

EFFECTS OF SEED TREATMENT AND ENVIRONMENTAL STRESS ON GERMINATION AND ESTABLISHMENT OF 'SABINE' ILLINOIS BUNDLEFLOWER

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ABSTRACT

Germination responses of scarified (hot water soak at 175° F for 3 minutes) and unscarified seeds of 'Sabine' Illinois bundleflower (*Desmanthus illinoensis*) were investigated at substrate water potentials of 0, -2.5, -5.0, -7.5, and -10.0 atmospheres in 50/68, 60/78, and 68/86°F temperature regimes. Scarification and warmer temperature regimes (60/78 and 68/86°F) increased cumulative germination and decreased mean germination time, whereas low water potentials decreased cumulative germination and increased mean germination time. A degraded native pasture in the Post Oak Savannah region was sod-seeded with scarified, unscarified, and a mixture of scarified and unscarified seeds following suppression (herbicide, disking, or untreated) of resident vegetation. Seedling densities were greatest for scarified seeds during the first growing season because of the breaking of seed coat dormancy. However, plant densities were greatest for unscarified seeds by the third growing season because of continual establishment from a dormant seed bank. Seedling and plant densities were greatest for the herbicide (glyphosate) suppression treatment that created vegetation gaps without disturbing the mulch layer or the soil surface.

Key words: *Desmanthus illinoensis*, seed dormancy, seed scarification, sod-seeding, water stress, temperature stress

INTRODUCTION

Native legumes are either nonexistent or make minor contributions to the forage base and nitrogen economy of millions of acres of rangelands in the Southern Great Plains because of selective removal by past livestock grazing (Sims et al., 1980). Several native legumes, including Illinois bundleflower (*Desmanthus illinoensis*), have been recently released or are currently 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. Illinois bundleflower, a deep-rooted, warm-season, perennial legume, occurs in nearly all of Texas except the extreme east, west, and south (Rechenthin, 1972). This plant is drought-resistant, winter-hardy, and adapted to a wide variety of soils (Phillips Petroleum Co., 1963).

Recently, considerable effort has been directed toward establishing legumes in grass stands by no-till (sod-seeding) methods (Bryan, 1985; Burton, 1976; Mueller and Chamblee, 1984; Olsen et al., 1981; Skousen and Call, 1987). Most sod-seeding research has emphasized the establishment of introduced, cool-season, annual legumes in cool- or warm-season, perennial grass stands. Consequently, little is known about the establishment of warm-season, perennial legumes in warm-season, perennial grass stands.

Warm-season legume establishment has been beset by seed dormancy problems and fluctuating weather conditions at the time of planting, so good stands are often difficult to achieve (Burton, 1976). Seeds of Illinois bundleflower develop an impermeable seed coat with age (Call, 1985; Latting, 1961). Germinability can be enhanced under controlled environment conditions following chemical and mechanical scarification treatments (Call, 1985). However, scarified

and unscarified seeds of Illinois bundleflower have not been germinated under a gradient of temperature and substrate water potentials representing the range of environmental conditions that may be encountered at planting time.

Our objectives were to characterize the germination of scarified and unscarified seed of 'Sabine' Illinois bundleflower under different temperature and substrate water potential regimes in a controlled environment and evaluate the establishment of similarly treated seeds when sod-seeded in a native pasture in the Post Oak Savannah region.

MATERIALS AND METHODS

Controlled Environment Study

Seeds of Sabine Illinois bundleflower were obtained from the SCS Plant Materials Center, Knox City, Texas. Seeds were harvested in 1983 and stored at 60°F and 40% relative humidity before germination trials in July through December 1984. Three replicates of 50 undamaged seeds, preconditioned by cutting the seed coat with a razor blade at the end opposite the micropyle, were placed in a 1.0% solution of triphenyl tetrazolium chloride for 24 hours at 78°F in complete darkness to determine viability. Percent viability was determined by evaluating intensity of staining and staining patterns under a 10 x lens (Grabe, 1970).

Lots of 100 scarified (hot water soak at 175°F for 3 minutes) or unscarified seeds were placed on one piece of Whatman No. 1 chromatography paper in 5 x 5.5 x 1.5-inch plastic trays. The chromatography paper was supported by a 0.25-inch thick piece of polyurethane foam with five cotton wicks which extended into a 7.15 ounce reservoir of solution. Solutions had water potentials of 0, -2.5, -5.0, -7.5, and -10.0 atmospheres, which were derived by mixing 20,000 MW polyethylene glycol (PEG) with distilled water. Solution water potentials were measured by saturating chromatography paper discs (0.12 inch diameter) and placing them into a Wescor Model C-52 psychrometer sample chamber. Readings were recorded with a Wescor HR 33-T microvotlmeter following a 1 min equilibration period and a 15 sec cooling period. Trays were wrapped with clear polyethylene film to reduce evaporation and stabilize relative humidity.

