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Propagation of Blowout Penstemon (*Penstemon haydenii* S. Watson): Germination-enhancing Treatments

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Development of germination-enhancing treatments will be essential for the recovery of Nebraska's only officially endangered plant species, blowout penstemon (*Penstemon haydenii* S. Watson). Effects of cold-moist stratification, mechanical or chemical scarification, and presence or absence of light in combination with alternating temperature regimes on seed germination of blowout penstemon was determined by laboratory studies. Stratified seeds exhibited greater mean germination percentages (21%) than nonstratified seeds (8%). Effects of mechanical scarification on germination varied with trial. Seeds chemically scarified with concentrated sulfuric acid exhibited greater mean germination percentages (48%) than controls (12%); such scarification may substitute for the effective, but time-consuming, process of hand-scarification. Seeds were insensitive to light and alternating temperatures. Germination of freshly harvested seeds was not affected by storage of up to 15 weeks. Sulfuric acid and hand-scarification may be used to effectively enhance germination and, in turn, aid the recovery of this species.

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INTRODUCTION

Blowout penstemon (*Penstemon haydenii* S. Watson) is the only federally endangered plant species in Nebraska (Nebraska Game and Parks Commission, 1986; U.S. Fish and Wildlife Service, 1987). This perennial, multistemmed forb occurs naturally in only a few blowouts in the Nebraska Sand Hills (Weedon et al., 1982b). Even though it is protected by law, its continued existence is not assured. Large fluctuations in plant numbers have been noted from one year to another (Stubbendieck and Weedon, 1984). The development of propagation methods will be essential to increase plant numbers, sites of establishment, and recovery of this species.

Blowout penstemon primarily reproduces by rhizomes, and naturally-occurring seedlings are relatively rare (Stubbendieck et al., 1983, 1984; Stubbendieck and Weedon, 1984). The supply of blowout penstemon seed is limited and germinability is usually low (Weedon et al., 1982a; Stubbendieck et al., 1982a, b; Stubbendieck et al., 1983; Stubbendieck and Weedon, 1984; Stubbendieck et al., 1984). Researchers have been able to increase germination to greater than 90% through a combination of hand-scarification and removal of soluble inhibitors; however, this process is time-consuming and not practical (Stubbendieck et al., 1982b; Stubbendieck et al., 1983; Stubbendieck and Weedon, 1984; Stubbendieck et al., 1984). Stratification (of *Penstemon* spp.) does not insure germination (Stubbendieck et al., 1982b). Therefore, the effects of cold-moist stratification, mechanical and chemical scarification, light in combination with various alternating temperature regimes, and influence of afterripening on germination were determined in laboratory studies.

MATERIALS AND METHODS

Blowout penstemon seeds were collected in August 1986 and 1987, in Garden County, Nebraska. Seeds were removed from air-dried fruiting stalks, separated in an air column into heavy and light fractions, and stored at 3°C in plastic vials. Heavy seeds harvested in 1986 were used for all trials, except the afterripening study, within 18 months after harvest. Heavy seeds harvested in 1987 were used for the afterripening study.

Germination tests were conducted in 9-cm sterile plastic petri dishes containing three germination blotters moistened with 14 ml of distilled water. Twenty-five seeds were placed in each petri dish. Petri dishes were labelled and placed in a germinator set at 30°C for 8 hours (with light) and 20°C for 16 hours (without light) out of each 24-hour period. Light and temperature conditions of the germinator varied with treatment in the light and temperature study. Blotters were kept moist by adding 2–3 ml of distilled water every three days, or as needed. Seeds were considered to have germinated with the emergence of the radical or epicotyl. Counts were recorded at 7, 14, and 21 days after trial initiation. Germinated seeds were removed as counted. Number of damaged (broken or chipped) seeds was recorded upon initiation of germination tests for mechanical scarification trials. Due to time constraints, the light and temperature and afterripening studies were not repeated; however, all other germination studies were duplicated.

