Enhancing Germination of Spiny Hackberry Seeds

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Abstract

Establishment of spiny hackberry (*Celtis pallida* Torr.) in plantings for wildlife has been hampered by low seed germination. We evaluated methods for enhancing germination of spiny hackberry by subjecting seeds to: (1) chemical scarification with 2.9 mol liter⁻¹ H₂O₂, 0.71 mol liter⁻¹ NaOCl, and concentrated (18 mol liter⁻¹) H₂SO₄; (2) rinsing with water; (3) 0, 0.3, 1.4, 2.9, and 4.3 mmol liter⁻¹ gibberellic acid (GA); (4) 0.02 mol liter⁻¹ KNO₃; (5) mechanical scarification, or (6) moist heat (30° C) followed by moist prechilling at 7° C for 2 weeks. Untreated seeds exhibited higher percent germination in the light than in the dark. Percentage germination at 30° C and germination rate were increased in the light and in the dark by either 1.4 mmol liter⁻¹ GA or 3 days of moist heat (30° C) followed by a 2-week moist prechill at 7° C. A combination of mechanical scarification + 1.4 mmol liter⁻¹ GA + 3 days moist heat (30° C) followed by moist prechilling for 2 weeks at 7° C increased germination from 1% (controls) to 49%. Germination varied slightly with seed source, but a large proportion of all lots was dormant. Spiny hackberry seeds can be treated with a combination of mechanical scarification + 1.4 mmol liter⁻¹ GA + 3 days moist heat (30° C) followed by moist prechilling for 2 weeks at 7° C before planting to increase percentage and rate of germination.

Spiny hackberry (*Celtis pallida* Torr.), a densely branched evergreen shrub, occurs from central Texas to Arizona, and south to Mexico (Vines 1960). Spiny hackberry is browsed by white-tailed deer (*Odocoileus virginianus* Raf.) (Everitt 1983c), and its fruits are relished by birds (Vines 1960, Scifres 1980). The shrub is highly favored as a nesting site by white-winged dove (*Zenaidura macroura* L.) (Brown et al. 1977), and it provides excellent cover for quail (*Colinus virginianus* L., *Lophortyx gambelii* Gambell) (Warnock 1977, Thornburg 1982, Lehmann 1984). Thus, there is considerable interest in establishment of spiny hackberry for wildlife food and cover.

Several species of *Celtis*, including spiny hackberry, exhibit seed dormancy. Hackberry (*C. occidentalis* L.) germination is greatly enhanced through fermentation of the fruits for 3 days at room temperature and subsequent removal of pulp before stratification (Bonner 1974). Hackberry and sugar hackberry (*C. laevigata* Willd.) seeds require 60 to 90 days of stratification at 5° C, while netleaf hackberry (*C. reticulata* Torr.) seeds require 120 days of stratification at 5° C (Bonner 1974). The objective of this study was to determine if germination of spiny hackberry seeds could be enhanced by selected treatments.

Methods

Spiny hackberry fruits were collected from native populations in Cameron, Kleberg, and Hidalgo Counties, Texas. Soils of the Cameron County collection site were Willacy fine sandy loam, a fine-loamy mixed, hyperthermic Udic Arguistoll, and Raymondville clay loam, a fine, mixed, hyperthermic Vertic Calciustoll. Soils of the Kleberg County site were Orelia fine sandy loam, a fine-loamy, mixed, hyperthermic Typic Ochraqulf and Willacy fine sandy loam. Soil of the Hidalgo County site was Rio Grande silt loam, a coarse-silty, mixed (calcareous), hyperthermic Typic Ustifluvent. Fruits were macerated in a fruit press to separate seeds from pulp (Young et al. 1981). Care was taken not to damage seeds.

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Seeds were air-dried at 25° C and stored in cloth bags at 15° C and 40% relative humidity. Seeds from Cameron County were used in all experiments except when sources were compared.

Seeds were germinated on blotter paper underlain by a layer of creped cellulose (kimpack) placed in plastic boxes measuring 13.0 by 13.5 by 3.5 cm with tightly fitting lids (Fulbright et al. 1983). Substrata were moistened with 100 ml of distilled water or the appropriate test solution. Seeds were treated with thiram [bis dimethylthiocarbamoly] disulfide] to minimize fungal growth.

Seeds were considered germinated when part of at least 1 cotyledon was visible and radicles extruded to 5 mm. Counts were made weekly for 14 days in pilot experiments and daily in final experiments. Germination rate (GR) was calculated by the equation of Maguire (1962) where GR is the sum of the quotients of the number of seeds germinated each day divided by the number of days for germination.