Trays were placed in controlled environment chambers with night/day temperature regimes of 50/68, 60/78, and 68/86°F and 12-hour photoperiods. During the day period, a light intensity of 1,000 foot-candles was maintained at tray level. Germination was evaluated every other day over a 21-day period. Germination was considered complete when radicle length was 0.25 inches or greater and at least one of the cotyledons was exposed (Copeland, 1978). Germination rates were estimated by calculating the mean time in days taken for viable seeds to germinate (Ellis and Roberts, 1978).

The experiment was arranged in a completely randomized design with three replicates per treatment. The entire experiment was repeated, and data from both trials were combined before statistical analysis. The effects of substrate water potentials and alternating temperature regimes on cumulative germination percentages (adjusted by an arcsine transformation before analysis) and mean germination time values were analyzed by the use of a quadratic response surface ($P < .05$) (Evans et al., 1982).

Sod-seeding Study

The study site was located on the Texas A&M Native Plant and Animal Conservancy, 3 miles west of College Station in the Post Oak Savannah region. The site was dominated by brownseed paspalum (*Paspalum plicatulum*) and little bluestem (*Schizachyrium scoparium*). Texas wintergrass (*Stipa leucotricha*), knot-root bristlegrass (*Setaria geniculata*), silver bluestem (*Bothriochloa saccharoides*),

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and several annual grasses and forbs were minor components of the plant community. Mean minimum temperature in January is 40°F while mean maximum temperature in July is 95°F. Mean annual precipitation is 38.5 inches, with peak rainfall in May and September. Soils are of the Tabor series (fine, montmorillonitic, thermic Udertic Paleustalf), but have been so severely eroded that the fine sandy loam surface horizon has been removed, leaving a heavy clay subsoil exposed. Domestic grazing animals have been excluded from the site for at least 10 years.

Sod suppression treatments (10 x 50 foot plots) imposed on the site 1 week prior to seeding were: 1) single pass with a tandem offset disk to disrupt the sod; 2) 50% suppression (10- inch wide sprayed bands separated by 10-inch wide unsprayed bands) with glyphosate herbicide (N-(phosphomethyl) glycine) applied at a rate of 5 lbs. a.i./acre; and 3) untreated control. Sabine Illinois bundleflower was seeded into the three sod suppression treatments on April 5, 1985 at a 0.5-inch depth on 20-inch wide row spacings (rows located in middle of 10-inch wide herbicide bands) with a Tye Pasture Pleaser. The following seed treatments were used for each sod suppression treatment: 1) 100% of seed lot scarified (hot water at 175°F for 3 minutes), planted at recommended seeding rate (1.5 lbs. pure live seed/acre); 2) 50% of seed lot hot water scarified/50% unscarified, planted at recommended seeding rate; 3) 100% of seed lot scarified, planted at recommended seeding rate; and 4) 100% of seed lot unscarified, planted at twice the recommended seeding rate. All seed treatments were inoculated with *Desmanthus* spec. 1 inoculum prior to seeding. Phosphorus (as triple superphosphate, 46% P₂O₅) and potassium (as potassium sulfate, 53% K₂O) were applied along the seeded row at 180 and 90 lbs./acre, respectively.

The sod suppression and seed treatments were combined into a 3 x 4 factorial experiment arranged in a completely randomized block design with four replications per treatment combination. Plant density and standing crop were determined from five permanent subplots (40 inches of row length) within each replication of each treatment combination. Plant growth parameters were tested for significance (P<.05) by using the SAS general linear model analysis of variance procedure, and treatment means were separated by Duncan's multiple range test at P<.05 (Ray, 1982).

RESULTS AND DISCUSSION

Controlled Environment Study

In general, hot water scarification and warmer temperature regimes increased cumulative germination (Table 1) and decreased mean germination time (Table 2) of Sabine Illinois bundleflower seed. Low substrate water potentials decreased cumulative germination (Table 1) and increased mean germination time (Table 2), regardless of seed treatment and treatment regime. Highest cumulative germination percentages and most rapid mean germination times were observed at the 0 atmosphere water potential level in the 60/78 and 68/86°F temperature regimes. Under these moisture and temperature conditions, cumulative germination percentages of scarified seeds approached the percent viability of the seedlot (95%), while those of unscarified seeds were significantly (P<.05) lower. Lowest cumulative germination percentages and slowest mean germination times were observed at the -7.5 and -10.0 atmosphere water potential levels in the 50/68°F temperature regime.

Table 1. Cumulative germination (%) of unscarified (UNSC) and scarified (SC) Sabine Illinois bundleflower seeds after a 21-day imbibition period in five osmotic potential solutions under three alternating temperature regimes.