Cold-moist Stratification

To determine the effect of cold-moist stratification on germination, 600 seeds (24 lots of 25 seeds each) were soaked in nylon bags under cold running water for 24 hours, and 600 dry seeds were placed in separate plastic bags containing 10 ml of either moist, autoclaved (120°C, 60 min.) washed sand or vermiculite (coarse grade). Approximately 3 ml of distilled water were added to the vermiculite and 1.5 ml to the sand. Plastic bags were closed and then refrigerated at 3°C for 6, 12, or 18 weeks. Remoistening of seed/medium mixture was not necessary. For comparison, 100 seeds, soaked in nylon bags under cold running tap water for 24 hours, and 100 dry seeds were also subjected to a germination test at 6, 12, and 18 weeks as controls, or nonstratified seeds.

At completion of each stratification period, the seed/medium mixture was placed in a sieve (840 microns), and the medium was forced

through the sieve with a stream of cold water. Seeds retained on the sieve were placed in petri dishes. The study was arranged as a 2x2x3 factorial. Treatments consisted of two soaking regimes before stratification (soaked and not soaked), two types of media (sand and vermiculite), and three stratification periods (6, 12, and 18 weeks).

Mechanical Scarification

To determine the effect of mechanical scarification on germination, 800 blowout penstemon seeds (32 lots) were scarified in a commercial alfalfa scarifier at 80 cycles per second (cps) for 0, 1, 2, or 3 times with or without a final scarification at 120 cps. Once scarified, the seeds were placed in nylon bags and soaked under cold running water for 24 hours to remove soluble inhibitors before initiating the germination test (Stubbenieck et al., 1982b). The study was arranged as a 4x2 factorial. Treatments consisted of four levels of scarification at 80 cps (0, 1, 2, and 3 times) followed by two levels of final scarification at 120 cps (0 and 1).

Chemical Scarification

Blowout penstemon seed lots (16 lots) were chemically scarified in 50 ml of concentrated (18 M) sulfuric acid (H₂SO₄), for 15, 30, 45, or 60 minutes. Four lots of 25 nonscarified seeds were used as the untreated controls. The sulfuric acid/seed mixture was gently swirled at the beginning, middle, and end of each treatment period. After treatment, the sulfuric acid/seed mixture was poured through a sieve (840 microns) into a glass beaker. The treated seeds, retained on the sieve, were rinsed under cold distilled water for 90 seconds and placed into petri dishes. Another 100 seeds were soaked in nylon bags under cold running tap water for 24 hours and hand-scarified. Seeds were hand-scarified by removing the seed coat at the distal end of the seed with a razor blade while under a dissecting microscope (Stubbenieck et al., 1982b). The hand-scarification treatment was included for comparison as it enhances germination (Stubbenieck et al., 1982a, b; Stubbenieck et al., 1983; Stubbenieck and Weedon, 1984; Stubbenieck et al., 1984). Thus, treatments consisted of five levels of sulfuric acid scarification (0, 15, 30, 45, and 60 minutes) and hand-scarification.

Light and Temperature

To determine the effect of light and various alternating temperature regimes on germination, seeds of blowout penstemon (soaked under cold running tap water for 24 hours) were placed under one of the following 8 hour/16 hour temperature regimes: 20/10, 25/15, 30/20, or 35/25°C, with or without light during the warm period.

Darkness was maintained during the soaking period by placing the seeds in a plastic container lined with a nylon bag. The container was attached to a water faucet, and light was not allowed to enter the container. After soaking, the seeds were placed in petri dishes in a dark room. Darkness was maintained during germination by wrapping the petri dishes in two layers of aluminum foil. Germination counts and periodic moistening of blotters were conducted in a dark room. Thus, treatments consisted of four alternating temperature regimes (20/10, 25/15, 30/20, and 35/25°C) and two light conditions (light and dark) during the warm period. As only one germinator was available for use, the effect of one alternating temperature regime with or without light during the warm period (two treatments) on germination was tested at one time.

