Temperature and Scarification Effects on Germination of Prostrate Bundleflower Seeds

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Abstract

A hard seed coat restricts germination of prostrate bundleflower [*Desmanthus virgatus* var. *depressus* (Humbolt and Bonpland ex Willd.) Turner] seeds. Our objectives were to determine: (1) the effects of temperature on germination of scarified and untreated seeds in the light and dark and (2) the efficacy of various presowing treatments in increasing germination. Scarified (nicked with a razor blade) and untreated seeds were germinated at 5–15, 10–20, 15–25, 20–30, 25–35, and 30–40° C (12 hours - 12 hours) in the dark or with light during the warmer temperature. Effects of scarification with 17 M H_2SO_4, hot (80° C) water, 0.7 mol liter^{-1} NaOCl, 2.9 mol liter^{-1} H_2O_2, and nicking with a razor blade on germination were compared. Maximum germination of untreated seeds was only 6%. Germination of scarified seeds exceeded 90% at 15–25° C and higher temperatures. Light did not affect germination at optimal temperatures for germination. Nicking seeds with a razor blade, soaking 40 minutes in 17 M H_2SO_4, and soaking 25 minutes in hot (80° C) water resulted in 91, 88, and 78% germination, respectively, compared to 3% for controls. Our results indicated that, for best germination, seeds should be soaked 40 minutes in 17 M H_2SO_4 or nicked with a razor and planted when mean minimum-maximum soil temperatures exceed 15–25° C.

Key Words: seed dormancy, *Desmanthus virgatus* var. *depressus*, range seeding, presowing seed treatments

Prostrate bundleflower [*Desmanthus virgatus* var. *depressus* (Humbolt and Bonpland ex Willd.) Turner] ranges from the Rio Grande Plains north to central Texas and west to New Mexico (Vines 1960). The native legume is eaten by white-tailed deer (*Odocoileus virginianus* Raf.) (Drawe 1968) and its seeds are consumed by bobwhites (*Colinus virginianus* L.) (Wood et al. 1986). The plant has been evaluated by the USDA Soil Conservation Service (SCS) at Knox City, Texas, for use in range seeding and erosion control (Richard Heizer, State Plant Materials Specialist, USDA-SCS, pers. commun.).

The hard coat of *Desmanthus* seeds restricts germination. Lating (1961) reported that germination of Illinois bundleflower [*D. illinoensis* (Michx.) MacM. ex Robins. and Fern.] was increased by soaking seeds for 10 minutes in concentrated sulfuric acid (H_2SO_4) and by clipping seeds at the end opposite the micropyle with a knife. Haferkamp et al. (1984) found that velvet bundleflower (*D. velutinus* Scheele) germination was increased by either cutting the seed coat with a razor, immersion of seeds in hot (80° C) water for 3 minutes, or soaking seeds for 17 minutes in concentrated H_2SO_4. Our objectives were to determine: (1) the effects of 6 alternating temperature regimes on germination of scarified and untreated seeds in the light and dark and (2) the efficacy of various presowing treatments in increasing germination of prostrate bundleflower.

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Methods

Seeds of accession 436898 prostrate bundleflower were obtained from the USDA-SCS Plant Materials Center at Knox City, Texas, and were stored in a cloth bag at 15° C and 40% relative humidity. They were 3 years old when used in experiments.

Seeds were germinated in blotter paper underlain by a layer of creped cellulose placed in 13.0 by 13.5 by 3.5-cm plastic boxes with tightly fitting lids. The substratum was moistened with 100 ml of tap water and remoistened when necessary. Seeds were treated with thiram [bis (dimethylthiocarbamoyl) disulfide] to minimize fungal growth.

Experiments were conducted with 4 boxes of 100 seeds each per treatment. Plastic boxes were arranged in controlled environment chambers in a randomized complete-block design. Each experiment was conducted twice except when otherwise stated. Data for the 2 experiments were combined for analysis and values reported in the text are the means of the experiments.

