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Effects of Storage and Germination Conditions on the Germination of Four Species of Wild Flowers[1]

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Abstract. Seeds of 4 ornamentally useful species of native Nebraska wild flowers (butterfly milkweed, *Asclepias tuberosa* L.; rough gayfeather, *Liatris aspera* Michx; thickspike gayfeather, *Liatris pycnostachya* Michx.; and shell-leaf pentstemon, *Pentsemon grandiflorus* Nutt.) were subjected to 3 storage conditions, 6 periods of storage, and 4 germination temp and photoperiod regimes in a germination study. Seeds of all species germinated best and most rapidly when stratified at 4C prior to germination although response to length of storage was generally variable. Depending on the species, germination incubators simulating Nebraska growing seasons of late spring or early fall, midsummer, and 1 with alternating temp provided the best germination conditions following an appropriate pregermination treatment.

The use of native wild flowers for roadside vegetation establishment is being explored because they enhance natural beauty, and have a significant role in preventing soil erosion. Seeding is the most economical method of establishment, but results obtained from preliminary field trials were inconsistent, and showed poor plant stands in many instances. The seeds may have low viability, or some type of dormancy which should be overcome before seeding.

There is limited information on the germination of wild flower seeds. Conditions essential for maximum seed germination and mechanism of dormancy involved are unknown for most wild species. Macguire and Overland (2) germinated seeds of 3 pentstemon species at 15C in darkness, 15C with alternating light and darkness, 20C in darkness, 20 and 30C alternating in darkness, and 20 and 30C alternating with alternating light and darkness. *Pentstemon procerus* Dougl. ex R. Grah. did not germinate at 15C in darkness while the remaining treatments gave 72% germination. *Pentstemon wilcoxii* Rydb. did not germinate at any of the 5 treatments. *Pentstemon speciosus* Dougl. ex Lindl. seeds germinated 38% at 20 and 30C alternating in darkness, but no germination was observed when these alternating temp were combined with alternating light and darkness. Prechilling the seeds of *P. speciosus* at 3C for 1 and 4 weeks gave highest germination at 20C in darkness.

Mitchell (3) and Nichols (5) reported that the germination of *Asclepias tuberosa* L. is affected more by temp than by light. Seeds germinated at 20–25C in diffuse light and in darkness gave 23 and 24% germination, respectively (3). Nichols (5) found that seeds held 71 days in most soil in a cold frame in winter gave 91% germination over a period of 21 and 49 days after transfer to the greenhouse, while those held continuously in the greenhouse germinated only 46% over a period of 34 to 123 days.

Blake (1) collected seeds of several species of NB prairie plants in fall and planted them in the greenhouse periodically. *Liatris punctata* Hook. seeds planted in Oct., Dec., Jan., May, July, and Sept. germinated 3, 40, 47, 41, 15, and 21%, respectively. Those stored dry at room temp, and tested in the greenhouse during succeeding years germinated 74% after 2.5 years, 53% after 3.5 years, and none after 4.5 years. *Echinacea pallida* Nutt. had less than 10% germination during any month, but those stratified in moist soil in a cold frame had as high as 38% germination. *Pentstemon grandiflorus* Nutt. seeds did not germinate after periods of dry storage at freezing and room temp, but stratification gave 38% germination.

The present study was conducted to identify the germination problems of 4 species of NB wild flowers and determine the conditions that will best overcome the problems and promote maximum germination.

Materials and Methods

Seeds of butterfly milkweed, rough gayfeather, thickspike gayfeather and shell-leaf pentstemon were collected in the fall of 1972 from wild flower plots at the University of Nebraska Field Laboratory near Mead, NB. After drying at room temp and cleaning, the seeds were placed in cloth bags and stored at room temperature until the test began on January 18, 1973.

Three storage conditions, 6 storage periods, and 4 germination conditions were used as experimental treatments for each species. The storage conditions used were room temp at 20 ± 4C, dry storage at 4C, and stratification or moist cold storage at 4C. The storage periods were 0, 21, 42, 63, 84, and 105 days, respectively. Four germination conditions were selected to simulate mean soil temp, and daylengths for early spring, late spring or early fall, and midsummer in NB. Mean soil temp were calculated by assuming bare soil of less than 2.54 cm depth and using the equation derived by Neild (4): $Y = a + bX_1 + cX_2$, where; X_1 = weekly average air temp; X_2 = week number of the year beginning on March 1; Y = weekly average soil temp: and a, b, c, = constants which vary according to soil depth and cover condition. Photoperiod conditions were determined from a chart of the sunrise and sunset hrs in Lincoln, NB. The data obtained from these calculations were used in assigning the following temp and daylengths to each of 4 germination incubators:

Incubator A = 19C with 12 hr light and 12 hr darkness to represent early spring.

[1] Received for publication September 2, 1974. Published as paper No. 3856, Journal Series, NB Agr. Exp. Sta. Research conducted for the NB Dept. of Roads by the Dept. of Horticulture, University of Nebraska, in cooperation with the U. S. Dept. of Transportation, Federal Highway Administration. The opinions, findings, and conclusions expressed in this paper are those of the writers and not necessarily those of the NB Dept. of Roads or the Federal Highway Administration.
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Incubator B = 26C with 14.5 hr light and 9.5 hr darkness to represent late spring and early fall.