Afterripening

To determine if seeds undergo a period of afterripening after harvest, lots of freshly-harvested seeds were divided into four replications, removed from storage at five three-week intervals, and placed in the germinator. Other seeds were hand-scarified or chemically scarified with concentrated sulfuric acid for 30 minutes before placement in the germinator, following the procedures as outlined above. Treatments consisted of hand-scarification, chemical (sulfuric acid) scarification, and a control.

The viability of 100 seeds was determined with a 2,3,5-triphenyltetrazolium chloride (TTC) test at 3 and 15 weeks, following procedures developed by Hartmann and Kester (1975) and Copeland (1976). Two lots of 50 seeds each were soaked in nylon bags under cold running tap water to soften seed coats. After soaking, each seed was cut transversely with a razor blade under a dissecting microscope to expose the embryo. Each group of 50 scarified seeds was soaked in 50 ml of 0.25% TTC solution for 2 hours in a germinator set at 30°C without light. After soaking, the TTC solution was poured off, and the seeds were placed in 50 ml of distilled water. To evaluate viability, each seed was then examined under a dissecting microscope and classified as germinable or nongerminable, according to the amount of red (living) tissue. Embryos of germinable seeds turned completely red or pink and those that were nongerminable were white after soaking in the TTC solution.

Statistical Analysis

In studies determining the effect of stratification, scarification, and afterripening on germination, a randomized complete block design with four replications was used. Blocking was done to reduce any variation in light intensity from the front to the back of the germinator. Each petri dish was considered as an experimental unit. The light and temperature study was designed as a randomized complete block arranged as a split-plot with two replications. Temperature was the whole-plot effect, and light treatment was the subplot effect. In this study, a group of four petri dishes was considered as an experimental unit.

Statistical analysis was performed with Statistical Analysis System (SAS) using the general linear model procedure (SAS Institute Inc., 1985). Univariate analysis of variance (ANOVA) on germination percentage at 21 days (final) and initial percentage damaged (mechanical scarification study) with specific linear contrasts were performed to partition the variance into main effects and interactions between the factors (Steel and Torrie, 1980). Final germination percentage data obtained from the light and temperature study were transformed (square root) before statistical analysis. Duncan's multiple-range test was used where appropriate to separate means at the 0.05 level of probability.

Repeated measures analysis of variance was used to detect differences among treatments and storage periods for final germination percentages obtained from the afterripening study (Steel and Torrie, 1980). Data selected from the chemical scarification trials were subjected to regression analysis to obtain equations predicting the effect of number of minutes soaking in sulfuric acid on mean final germination percentage.

RESULTS AND DISCUSSION

Cold-moist Stratification

Cold-moist stratification enhanced germination in both trials. Stratified seeds had an overall mean final germination percentage of

21%, whereas nonstratified (control) seeds germinated to 8%. Soaking prior to stratification and length of stratification period interacted to affect germination in the first trial of this study ($p=0.01$). Increasing the length of the stratification period increased the germination of soaked seeds (Fig. 1). However, increasing the length of the stratification period of nonsoaked seeds decreased their mean final germination percentage (Fig. 1). Only the linear effect of length of stratification period was significant ($p=0.01$). Media type had no effect on the germination of stratified seeds ($p=0.14$).

Only soaking prior to stratification affected germination in the second stratification trial ($p=0.02$). Soaking increased mean germination from 12% to 18%. The length of the stratification period and media type did not affect germination ($p>0.05$). Cold-moist stratification did not consistently enhance germination, substantiating results obtained from earlier observations (Stubbendieck et al., 1982b).

Mechanical Scarification

In the first trial, scarification at 80 cps affected germination. Only the quadratic effect of increasing number of scarifications at 80 cps was significant ($p=0.01$). One and two scarifications at 80 cps decreased germination as compared to the control (Fig. 2). Three scarifications, however, were not different from the control. It is hypothesized that one and two scarifications merely fractured the seed coat, allowing the seed to dry, but not germinate, whereas three scarifications could have removed enough of the seed coat to allow germination to occur. The mean final percentage of damaged seeds observed in the first trial of this study was not affected by number of scarifications at 80 cps or the final scarification at 120 cps ($p>0.05$).