Prostrate bundleflower seeds were considered germinated when at least 1 cotyledon was exposed and radicles were 5 mm or greater in length (Kissock and Haferkamp 1983). Seedlings not meeting these requirements at the end of an experiment were considered abnormal. Counts were made daily for 28 days in temperature experiments and 14 days in presowing-treatment experiments. Germination rate index (GRI) was calculated as the sum of the quotients of the number of seeds germinated divided by the number of days for germination (Maguire 1962). Corrected germination rate index (CGRI) was obtained by dividing GRI by the final germination percentage and multiplying by 100 (Evetts and Burnside 1972).

Temperature, Light, and Scarification

Effects of temperature, light, and scarification on germination were determined by germinating scarified and untreated seeds at alternating temperatures of 5–15, 10–20, 15–25, 20–30, 25–35, and 30–40° C (12 hours - 12 hours) in the dark or with light during the warmer temperatures. Seeds were scarified by nicking the distal end with a razor blade (Kissock and Haferkamp 1983, Haferkamp et al. 1984). For the light treatment, cool-white fluorescent light with an average photosynthetic photon flux density of 24 μmol $m^{-2}s^{-1}$ was provided. To maintain constant darkness, plastic boxes were wrapped in 3 layers of aluminum foil. A green light was used for counts.

Table 1. Effects of 6 alternating (12 hours–12 hours) temperatures (° C), light (12 hours daily), and scarification (nicked with a razor blade) on percentage mean germination and standard deviations, corrected germination rate index (CGRI), and percent abnormal seedlings of prostrate bundleflower[1].

Seed treatments	Temperature 5–15	10–20	15–25	20–30	25–35	30–40
Percent germination						
Light						
Scarified	3(1.8)	67(14.8)	94(3.4)	95(1.8)	96(2.4)	92(2.4)
Untreated	0	2(0.8)	4(2.4)	6(2.6)	5(1.5)	6(2.9)
Dark						
Scarified	18(11.3)	81(6.6)	91(5.0)	95(1.9)	93(3.0)	93(4.0)
Untreated	0	2(2.1)	4(2.3)	5(1.2)	5(1.6)	4(2.3)
CGRI						
Light						
Scarified	5(2.4)	14(2.2)	30(1.3)	53(6.0)	50(1.0)	64(11.7)
Untreated	0	12(3.5)	18(10.2)	29(10.6)	26(3.5)	36(14.4)
Dark						
Scarified	6(1.0)	17(2.1)	27(1.1)	50(15.3)	38(4.1)	59(10.5)
Untreated	0	10(3.1)	14(4.3)	25(5.8)	26(11.9)	20(15.1)
Percent abnormal seedlings						
Light						
Scarified	16(11.1)	5(3.0)	1(0.6)	1(0.8)	1(0.7)	1(1.2)
Untreated	<1(0.7)	<1(0.5)	<1(0.5)	<1(0.5)	<1(0.4)	<1(0.4)
Dark						
Scarified	23(8.8)	3(1.6)	3(1.4)	1(0.7)	1(0.8)	1(1.0)
Untreated	0	1(0.7)	<1(0.5)	1(0.8)	1(1.1)	1(1.0)

[1]Values are means and standard deviations (in parentheses), n = 8.

Response curve analysis was used to determine the relationship between temperature and germination (Snedecor and Cochran 1967). Analysis of variance was used to determine if significant differences existed between light and scarification treatment means within each temperature. An arcsine transformation was used on percent germination and abnormal seedling data for analysis.

Presowing Treatments

Seeds were: (1) soaked 0, 20, 40, 60, and 80 minutes in concentrated (17 M) sulfuric acid (H_2SO_4) (Young et al. 1981); (2) soaked 20, 40, 60, and 80 minutes in hot water (80° C) (Kissock and Haferkamp 1983, Haferkamp et al. 1984); (3) soaked 0, 15, 30, 45, and 60 minutes in 0.7 mol liter^{-1} sodium hypochlorite (NaOCl), (4) nicked through the coat with a razor blade (Kissock and Haferkamp 1983, Haferkamp et al. 1984); and (5) soaked 0, 15, 30, 45, and 60 minutes in 2.9 mol liter^{-1} hydrogen peroxide (H_2O_2) (Stidham et al. 1980). Seeds were rinsed 5 minutes with tap water after soaking. These experiments were conducted once with 4 replications of 100 seeds per box.