Incubator C = 33C with 14.5 hr light and 9.5 hr darkness to represent mid-summer.

Incubator D = alternating 33C with 14.5 hr light and 19C with 9.5 hr darkness.

Thermographs continuously monitored the incubation temp. Fluorescent light emitting 2.5×10^{-3} to 3.2×10^{-3} E/cm/sec energy and placed about 36.6 cm from the seeds were used for illumination.

The total number of seeds used for each species in the different trials was as follows: 3 storage conditions × 6 periods of storage × 4 germination conditions × 2 replications × 20 seeds per trial = 2880 seeds. The seeds of each species were divided into 6 lots of 160 seeds each and placed in small brown manila envelopes. Five lots of each species were placed in each of the 3 storage rooms for the periodic germination trials. The seeds that were stored for stratification were arranged in their envelopes between layers of moist sand in a seed flat. Moisture in the sand was maintained by adding deionized water when needed.

Seeds of the 6th lot of each species were used for the first germination trial which constituted 0 day storage or check. The remaining 5 lots were used for the succeeding 5 tests which were conducted at intervals of 21 days.

Seeds were germinated in a sterilized (autoclave 20 min) covered Petri dish containing a single layer of blue blotting paper. The seeds were treated against pathogenic organisms by soaking in 1% NaClO for 5 min before placement on the blotting paper. The blotting paper was kept moist by adding deionized water when needed.

Each Petri dish represented a trial and 20 seeds were used. Placement of the Petri dishes in the germination incubators constituted the initial day of any trial. Germination counts were made daily for 21 days. Seeds with emerged radicles were considered germinated and discarded as they were counted. Percent germination and mean days to germination were calculated.

The experimental design was factorial split-split plot design with 2 replications. The data were analyzed accordingly.

Results

Butterfly milkweed. Table 1 shows that storage condition, lengths of storage, and germination conditions affected the germination of butterfly milkweed seeds. Germination was fastest and highest when seeds were stratified at 4C, held in storage for 105 days, and germinated at 33C and 14.5 hr light alternating with 19C and 9.5 hr darkness or at 26C with 14.5 hr light and 9.5 hr darkness. Germination rate also increased with the length of time in storage, but only 52.5% germinated even after 105 days which was the longest period tested. This suggests that a storage period longer than 105 days is needed for this species to yield its maximum germination potential. The results from the storage treatments agree with those of Nichols (5) regarding the need for pregermination stratification to break the dormancy of butterfly milkweed.

Rough gayfeather. Table 2 summarizes the effects of the different treatments on rough gayfeather seed germination. Seeds stratified at

Table 1. Effects of storage conditions, length of storage, and germination conditions on the germination of butterfly milkweed, *Asclepias tuberosa* L.

Treatment	% Germination	Mean days to germination
Storage condition		
Stratification, 4C	68.6a[z]	5.5b
Dry storage, 4C	30.8b	10.3a
Room temp, 20 ± 4C	40.5b	10.2a
Germination temp and photoperiod		
19C, 12 hr light + 12 hr darkness	41.6b	8.2b
29C, 14.5 hr light + 9.5 hr darkness	65.3a	4.4c
33C, 14,5 hr light + 9.5 hr darkness	11.4c	14.2a
33C, 14.5 hr light + 19C, 9.5 hr darkness	68.1a	6.2c
Length of storage (days)		
Check (0)	35.0b	7.6ab
21	43.3b	9.2a
42	44.5b	8.2ab
63	45.5b	9.2a
84	47.1b	8.3ab
105	52.5a	6.7b

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 2. Effects of storage conditions, lengths of storage, and germination conditions on the germination of rough gayfeather, *Liatris aspera* Michx.

Treatment	% Germination	Mean days to germination
Storage condition		
Stratification, 4C	77.4a[z]	8.6a
Dry storage, 4C	67.9b	9.0a
Room temp, 20 ± 4C	60.9c	7.9a
Germination temp and photoperiod		
19C, 12 hr light + 12 hr darkness	61.8b	7.0b
29C, 14.5 hr light + 9.5 hr darkness	77.2a	7.8b
33C, 14.5 hr light + 9.5 hr darkness	55.3c	11.9a
33C + 14.5 hr light with 19C, 9.5 hr darkness	80.7a	7.2a
Length of storage (days)		
Check (0)	72.3a	10.4b
21	75.3a	8.5bc
42	70.3a	7.6c
63	74.0a	7.1c
84	76.0a	6.5c
105	48.2b	12.6a

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 3. Effects of storage conditions, lengths of storage, and germination conditions on the germination of thickspike gayfeather, *Liatris pycnostachya* Michx.