In the second scarification trial, scarification at 80 cps interacted with a final scarification at 120 cps to affect germination ($p=0.05$). Only the cubic effect of increasing number of scarifications at 80 cps with or without a final scarification at 120 cps was significant (Fig. 3) ($p=0.05$).

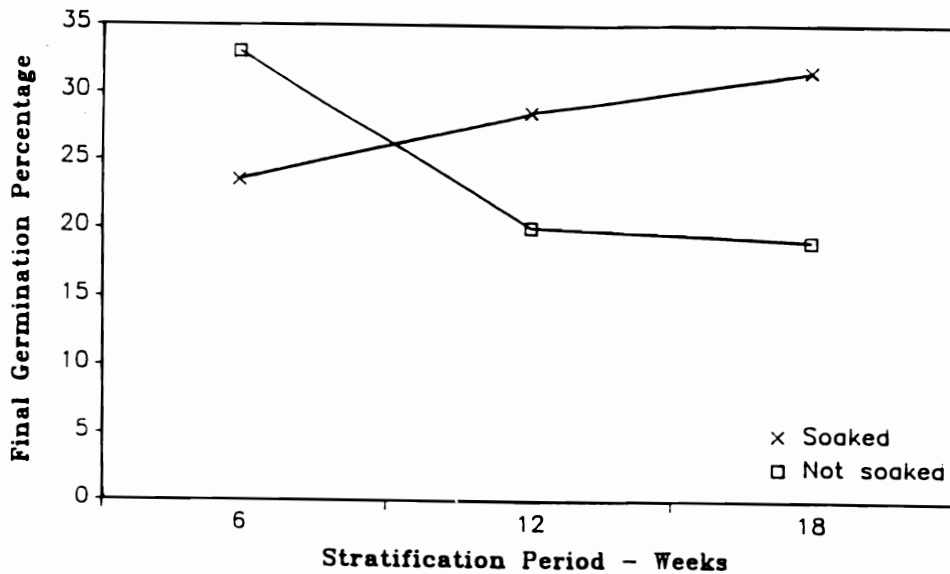


FIG. 1. Effects of soaking and stratification, Trial One.

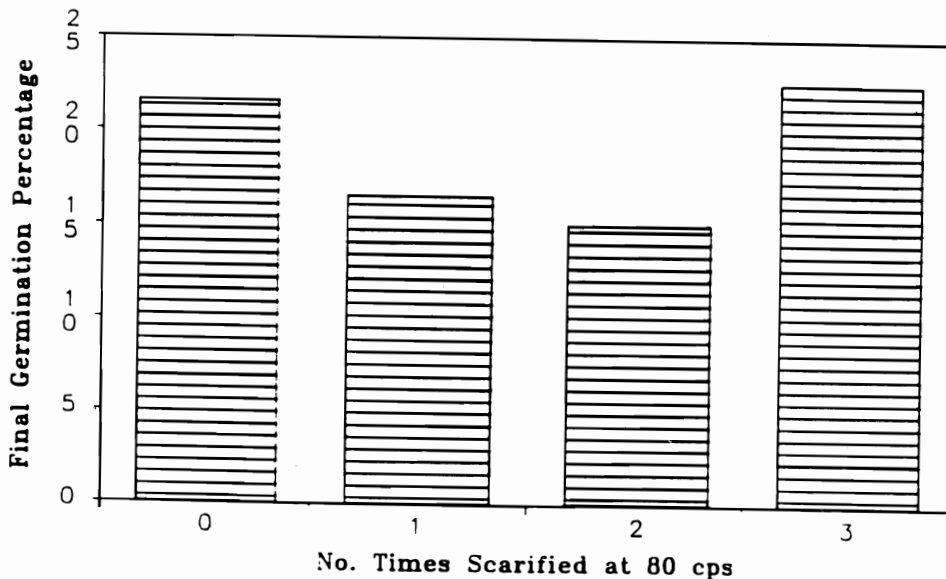


FIG. 2. Mechanical scarification in Trial Two.

