Storage Life of Illinois Bundleflower and Western Indigo Seed

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Abstract

Seed of Illinois bundleflower (*Desmanthus illinoensis* (Michs.) MacM.) and western indigo (*Indigofera miniata* var. *leptosepala* (relative humidity were imbibed in a controlled environment for 20 Turdays with a night/day temperature regime of 15/25° *C* and a 12-hour en stored for 1 to 4 years at 16° *C* and 40%photoperiod. Preimbibition treatments included acid scar-ification by immersion in concentrated sulfuric acid for 15 minutes, mechanical scarification by cutting the seed coat at the end opposite the micropyle, and an untreated control. Seed viability was determined by a triphenyl tetrazolium chloride test. Germination rate and cumulative germination decreased for untreated Illinois bundleflower seed and increased for untreated western indigo seed as the length of storage increased from 1 to 4 years. The decrease in germinability of Illinois bundleflower seed was related to the development of an impermeable seed coat, while the increase in germinability of western indigo seed was related to a decease in hard seededness and the fulfillment of after-ripening requirements. Scarification treatments increased cumulative germination and germination rates in each seed storage class for both species. Over the 4-year storage period, Illinois bundleflower seed viability decreased by approximately 10%, and western indigo viability remained relatively constant.

Native legumes are either nonexistent or make minor contributions on millions of hectares of rangeland in the southern Great Plains because of selective removal by past grazing (Sims et al. 1980). Few native legumes are being used in rangeland plantings even though they have a high forage quality and are a potential source of economical nitrogen for increasing the productivity of native and introduced grass plantings. However, several native legumes, including Illinois bundleflower (*Desmanthus illinoensis* (Michs.) MacM.) and western indigo (*Indigofera miniata* var. *leptosepala* (Nutt.) Turner), are being evaluated for range improvement uses by the Soil Conservation Service (SCS) in cooperation with the Texas Agricultural Experiment Station and the Texas Parks and Wildlife Department (Soil Conservation Service 1980).

Illinois bundleflower, a deep-rooted, warm-season, perennial legume, is widely but sparsely distributed throughout the prairies and plains in the midwestern and southwestern U.S. (Latting 1961). 'Sabine' Illinois bundleflower, recently released by the SCS Plant Materials Center at Knox City, Texas, appears best adapted to areas in Texas and Oklahoma, receiving 50 cm or greater mean annual precipitation (Soil Conservation Service 1983). Plants grow on a wide variety of soils, except coarse sands and dense clays (Wasser 1982). Western indigo, a deep-rooted, mat-forming, cool-season, perennial legume, occurs in the eastern two-thirds of Texas and Oklahoma (Graham 1941, Soil Conservation Service 1980). Both species are palatable to all classes of livestock and are used by wildlife for food and cover (Rechenthin 1972, Wasser 1982).

Illinois bundleflower and western indigo have the potential to be used in seeding mixtures for revegetation of disturbed and deteriorated rangelands in Texas and Oklahoma. However, as with many native forb species, we are lacking information relative to the longevity and germinability of stored seeds (Hanelt 1977). Seed of many native forb species used in rangeland revegetation programs is available only from wildland sources and/or a limited number of commercial growers. Unfavorable environmental conditions during flowering or seed development periods may prevent yearly production of good seed crops (Mayer and Poljakoff-Mayber 1982, Stevens et al. 1981). Therefore, seed may need to be stored under controlled conditions to have an adequate amount available for future plantings. The objective of this study was to evaluate the effects of short-term storage (1 to 4 years) on the germinability and viability of Illinois bundleflower and western indigo seed.

Materials and Methods

Seed of Sabine Illinois bundleflower and accession PMT-1051 of western indigo were obtained from the SCS Plant Materials center, Knox City, Texas. Seed of Illinois bundleflower had been collected in 1978, 1979, 1980, and 1981; and seed of western indigo had been collected in 1978, 1980, and 1981. All seed had been stored at 16°C and 40% relative humidity prior to germination trials which took place from July to December, 1982. Seed collected in 1981, 1980, 1979, and 1978 were respectively designated 1-year-old, 2-year-old, 3-year-old and 4-year-old seed in terms of storage age class.