Treatment	% Germination	Mean days to germination
Storage condition		
Stratification, 4C	71.0a[z]	6.5a
Dry storage, 4C	70.6a	9.1b
Room temp, 20 ± 4C	56.1b	10.0b
Germination temp and photoperiod		
19C, 12 hr light + 12 hr darkness	54.2c	11.7a
29C, 14.5 hr light + 9.5 hr darkness	62.1b	8.1b
33C, 14.5 hr light + 9C, 9.5 hr	72.8a	7.0b
33C + 14.5 hr light + 19C, 9.5 hr darkness	74.4a	7.4b
Length of storage (days)		
Check (0)	65.0b	10.1a
21	60.1b	10.0a
42	59.8b	10.0a
63	74.3a	8.2ab
84	62.4b	7.8b
105	72.9a	7.0b

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 4. Effects of storage conditions, lengths of storage, and germination conditions on the germination of shell-leaf pentstemon, *Pentstemon grandiflorus* Nutt.

Treatment	% Germination	Mean days to germination
Storage condition		
Stratification, 4C	90.0a[z]	4.1a
Dry storage, 4C	10.0b	7.9b
Room temp, 20 ± 4C	66.7b	8.5b
Germination temp and photoperiod		
19C, 12 hr light + 12 hr darkness	54.0b	10.0a
29C, 14.5 hr light + 9.5 hr darkness	94.7a	4.3c
33C, 14.5 hr light + 19C, 9.5 hr	58.6b	7.8b
33C + 14.5 hr light with 19C, 9.5 hr darkness	95.4a	5.1c
Length of storage (days)		
Check (0)	1.3	13.0a
21	79.8	6.8b
42	70.5	6.9b
63	80.0	6.7b
84	71.5	7.1b
105	76.5	6.5b

[z] Mean separation by Duncan's multiple range test, 5% level.

4C germinated 77.4%, while those stored dry at 4C and at 20 ± 4C germinated 67.9 and 60.9%, respectively. The best temp and photoperiod regimes for germinating rough gayfeather seeds following storage were those at 26C or at 33C alternating with 19C, both being combined with a long daylength. Length of storage had no beneficial influence on germination because seeds of the non-stored check germinated as well as those stored for 21 to 84 days. The favorable germination response noted when seeds were stored wet or dry at 4C was not necessarily an indication of the presence of dormancy in the seeds. Low temp probably preserved the viability of the seeds because the initial germination compared favorably with those held in storage for as long as 85 days. The drop in germination after 105 days in storage indicated deterioration of viability with time regardless of wet or dry conditions among the storage temp tested.

Thickspike gayfeather. Table 3 shows that moist and dry storages at 4C were equally effective in promoting the germination of thickspike gayfeather. The stratified seeds, however, reached maximum germination in fewer days than those stored dry. Percentages of germination increased and mean days to germination decreased as incubator temp increased. Best germination responses were obtained when seeds were germinated at 33C and 14.5 hr light alternating with 19C and 9.5 hr darkness or at 33C with 14.5 hr light and 9.5 hr darkness. Data comparing the effects of periods of storage were inconsistent, although seeds stored for 63 and 105 days germinated better than those of the non-stored check.

Shell-leaf pentstemon. Seeds of shell-leaf pentstemon that were stratified at 4C germinated faster and in greater numbers than those stored dry at 4C or at 20 ± 4C. The best temp and photoperiod regimes for germinating the seeds following an appropriate storage treatment were those simulating late spring and early fall or combination of early spring and mid-summer in eastern NB. A storage period of 21 days was sufficient to raise the germination rate above those of the non-stored check. This favorable response to a short period of pregermination stratification indicates that the dormancy of this species may be purely physical rather than physiological or chemical. Since this species has a hard seed, the moist storage apparently provided the moisture necessary to overcome its resistance to water penetration. Soaking in water might be better than stratification as a pregermination treatment for shell-leaf pentstemon seeds (Table 4).

Storage condition, length of storage, and germination condition interactions. These experimental variables showed several significant 2-way interactions among each of the 4 species studied. Graphs of these interactions were not included in this report because they showed effects that were similar to those of the primary experimental variables.

Discussion

Seeds of the 4 species of wild flowers studied germinated best when subjected to temp and photoperiod regimes simulating those predominant during certain periods of the growing season in NB. If facilities for proper pre-planting treatment of seeds are available, butterfly milkweed, rough gayfeather, and shell-leaf pentstemon will probably give best stands when seeded in late spring or early fall as compared to mid-summer for thickspike gayfeather. If seed pre-planting treatment facilities are not available, a late fall seeding would be another possibility to provide the natural cold treatment during winter.

All 4 species also gave excellent germination when the day and night temp of a long photoperiod were alternated between 33 and 19C. Macguire and Overland (2) reported similar results in a germination study conducted on seeds of 3 pentstemon species. The beneficial effects of alternating temp to germination of these species is difficult to explain on the basis of the data presented. Seed germination, however, is a complex physiological phenomenon involving many metabolic processes. These different processes are probably promoted by changing rather constant temp.

Seeds of these species also germinate rather poorly in nature. Volunteer plants in the vicinity of natural stands are rarely observed. Harmful effects of competition from existing vegetation, adverse soil and climatic conditions, and invasion of pathogenic or decay organisms are probably the causes of this occurrence. Seeds collected from natural stands also germinate considerably lower than those collected from cultivated plants of the same species.

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