The mean final percentage of damaged seeds was affected by a final scarification at 120 cps in trial two ($p=0.01$). A final scarification at 120 cps increased damage on the average from 8% to 22%. Perhaps higher numbers of damaged seeds were responsible for lower germination percentages observed for seeds scarified at 80 cps for three times and at 120 cps (Fig. 3). Mechanical scarification may not be consistent or desirable in enhancing the germination of blowout penstemon seeds.

Chemical Scarification

Scarifying seeds with concentrated sulfuric acid enhanced germination in both trials. Linear (trial one only) and quadratic (both trials) germination responses to increasing number of minutes soaked in sulfuric acid (Fig. 4) were significant ($p<0.05$). The following equations were determined to predict the effect of length of soaking period (minutes) on mean final germination percentage of blowout penstemon seeds from the results of regression analysis: $y = 10.37 + 2.88x - 0.04x^2$ ($R^2=.59$), and $y = 15.94 + 2.09x - 0.03x^2$ ($R^2=.49$), for trial one and trial two, respectively, where y = mean final germination percentage and x = number of minutes.

Mean final germination percentage of seeds soaked in sulfuric acid was greater than nonscarified, but less than hand-scarified seeds (Table I). Chemical scarification with concentrated sulfuric acid may substitute for hand-scarification in enhancing the germination of blowout penstemon seeds, as the resulting germination percentages were adequate, and the procedure was less time-consuming. However, if seed supplies are extremely limited, hand-scarification may be the most viable option.

Light and Temperature

Light and the range of alternating temperatures evaluated in this study did not affect germination ($p>0.05$). However, with just two replications per treatment, the power of the statistical test was inadequate

($p<80\%$). Further studies examining the effect of alternating and constant temperatures with or without light on final germination (with adequate replication) would provide more information on light and temperature combination(s) required for maximum germination.

Afterripening

Afterripening is not a factor in dormancy as indicated by germination of freshly harvested dry (controls), hand-scarified, or chemically scarified seeds following up to 15 weeks of cold storage (Fig. 5) ($p=0.09$). Others found germination of freshly harvested seeds was not affected by up to 18 weeks of storage, or storage temperature (Stubbendieck et al., 1982b).

Both hand- and chemical scarification greatly increased seed germination ($p<0.01$). However, the mean final germination percentage of seeds chemically scarified varied with each storage period ($p<0.01$) (Fig. 5). Mean final germination percentages were highest at week 9 and lowest at weeks 3 and 15. These results indicate that the effects of sulfuric acid on germination were inconsistent. Changes in the structure of the coat of freshly harvested seeds over time may explain the observed response to chemical scarification.

The mean percentage of germinable seeds at 3 weeks according to the TTC test, 66%, was not different than the mean percentage of seeds germinable at 15 weeks, 70% ($p>0.05$). These results suggest that germinability does not rapidly decline shortly after harvest.

CONCLUSIONS

Hand- and sulfuric acid scarification significantly enhanced the germination of blowout penstemon seeds. Sulfuric acid scarification may substitute for hand-scarification, as the resulting germination percentages were adequate, and the procedure was much less time-consuming. Cold-moist stratification and mechanical scarification