Experiments were conducted at 30° C in controlled environmental chambers. Several authors have reported 30° C to be within the optimum temperature range for germination of various woody plant species in South Texas (Scifres 1974; Alaniz and Everitt 1978, 1980; Everitt 1983a, 1984). Seeds were exposed to cool-white fluorescent lights for 12 hours daily or were germinated in the dark. Photosynthetic photon flux density averaged 26 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Dark conditions were maintained by wrapping plastic boxes in aluminum foil and seeds were briefly exposed to light during counts.

Treatments evaluated in pilot experiments included: (1) soaking seeds in 2.9 mol liter⁻¹ hydrogen peroxide (H₂O₂) for 10, 20, and 30 min followed by a 5-min rinse in large volumes of tap water (Young et al. 1981); (2) soaking seeds in 0.71 mol liter⁻¹ sodium hypochlorite (NaOCl) for 10, 20, and 30 min followed by a 5-min rinse in large volumes of tap water (Stidham et al. 1980); (3) soaking seeds in concentrated (18 mol liter⁻¹) sulfuric acid (H₂SO₄) for 2, 4, 6, and 10 min followed by a 5-min rinse in large volumes of tap water (Young et al. 1981); (4) rinsing seeds in running water for 12, 24, 36 and 48 hours to remove soluble inhibitors (Young et al. 1981); (5) germinating seeds on substrata moistened with 0.3, 1.4, 2.9, and 4.3 mmol liter⁻¹ solutions of gibberellic acid (GA) (Mayer and Poljakoff-Mayber 1975); (6) germinating seeds on substrata moistened with 0.02 mol liter⁻¹ potassium nitrate (KNO₃) (Young et al. 1981); (7) nicking seeds with a razor (mechanical scarification) (Kissock and Haferkamp 1983); (8) moist prechilling seeds for 2 weeks at 7° C (Young et al. 1981); and (9) incubating imbibed seeds at 30° C for 1 week followed by moist prechilling at 7° C for 2 weeks (Young et al. 1981).

Two final experiments were conducted following pilot experiments. In the first experiment, effects of (1) mechanical scarification, (2) 1.4 mmol liter⁻¹ gibberellic acid (GA), (3) 3-day moist heat treatment followed by 2-weeks of moist prechilling at 7° C, and (4) light (12 hours daily) were determined in a 2 by 8 factorial experiment. A 3-day moist heat treatment was used in final experiments because a few seeds germinated after 3 days at 30° C. The experiment was conducted twice, once in December 1984 and again in early January 1985 with seeds harvested in late May and early June 1984.

Effects of seed source and seed treatments on germination were determined in the second experiment. Seeds from the 3 sources were either untreated or were scarified, subjected to a 3-day moist heat treatment followed by a 2-week moist prechill at 7° C, and germinated on substrata moistened with 1.4 mmol liter⁻¹ GA. The experiment was conducted in December 1984 and early January

1985 with seeds harvested in mid-July 1984.

A randomized complete-block design was used in all experiments. Each treatment was assigned to 4 plastic boxes containing 25 seeds each in pilot experiments. In other experiments, each treatment was assigned to 4 plastic boxes containing 50 seeds each and experiments were repeated twice. Data from the pilot GA experiment were analyzed by response curve analysis to examine the relationship between percent germination and GA concentration (Snedecor and Cochran 1967). Data from other experiments were analyzed by analysis of variance (Snedecor and Cochran 1967). Data were subjected to arcsine $\sqrt{0.01 \times \%}$ transformation before analysis of variance. Tukey's test was used at the 0.05 level of probability to identify mean differences when F values for treatments were significant. Untransformed data are reported in the text and in tables 1, 3, and 4. All differences discussed in the following section were significant at the 0.05 level of probability unless other stated.

Results and Discussion

Chemical scarification (with 2.9 mol liter⁻¹ H₂O₂, 0.71 mol liter⁻¹ NaOCl, and concentrated H₂SO₄), rinsing seeds in running tap water to leach out water soluble inhibitors, and germinating seeds on substrata moistened with 0.02 mol liter⁻¹ KNO₃ did not increase germination (data not shown). Germinating seeds on substrata moistened with 0, 0.3, 1.4, 2.9, and 4.3 mmol liter⁻¹ GA resulted in 10, 17, 27, 23, and 36% germination ($Y=14.0 + 4.8X$, $r = 0.62$), respectively, for intact seeds, and 9, 45, 40, 52, and 52% germination ($Y = 27.2 + 7.0X$; $r = 0.60$), respectively, for mechanically scarified seeds. Moist prechilling seeds for 2 weeks at 7° C did not increase germination. Placing seeds on moist blotter paper at 30° C for 1 week followed by moist prechilling 2 weeks at 7° C increased germination from 21% (controls) to 43%.

Percent germination of seeds treated 3 days with moist heat (30° C) followed by moist prechilling at 7° C for 2 weeks (hereafter referred to as "heat/chill") did not differ significantly ($P>0.05$) from percent germination of seeds treated with 1.4 mmol liter⁻¹ GA or mechanical scarification (Table 1). Percent germination of heat/chilled seeds and seeds treated with GA was higher than controls in

Table 1. Effects of selected presowing seed treatments on percentage germination of spiny hackberry seeds at 30° C.

Seed treatment	Light regime	
	Light	Dark
Control	32e ¹	20e
Mechanical scarification (SC)	35de	34cd
1.4 mmol liter ⁻¹ gibberellic acid (GA)	42cd	35bcd
3-Day moist heat (30° C)/2-week moist prechill (7° C) (heat/chill)	43bcd	35cd
SC + GA	41cde	27de
SC + heat/chill	49bc	44ab
GA + heat/chill	52ab	38abc
SC + GA + heat/chill	62a	45a

¹Means in a column followed by the same letter are not significantly different at the 0.05 level of probability according to Tukey's test. Data for light and dark regimes were analyzed separately because the light by seed treatment interaction was significant ($P<0.01$).

the light and in the dark. For mechanically scarified seeds, percent germination was higher than controls in the dark but not in the light.

Heat/chilling combined with mechanical scarification or GA increased germination more in the light than did mechanical scarification or GA applied individually (Table 1). Percent germination of seeds treated with mechanical scarification + GA + heat/chill was about twice that of controls regardless of light treatments.

The light by seed treatment interaction was significant for percent germination (Table 2). Percent germination was similar between light and dark for seeds that were mechanically scarified, treated with GA, heat/chilled, or mechanically scarified + heat/

Table 2. Analysis of variance for the effects of selected presowing seed treatments on percentage germination of spiny hackberry seeds at 30° C.

Source of variation	df	Mean square ¹	F	Probability of a >F
Replication (R)	7	0.020		
Seed treatments (T)	7	0.133	28	0.0001
Error a (RXT)	49	0.005		
Light (L)	1	0.317	103	0.0001
Error b (RXL)	7	0.003		
TXL	7	0.013	4	0.0015
Error (RXTXL)	49	0.003		

¹Arcsine $\sqrt{\% \times 0.01}$ transformed percent germination.

chilled. For untreated seeds and seeds treated with mechanical scarification + GA, GA + heat/chilling, and mechanical scarification + GA + heat/chilling, percent germination was higher in the light than in the dark. Our results were not consistent with findings for seeds of several other woody plants of South Texas, which germinated equally well in darkness and light (Scifres and Brock 1969; Scifres 1974; Everitt 1983 a,b, 1984).

Germination rate was similar among seeds treated with mechanical scarification alone, GA alone, and heat/chill alone (Table 3).

Table 3. Effects of selected presowing seed treatments on germination rate of spiny hackberry seeds at 30° C.

Seed treatment	Light regime	
	Light	Dark
Control	1.8 c ¹	1.2 e
Mechanical scarification (SC)	2.1 bc	2.1 cd
1.4 mmol liter ⁻¹ gibberellic acid (GA)	2.6 bc	2.2 c
3-Day moist heat/2-week prechill (heat/chill)	2.8 b	2.2 c
SC + GA	2.7 bc	1.5 de
SC + heat/chill	3.9 a	3.1 ab
GA + heat/chill	4.5 a	2.7 bc
SC + GA + heat/chill	4.7 a	3.4 a

¹Column means followed by the same letter are not significantly different at the 0.05 level of probability according to Tukey's test. Data for light and dark regimes were analyzed separately because the light by seed treatment interaction was significant ($P<0.01$).

Germination rate of mechanically scarified seeds was higher than that of controls in the dark but not in the light. The light by seed treatment interaction was significant ($P<0.01$) for germination rate. Germination rate was higher in the light than in the dark for seeds treated with mechanical scarification + GA, GA + heat/chill, and mechanical scarification + GA + heat/chill. Germination rate was similar under light and dark conditions for other treatments. Treating seeds with either mechanical scarification + heat/chill, GA + heat/chill, or mechanical scarification + GA + heat/chill produced higher germination rates in the light than applying mechanical scarification alone, GA alone, or heat/chill alone (Table 3). In the dark, mechanical scarification + heat/chill and mechanical scarification + GA + heat/chill produced higher germination rates than any treatment applied singly.

Ease of establishment would be increased if populations of spiny hackberry with inherently low seed dormancy were available. Seeds of sources examined in the second final experiment were highly dormant. Mechanical scarification + 1.4 mmol liter⁻¹ GA + heat/chill enhanced germination of seeds from all sources (Table 4). Seeds from Cameron County exhibited higher percent germination than seeds from Hidalgo County, but germination rates were similar. Germination rate of seeds from Cameron County was higher than that of seeds from Kleberg County, but percentage germination was similar between sources.

Differences in percentage of seeds germinated and rate of germi-

Table 4. Effects of seed source and a combination of mechanical scarification + 1.4 mmol liter⁻¹ GA + 3-day moist heat (30° C) treatment followed by 2-weeks of moist prechilling at 7° C (SC+GA+heat/chill) on spiny hackberry germination at 30° C with 12 hours of light daily.

Parameter and treatment	Seed source			Seed treatment means
	Cameron	Kleberg	Hidalgo	
Germination (%)				
Control	3	1	1	1
SC+GA+heat/chill	52	49	47	49**
Seed source means	27a ¹	25ab	24b	
Germination rate				
Control	0.09	0.05	0.02	0.05
SC+GA+heat/chill	4.27	3.70	3.78	3.92**
Seed source means	2.18a	1.87b	1.90ab	

**Significantly different from controls ($P < 0.01$).

¹Means in a row followed by the same letter were not significantly different at the 0.05 level of probability according to Tukey's test.

nation for the same treatment between experiments were attributed to differences in seed age and time of harvest. Untreated seeds harvested in late May and early June in Cameron county exhibited 10% germination in July (pilot GA experiment) and 20% germination in December and early January in the dark (Table 1). Untreated Cameron County seeds harvested in mid-July produced only 3% germination in the light in December and early January (Table 4) compared to 32% for seeds harvested in late May and early June germinated during the same time period (Table 1). Differences in germination of different aged seeds may have resulted from afterripening.

Our results indicate treating spiny hackberry seeds with mechanical scarification + 1.4 mmol liter⁻¹ GA + 3 days moist heat (30° C) followed by moist prechilling for 2 weeks at 7° C before planting will increase percentage and rate of germination. This treatment is similar to 3-day fermentation at room temperature followed by scarification reported by Bonner (1974) to enhance germination of hackberry. Enhancement of germination by GA and moist heat followed by moist prechilling indicates that dormancy in spiny hackberry seeds may be caused partly by physiological mechanisms, but further research is needed to determine exact causes of dormancy.

Literature Cited

- Alaniz, M.A., and J.A. Everitt. 1978. Germination of Texas ebony seeds. J. Rio Grande Valley Hort. Soc. 32:95-100.
 Alaniz, M.A., and J.H. Everitt. 1980. Germination of anacua seeds. J. Rio Grande Valley Hort. Soc. 34:75-80.

- Bonner, F.T. 1974. *Celtis* L. Hackberry, p. 298-300. In: C.S. Schopmeyer (technical coordinator), Seeds of Woody Plants in the United States. USDA, Agr. Handb. 450.
 Brown, D.E., D.R. Blankenship, P.K. Evans, W.H. Kiel, Jr., G.L. Wag-german, and C.K. Winkler. 1977. White-winged dove (*Zenaida asiatica*), p. 246. 272. In: G.C. Sanderson (ed.), Management of Migratory Shore and Upland Game Birds in North America. Internat. Ass. of Fish and Wildlife Agencies, Washington, D.C.
 Everitt, J.H. 1983a. Seed germination characteristics of 3 woody plant species from southern Texas. J. Range Manage. 36:246-249.
 Everitt, J.H. 1983b. Seed germination characteristics of 2 woody legumes (Retama and Twisted Acacia) from South Texas. J. Range Manage. 36:411-414.
 Everitt, J.H. 1983c. Effects of plant shredding on nutrient content of 4 south Texas deer browse species. J. Range Manage. 36:779-781.
 Everitt, J.H. 1984. