but reached 82% after 49 days.

Conclusions

A number of factors affect both the rate and total germination of seed of spotted locoweed. These factors include temperature, moisture, soil water potential/salinity/ion toxicity (factors not separ-

ated in this study), the source of the seed, the condition of the seed coat, and various interactions among these factors. The following model is proposed, not as a final description of germination, but as a guide to future investigations of the influence of these factors and their interactions on germination:

$$Y = f [IT_p (S_p \text{ or } W_p) (ta)^{-1}]$$

Where:

Y = number of germinations per unit time in a given area.

f = a function of.

I = number of sufficiently leached seed that have imbibed water.

T = percent of germination at a given soil temperature.

S = percent germination at a given soil water potential in presence of toxic ions.

W = percent germination at a given soil water potential in the absence of toxic ions.

p = local population of spotted locoweed with its characteristics of seed produced.

t = unit of time.

a = unit area.

Most of the components of this model will require further investigation before the model can be used to define the germination process. Component "I" is defined as the number of seed from which the water-soluble inhibitors have been leached and that have imbibed water. These seed are only a part of the soil seed reserve for which no quantitative data are available. Although this study has indicated some of the factors controlling dormancy of spotted locoweed seed, the factors in the soil environment that contribute to breaking seed dormancy remain a matter of speculation.

The methods used in this study do not differentiate between soil water potential and ion toxicity. The results reported here must be considered to be an interaction between soil water potential and ion toxicity until further research defines their roles in the germination process.

The major barrier to developing a practical model for germination that would be useful to the livestock industry appears to be the intravarietal variations indicated by the data presented here. The model would have to account for significant variations in the responses to the various seed sources to the factors influencing germination. However, if a model can be developed to accept these variations, it would eliminate the difficult task of identifying the taxa below the species level. Identification of spotted locoweed varieties should be left to taxonomists thoroughly familiar with the morphological variations within these taxa.

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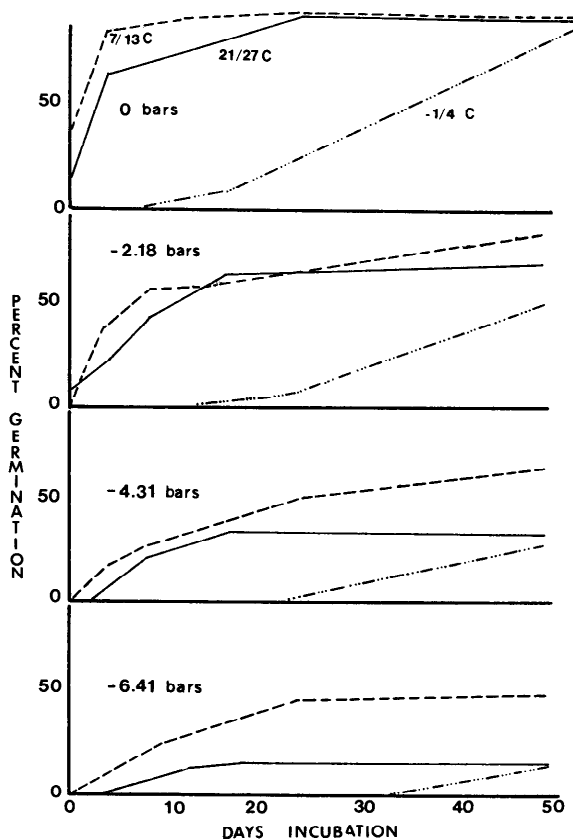


Fig. 4. Cumulative average percent germination for four seed collections (three varieties) of *Astragalus lentiginosus* at three diurnal temperature alterations as influenced by four salinity levels.