Response curve analysis was used to predict the optimum level of each presowing treatment. The optimum level of each treatment was then compared in a final experiment to determine the presowing treatment most effective in enhancing germination.

Data from the final experiment were analyzed by analysis of variance (Snedecor and Cochran 1967). Tukey's test was used at the 0.05 level to identify significantly different means when significant F values were found (Kleinbaum and Kupper 1978). An arcsine transformation was used on percent germination and abnormal seedling data for analysis.

Results

Temperature, Light, and Scarification

Scarified and untreated seeds exhibited a quadratic response to temperature for percent germination in the light and in the dark (Tables 1 and 2). Germination of scarified seeds exceeded 90% at 15–25° C and higher temperatures. Maximum germination of untreated seeds was only 6%.

CGRI of scarified and untreated seeds increased with increasing temperature in the light and dark (Tables 1 and 2). Maximum CGRI of scarified seeds was at 30–40° C. CGRI was 13 times higher at 30–40° C than at 5–15° C for scarified seeds in the light.

Table 2. P- and R^2 values for response curve analyses of the relationship between percent germination, corrected germination rate index (CGRI), and percent abnormal seedlings and temperature for prostrate bundleflower. R^2 values are for the quadratic model when it was significant ($P<0.05$); otherwise values are for the linear model[1].

	Treatment: Light Scarified	Light Untreated	Dark Scarified	Dark Untreated
Percent germination				
Linear	<0.01	<0.01	<0.01	<0.01
Quadratic	<0.01	<0.01	<0.01	<0.01
R^2	0.93	0.71	0.85	0.72
CGRI				
Linear	<0.01	<0.01	<0.01	<0.01
Quadratic	0.03	0.13	0.25	<0.01
R^2	0.90	0.64	0.75	0.53
Percent abnormal seedlings				
Linear	<0.01	0.63	<0.01	0.14
Quadratic	<0.01	0.21	<0.01	0.63
R^2	0.69	0.01	0.76	0.05

[1]Data for percent germination and percent abnormal seedlings were arcsine transformed.

Percent germination was similar ($P>0.05$) in the light and dark at 15–25° C and warmer temperatures (Tables 1 and 3). The light × scarification interaction was significant ($P<0.01$) at 5–15 and 10–20° C. At these temperatures, percent germination of scarified seeds was higher in the dark than light, whereas germination of untreated seeds was similar. For CGRI, the light × scarification interaction was significant ($P<0.05$) only at 10–20° C. CGRI of scarified seeds was higher in the dark than light, while that of untreated seeds was higher in the light. At other temperatures CGRI was similar ($P>0.05$) in the light and dark except at 30–40° C where it was lower in the dark.

Percent germination and CGRI of scarified seeds exceeded ($P<0.01$) that of untreated seeds under all temperature and light conditions (Tables 1 and 3). Scarified seeds exhibited 13–41 times higher percent germination than untreated seeds. At temperatures

Table 3. P- values for the analysis of variance of the effects of light (12 hours daily) and scarification (nicked with a razor blade) on percent germination, corrected germination rate index (CRRI), and percent abnormal seedlings of prostrate bundleflower for each temperature (° C)[1].

Seed treatment	Temperature					
	5–15	10–20	15–25	20–30	25–35	30–40
Percent germination						
Light (L)	<0.01	<0.01	0.48	0.62	0.36	0.88
Scarification (S)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
LXS	<0.01	<0.01	0.26	0.51	0.37	0.23
CGRI						
Light (L)	0.21	0.65	0.11	0.20	0.06	0.03
Scarification (S)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
LXS	0.21	0.03	0.71	0.94	0.08	0.18
Percent abnormal seedlings						
Light (L)	0.19	0.71	0.11	1.00	0.08	0.07
Scarification (S)	<0.01	<0.01	<0.01	0.12	0.03	0.02
LXS	0.07	<0.01	0.03	0.78	0.97	0.58

[1]Data for percent germination and percent abnormal seedlings were arcsine transformed.

above 15–25° C, germination of scarified seeds was 1.5 to 3 times more rapid than that of untreated seeds.