Water potential (atmospheres)	Temperature regime					
	50/68°F		60/78°F		68/86°F	
	UNSC	SC	UNSC	SC	UNSC	SC
0	41(9) ¹	80(10)	50(10)	85(9)	57(10)	91(9)
-2.5	44(8)	47(9)	39(8)	68(7)	36(9)	79(7)
-5.0	13(10)	15(10)	22(10)	50(8)	25(9)	52(8)
-7.5	1(12)	3(12)	9(11)	14(11)	28(10)	23(9)
-10.0	0(13)	12(10)	2(13)	8(12)	10(12)	13(12)

¹Germination estimates derived by quadratic response surface analysis; values in parentheses following means are one-half the calculated confidence intervals (P<.05).

Table 2. Mean germination time (days) of unscarified (UNSC) and scarified (SC) Sabine Illinois bundleflower seeds after a 21-day imbibition period in five osmotic potential solutions under three alternating temperature regimes.

Water potential (atmospheres)	Temperature regime					
	50/68°F		60/78°F		68/86°F	
	UNSC	SC	UNSC	SC	UNSC	SC
0	4.2(1.1) ¹	3.5(0.9)	2.0(1.0)	1.4(0.8)	1.8(0.9)	1.0(0.8)
-2.5	5.6(0.9)	7.3(0.8)	3.7(0.8)	3.9(0.7)	3.1(0.8)	2.6(0.8)
-5.0	10.0(1.0)	7.9(0.8)	7.5(0.8)	4.4(0.6)	5.0(0.8)	4.0(0.6)
-7.5	14.4(1.3)	18.4(1.6)	7.2(0.9)	6.0(0.9)	4.9(1.0)	5.2(0.8)
-10.0	14.1(1.4)	5.5(1.0)	6.6(1.1)	7.4(1.2)	5.6(1.2)	5.7(1.0)

¹Mean germination time estimates derived by quadratic response surface analysis; values in parentheses following means are one-half the calculated confidence intervals (P<.05).

Similar germination responses have been reported in other controlled environment studies with Illinois bundleflower and other warm-season, perennial bundleflowers found on Texas rangelands. Call (1985) observed 43 and 82% cumulative germination, respectively, for unscarified and acid scarified (concentrated sulfuric acid for 15 minutes), one-year-old seed of Sabine Illinois bundleflower at a 0 atmosphere substrate water potential in a 60/78°F temperature regime. Flenniken and Fulbright (1985) reported greater than 90% germination for acid scarified seed of prostrate bundleflower (*D. virgatus* var. *depressus*) at temperature regimes of 60/78 and 68/86°F. In the same study, germination was reduced by substrate water potentials of -9.0 atmospheres or lower. Haferkamp et al. (1984) improved the germination of velvet bundleflower (*D. velutinus*) seed at 0 atmosphere water potential in 50/68, 60/78, and 68/86°F temperature regimes with hot water (175° for 3 minutes) acid (concentrated sulfuric acid for 17 minutes), and mechanical (seed coat cut with razor blade) scarification treatments. Hot-water- scarified seeds

reached about 80% cumulative germination in 2 days in the 60/78 and 68/86°F regimes and in 10 days in the 50/68°F regime.

Sod-seeding Study

Because of the variability of the site and treatment responses, only main effects (seed treatments and sod suppression treatments) were statistically significant ($P < .05$) for plant density and standing crop data. Increases in standing crop were associated with increases in density. Thus, seed treatment and sod suppression treatment effects on plant density will be used to describe the results of the field study.

Illinois bundleflower seedlings emerged for mid-April to mid-May 1985 in response to four major precipitation events (1.0 inch or greater on April 13 and 30 and May 8 and 13) at the study site. Seedling emergence was most rapid for hot-water-scarified seeds, and by the May 17 sampling date, the 100% scarified seed treatment had a significantly ($P < .05$) greater seedling density than the 50% scarified/50% unscarified seed treatment, which in turn, had a greater density than the unscarified seed treatments (Table 3). Trends in seedling emergence from scarified and unscarified seed in the field followed those of germination of similarly treated seeds in 60/78 and 68/86°F temperature regimes in controlled environments (Table 1). Below-normal precipitation from mid-May through mid-July induced soil cracking (up to 2.5 inches wide), especially along drill rows. Seedling root systems were exposed to the atmosphere, resulting in the desiccation of many seedlings. Seedlings with adequate root-soil contact endured the dry period, but seedling numbers were further reduced by an outbreak of grasshoppers before the July 17 sampling date. Grasshoppers were controlled with diazinon [0,0-dimethyl 0-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate]. Remaining plant did not flower or set seed before leaf senescence in mid-July.