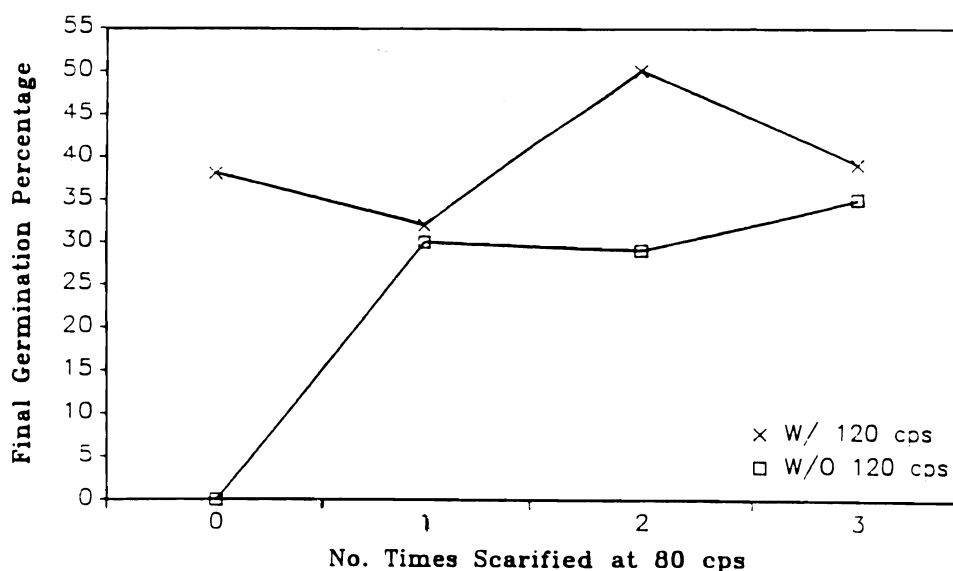


FIGURE 3. Effect of interaction of mechanical scarification at 80 cps with or without a final scarification at 120 cps on mean final germination percentage of blowout penstemon seeds in Trial Two.