Scarification increased ($P<0.05$) the percent abnormal seedlings at all temperatures except 20–30° C (Tables 1 and 3). For scarified seeds, the percentage abnormal seedlings was highest at lower temperatures (Tables 1 and 2). Averaged across light treatments, there were 20 times more abnormal seedlings at 5–15° C than at 20–30° C. Cotyledons extended through the nicked portion of seeds at 5–15° C, but radicles did not reach 5 mm in length. Poor radicle development probably resulted because seedling vigor was low at this temperature. For untreated seeds, there was no significant ($P>0.05$) relationship between percent abnormal seedlings and temperature. Percent abnormal seedlings did not differ significantly ($P>0.05$) between light and dark (Tables 1 and 3).

Presowing Treatments

Prostrate bundleflower germination was not increased by soaking seeds in 0.7 mol liter^{-1} NaOCl and 2.9 mol liter^{-1} H_2O_2 (data not shown). Soaking seeds in hot (80° C) water for 0, 20, 40, 60, and 80 minutes resulted in 3, 87, 75, 17, and 1% germination, respectively, ($y = 2.450 + 8.117x - 0.217x^2 + 0.001x^3$, $R^2 = 0.96$, $P<0.01$). Soaking seeds in 17 M H_2SO_4 for 0, 20, 40, 60, and 80 minutes resulted in 3, 98, 99, 95, and 90% germination, respectively ($y = 4.496 + 7.054x - 0.151x^2 + 0.001x^3$, $R^2 = 0.98$, $P<0.01$). Predicted optimum duration of soaking for the hot water and H_2SO_4 treatments was 25 and 40 minutes, respectively.

Table 4. Effects of soaking in hot (80° C) water for 25 minutes, soaking in 17 M H_2SO_4 for 40 minutes, and nicking with a razor blade on germination of prostrate bundleflower seeds at 20–30° C (12 hours with darkness - 12 hours with light)[1].

Treatment	Percent germination	CGRI	Percent abnormal seedlings
Control	3a	31a	0a
Hot water	78b	42b	1ab
H_2SO_4	88bc	46b	4b
Nicked	91c	41b	1a

[1]Values are means and standard deviations (in parentheses), n = 8. Means in a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's test.

Germination of seeds soaked 25 minutes in hot water and 40 minutes in H_2SO_4 was compared to that of seeds nicked with a razor and untreated in a final experiment. Nicking and soaking seeds in H_2SO_4 produced similar ($P>0.05$) results; however, percent germination of nicked seeds was higher ($P<0.05$) than that of seeds soaked in hot water (Table 4). CGRI was similar among presowing treatments. Although H_2SO_4 scarification resulted in a higher percentage of abnormal seedlings than other treatments, the number was low.

Discussion

May, June, and September are peak rainfall months in south Texas (Gould 1975). Planting before peak rainfall periods should aid in establishment of prostrate bundleflower and temperatures during these months should be within the optimum range for germination.

Planting scarified seeds when soil temperature are below 10–20° C may result in considerable abnormal germination. Planting under these conditions should be avoided because the quantity of viable seed will be lower when conditions become more favorable for germination.

Optimal temperatures for prostrate bundleflower germination are apparently higher than those for Illinois bundleflower. Townsend and McGinnies (1972) found that germination of Illinois bundleflower was 93% at 5–20° C (12 hours–12 hours) but was less than 35% at 15–25 and 20–35° C. Conversely, germination of velvet bundleflower was much higher at 10–20, 15–25, and 20–30° C than at 5–15° C (Haferkamp et al. 1984).

Methods effective in increasing germination of other *Desmanthus* species (nicking seeds, soaking in H_2SO_4, and soaking in hot water) also increased germination of prostrate bundleflower. Results indicated that nicking the seeds and soaking in 17 M H_2SO_4 are the most effective treatments. For large lots of seeds, scarifying with H_2SO_4 would be more practical than nicking.

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