Table 3. Density (plants/40 inches of row length) of Illinois bundleflower over three growing seasons (1985-1987) in a native pasture near College Station, Texas as influenced by seed treatment.¹

Seed treatment ²	Sampling date				
	1985		1986		1987
	May 17	July 17	May 21	July 22	May 20
Unscarified, recommended rate	0.6 d ³	0.3 c	1.6 d	1.8 b	2.3 b
Unscarified, double recommended rate	1.0 bc	0.5 bc	2.1 a	2.2 a	3.0 a
100% scarified, recommended rate	1.5 a	0.7 ab	1.0 c	1.0 c	1.4 c
50% scarified/50% unscarified recommended rate	1.2 b	0.8 a	1.9 ab	1.8 b	2.7 ab

¹Seed treatment x sod suppression treatment interaction was nonsignificant ($P < .05$), seed treatment data have been pooled across sod suppression treatments.

²Sacrificed = hot water at 175°F for 3 minutes; recommended seeding rate = 1.5 lbs. PLS/acre.

³Means followed by same letter for each sampling date are not significantly different ($P < .05$) using Duncan's multiple range test.

Established plants initiated new growth in mid-March 1986, and new seedlings established from remaining dormant seed in drill rows in response to favorable precipitation in May (2.0 inches on May 1, 0.6 inches on May 10, and 1.0 inch on May 15 and 17). The unscarified, double seeding rate treatment had the greatest remaining seed bank and thus the greatest increase in seedling density, followed

by the unscarified, recommended seeding rate treatment, the 50% unscarified seed treatment, and the 100% scarified seed treatment (Table 3). Above-normal precipitation in May and June maintained fairly constant plant densities until the end of the second growing season. By the July 22 sampling date, the majority of plants established in the first growing season had flowered and set seed.

Plant densities increased for all seed treatments during the third growing season (1987) due to seedling recruitment from seed produced by established plants and possibly from dormant seed remaining in the drill rows in vegetation gaps in all sod suppression treatments. The 100% scarified seed treatment, which had the highest plant density at the beginning of the study now had the lowest density, due in part, to the lack of a dormant seed bank.

The effects of sod suppression treatments were evident during all three growing seasons. Plant density was significantly ($P < .05$) greater for herbicide and disking treatments than the untreated control throughout the study period, and density on herbicide-treated plots was greater than that for disking plots during the second and third growing season (Table 4). Herbicide and disking treatments created larger vegetation gaps for plant establishment (Grime 1979) than those occurring naturally in the untreated control. Plant densities were greater in the herbicide treatment than in the disking treatment because of: 1) less soil disturbance and subsequent competition from invading species; and 2) conservation of moisture and moderation of temperature near the soil surface by leaving a mulch in place. Similar, gradual increases in plant density in vegetation gaps have been reported for Sabine Illinois bundleflower sod-seeded in Coastal bermudagrass (*Cynodon dactylon*) on reclaimed lignite overburden in central Texas (Skousen and Call 1987).

Table 4. Density (plants/40 inches of row length) of Sabine Illinois bundleflower over three growing seasons (1985-87) in a native pasture near College Station, Texas as influenced sod suppression treatment.¹

Sod treatment ²	Sampling date				
	1985		1986		1987
	May 17	July 17	May 21	July 22	May 20
Untreated	0.7 c ³	0.2 c	1.1 c	1.0 c	1.2 c
Disked	1.1 ab	0.6 ab	1.6 b	1.7 b	2.6 b
Herbicide	1.4 a	0.9 a	2.2 a	2.4 a	3.2 a

¹Sod suppression treatment x seed treatment interaction was nonsignificant ($P < .05$), sod suppression data have been pooled across seed treatments.

²Disked = one pass with tandem offset disk; Herbicide = 50% suppression with glyphosate.

³Means followed by same letter for each sampling date are not significantly different ($P < .05$) using Duncan's multiple range test.

Management Implications

Scarified and unscarified seeds of Sabine Illinois bundleflower germinate over a wide range of temperature and substrate moisture regimes. High cumulative germination and rapid germination rates at 0 and -2.5 atmosphere water potential levels in 60/78 and 68/86°F temperature regimes indicate that optimum germination would occur in April and May when adequate moisture was available (as in the sod-seeding study). Scarification accelerates the rate of germination

under transiently favorable water conditions that occur more often at the high end of the favorable temperature range for germination.

When renovating pastures with perennial legumes such as Illinois bundleflower, land managers should create vegetation gaps and persistent seed banks. Vegetation gaps that leave a mulch in place without disturbing the soil surface (e.g. banding of glyphosate herbicide) are most desirable since they conserve moisture and modify temperatures in the seeded and do not encourage colonization by invading species that could compete for available resources. The germinability of the seeded species should be high enough to allow for adequate establishment during the growing season, but some dormant seed should be included if the initial stand is diminished by unfavorable environmental conditions. A mixture of scarified and unscarified seed would meet these criteria.

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