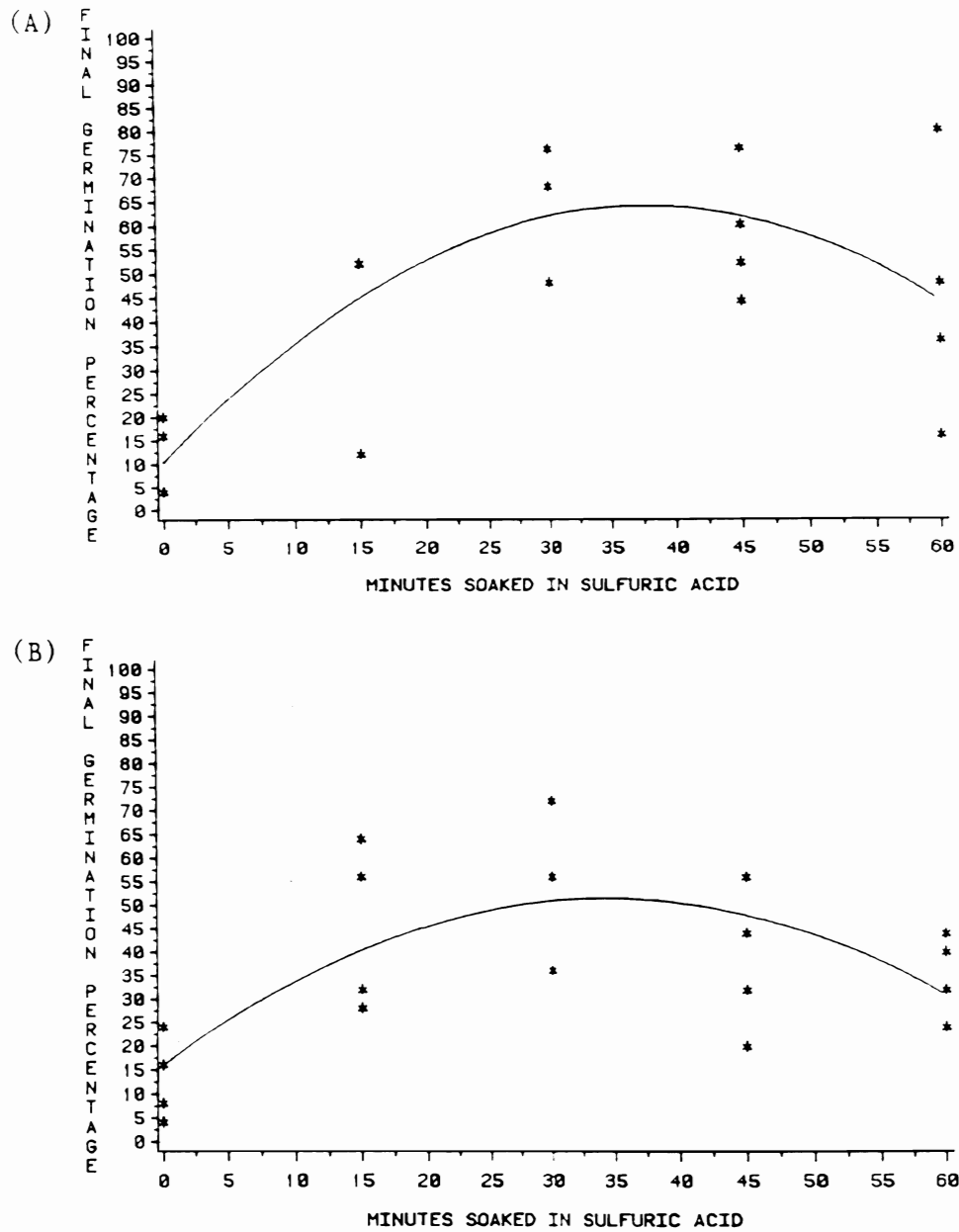


FIGURE 4. Quadratic regression of final germination percentage of blowout penstemon seeds soaked in sulfuric acid, as a function of time (minutes) for Trial One (A) and Trial Two (B).

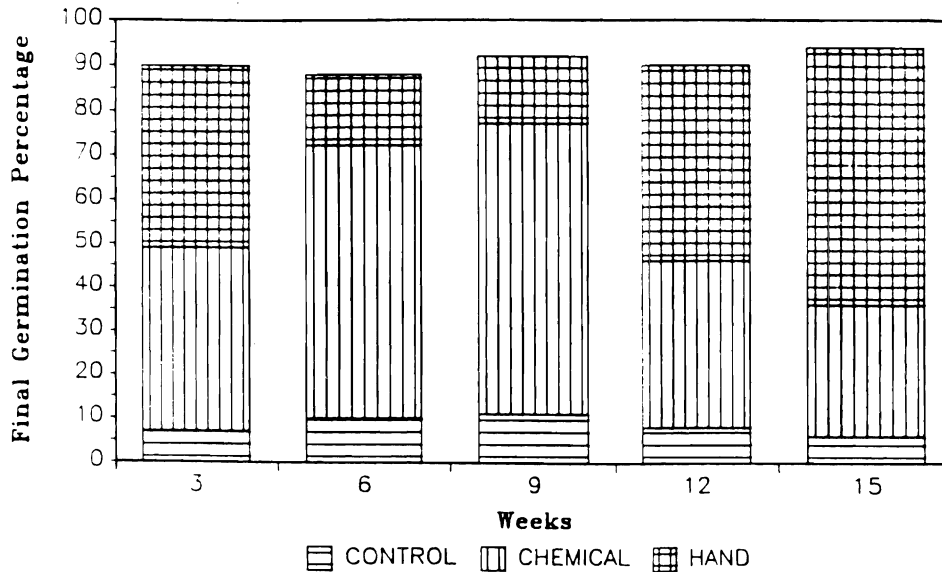


FIGURE 5. Effect of length of storage period (weeks) and chemical or hand-scarification treatments on mean final germination percentage of freshly-harvested blowout penstemon seeds.

did not consistently enhance germination. Germinability of seeds was not enhanced by light and alternating temperatures. Freshly harvested seeds did not exhibit afterripening requirements.

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