Seed viability was determined by a triphenyl tetrazolium chloride (TTC) test (Grabe 1970). Three replicates of 50 undamaged seed from each storage age class for each species, preconditioned by cutting the seed coat with a razor blade at the end opposite the micropyle, were placed in a 0.03M solution of TTC in phosphate

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buffer (0.06 M KH_2PO_4 - 0.6 M Na_2HPO_4) solution for 24 hours at 25°C in complete darkness. Percent viability was determined by evaluating intensity of staining and staining patterns under a 10x lens.

Germination trials were conducted with undamaged seeds from each storage age class. Pre-imbibition seed treatments included acid scarification, mechanical scarification, and an untreated control. Acid scarified seeds were immersed in 17.8 M H_2SO_4 for 15 minutes with continuous stirring and rinsed 3 times in distilled water. Seeds were mechanically scarified by cutting the seed coat with a razor blade at the end opposite the micropyle (Latting 1961). Due to a small number of seed, western indigo seeds did not undergo mechanical scarification.

Groups of 100 seeds each were dusted with captan (Cis-N-((trichloromethyl)) thio) – 4 cyclohexene – 1,2 – dicarbocimide) fungicide and placed on a single piece of Whatman No. 1 chromatography paper in 13 by 13.5 by 3.5-cm plastic trays. The paper was supported by a 5-mm thick polyurethane foam pad with 5 cotton wicks which extended into a 200-ml reservoir of distilled water (Berkat and Briske 1982).

Germination trials were conducted in a controlled environment chamber with a night/day temperature regime of 15/25°C and a 12-hour photoperiod. A light intensity of 450 $\mu mol \cdot m^{-2} \cdot sec.^{-1}$ (photosynthetically active solar radiation) was maintained during the high temperature period. An alternating temperature regime of 15/25°C was selected for both species on the basis of previous germination studies which indicated that germination was greater at 15/25°C than alternating temperature regimes of 10/20 and 20/30°C (C.A. Call, unpublished data; Kissock and Haferkamp 1983). A seed was considered to have germinated when it had at least 1 cotyledon exposed and radicle greater than or equal to 5 mm in length (Crosier 1970). Germinated seeds were counted and removed from trays every other day over a 20-day period, and cumulative germination data were reported as a percentage of the total number of seeds in each tray. Germination rates were estimated by calculating the mean time in days taken for nondormant viable seeds to germinate (Ellis and Roberts 1978). The mean germination time (MGT) was calculated as follows:

$$MGT = \frac{\Sigma(Dn)}{\Sigma n}$$

Where n is the number of seeds which germinate on day D, and D is the number of days counted from the beginning of the germination test.

Trays were arranged in a completely randomized design with 3 replications per seed treatment for each storage age. Analysis of variance and Duncan's multiple range test were utilized in data interpretation (Ray 1982). Cumulative germination data and viability data were transformed prior to analysis using an arcsine transformation (Steele and Torrie 1960).

Results and Discussion

Illinois Bundleflower

Under storage conditions of 16°C and 40% relative humidity, cumulative germination of untreated Illinois bundleflower seed decreased significantly ($P<0.05$) as the length of the seed storage period increased from 1 to 4 years (Table 1). The germinability of untreated 4-year-old seed was less than one-half that of untreated 1-year-old seed. The germinability of untreated Illinois bundleflower seed has also been shown to decline after 4 months of storage at room temperature (Latting 1961). Latting (1961) related the reduction in germination to the rapid development of an impermeable seed coat following maturity.

Breaking the seed coat by mechanical scarification or weakening the seed coat by acid scarification significantly ($P<0.05$) increased cumulative germination in each seed storage age class (Table 1). Mechanical scarification increased germination more effectively than acid scarification. The difference in germination between mechanically scarified seed and untreated seed indicated that the decrease in germination over time was due primarily to hard seededness, and not a loss of viability, since germination percentages of mechanically scarified seed were similar to viability percentages of seed scarified in the same manner prior to TTC testing. Seed viability was estimated at 97, 82, 94, and 86%, respectively, for 1-, 2-, 3-, and 4-year-old seed. Seed viability decreased by 11% over the 4-year storage period.

Table 1. Cumulative germination of Sabine Illinois bundleflower seed as influenced by length of seed storage period and a seed coat scarification treatment.

Year of collection	Storage period (years)	Scarification treatment	Germination[1] (%)
1981	1	untreated	43 c
		acid	82 b
		mechanical	96 a
1980	2	untreated	28 d
		acid	67 c
		mechanical	80 b
1979	3	untreated	29 d
		acid	84 b
		mechanical	94 a
1978	4	untreated	20 e
		acid	71 c
		mechanical	82 b

[1]Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's Multiple Range Test.

Cumulative germination data and viability data for seed collected in 1980 and stored for 2 years do not conform to the general trends that developed for the other seed storage age classes over the 4-year period (Table 1). Cumulative germination for untreated 2-year-old seed was slightly lower than that for untreated 3-year-old seed. Acid-scarified and mechanically-scarified 2-year-old seed had significantly ($P<0.05$) lower germination than similarly treated 3-year-old seed, and slightly lower germination than similarly treated 4-year-old seed. The viability of 2-year-old seed was significantly ($P<0.05$) lower than that of 3-year-old seed, and slightly lower than that of 4-year-old seed.

Seed viability and germinability are not only a function of seed storage, but also the environmental conditions to which the parent plant is exposed during seed formation and ripening (Mayer and Poljakoff-Mayber 1982). A heat stress during the seed maturation period, especially during the first part of the maturation period, can have marked effects on germinability (Koller 1972). Illinois bundleflower typically flowers in late May or early June and produces seed from mid-June to early July at the SCS Plant Materials Center at Knox City (personal communication, J.B. Muncreif, SCS, Knox City, Texas). During this 3-week period in 1980, the daily maximum temperature was between 40 and 45°C for 13 days and above 45°C for 2 days as compared to 1978 with 2 days between 40 and 45°C, and 1979 and 1981 with no days above 40°C (unpublished climatic data, SCS, Knox City, Texas). Even though the parent plants received supplemental irrigation to insure seed production, the higher temperature in 1980 would have increased transpirational losses and raised leaf, stem, and seed pod temperatures. Associated changes in parent plant metabolism could have had negative effects on seed development and subsequent germination and viability of stored seed.

Mean germination time, a measure of the germination rate of viable seeds, increased significantly for untreated seeds as the length of the storage period increased to 3 and 4 years (Table 2). Untreated, 4-year-old seed had an MGT of almost 2 days longer than 1-year-old seed. Germination rates in all seed storage age classes were accelerated when seed coats were weakened by acid

Table 2. Mean germination time of Sabine Illinois bundleflower seed as influenced by length of seed storage period and seed coat scarification treatment.

Year of collection	Storage period (years)	Scarification treatment	Mean germination[1] time (days)
1981	1	untreated	5.8 bc
		acid	5.2 cde
		mechanical	4.6 de
1980	2	untreated	5.9 bc
		acid	5.5 bc
		mechanical	5.1 cde
1979	3	untreated	7.0 a
		acid	5.4 bcd
		mechanical	4.6 e
1978	4	untreated	7.6 a
		acid	6.0 b
		mechanical	5.4 bcd

[1]Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's Multiple Range Test.

scarification or broken by mechanical scarification (Table 2). Significant ($P<0.05$) decreases in MGT, by as much as 2.4 days, were noted for mechanically scarified 3- and 4-year-old seed. The rate of germination can be a critical factor in the success or failure of a wildland seeding, especially when germination occurs under transiently favorable water conditions (Hillel 1972, McDonough 1977).

Western Indigo

Cumulative germination of untreated western indigo seed increased significantly ($P<0.05$) as the length of the seed storage period increased from 1 to 4 years (Table 3). Germination of untreated 4-year-old seed was almost twice that of untreated 1-year-old seed. This increase in germination with increasing storage time can be related to a decrease in hard seededness. Differences between acid scarified seed and untreated seed indicated that there were 42%, 32%, and 25% hard seed, respectively, from the 1-, 2-, and 4-year-old seed storage age classes (Table 3).

Table 3. Cumulative germination of western indigo seed as influenced by length of seed storage period and seed coat scarification treatment.

Year of collection	Storage period (years)	Scarification treatment	Germination[1] (%)
1981	1	untreated	36 e
		acid	77 b
1979	2	untreated	48 d
		acid	80 ab
1978	4	untreated	61 c
		acid	86 a

[1]Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's Multiple Range Test.

The increase in germinability may also be related to the fulfillment of after-ripening requirements during storage. Seed viability remained fairly constant over the 4-year storage period (92, 89, and 90%, respectively, for 1-, 2-, and 4-year-old seed), while germination of acid-scarified seed increased by 9% over the same period (Table 3). No anatomical or morphological differences were observed in seeds from different storage age classes. Therefore, it was assumed that the process of after-ripening was the result of chemical or physical changes in the seed or seed coat (i.e., an alteration of the composition of storage materials in the seed, a change in seed coat permeability, and/or the appearance of promoting substances or disappearance of inhibitory substances) (Copeland 1976). These changes usually result in a gradual relaxation in the degree of strictness of the environmental requirements for germination (Koller 1972).

Germinability did not decline in seed collected in 1980 and stored for 2 years as it did with Illinois bundleflower. Western indigo typically flowers from April through September, and produces the majority of its seed earlier in the season than Illinois bundleflower (Rechenthin 1972). The seed development period apparently was not adversely affected by above-normal high temperatures in June and July 1980 as it may have been for Illinois bundleflower.

Mean germination time decreased slightly for untreated seeds as the length of the storage period increased from 1 to 4 years (Table 4). Significant ($P<0.05$) decreases in MGT, by as much as 1.6 days, were observed for acid-scarified seed in all storage age classes.

Table 4. Mean germination time of western indigo seed as influenced by length of seed storage period and seed coat scarification treatment.

Year of collection	Storage period (years)	Scarification treatment	Mean[1] germination time (days)
1981	1	untreated	9.1 a
		acid	7.5 b
1980	2	untreated	8.7 a
		acid	7.2 b
1978	4	untreated	8.6 a
		acid	7.0 b

[1]Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's Multiple Range Test.

Although there is no doubt that an increase in water permeability is an essential part of the dormancy-breaking action of a scarification treatment, it is by no means certain that this is the only result of this treatment (Mayer and Poljakoff-Mayber 1982). Physical and/or chemical changes in the seed coat may also result in associated changes in gas exchange permeability, and light and temperature response.

Management Implications

Various mechanisms regulate the germination of seeds in their natural habitat and determine whether a given seed will germinate in a certain environment. Dormancy in seeds (hard seededness or an immature embryo or a combination of the two) leads to the accumulation of individuals in a persistent seedbank, which acts as a mechanism to increase the genetic base of a population by mixing cohorts from one year with another (Thompson 1981). Seeds produced by the same population of plants over several years may show considerable differences in response to environmental factors such as light, temperature, and moisture (Grime 1979). Such a regenerative strategy protects native species from eradication as a result of adverse environmental conditions which may follow germination.

Revegetation seedings on wildlands should simulate the seedbank concept to a certain extent. The germinability of the selected species should be high enough to allow for the establishment of an acceptable stand during the first season, but the seeding mix should contain some dormant seed to insure subsequent stand establishment if the first stand is diminished by unfavorable environmental conditions. When using native legumes, such as Illinois bundleflower and western indigo, the land manager might plant a mixture of scarified and untreated seed. The data suggested that Illinois bundleflower seed stored longer than 1 year at 16°C and 40% relative humidity should be scarified, since hard seededness apparently increases with the length of storage. Scarification is recommended for western indigo seed stored for 1 or 2 years under the same conditions, but not for seed stored for 4 years. Four-year-old seed has a relatively low hard seed content and provides a good mixture of readily germinable seed for initial stand establishment and dormant seed for subsequent stand establishment. The data also indicate that scarification treatments increase the rates of germination of these species, which should improve the potential for stand establishment under transiently favorable environmental conditions.

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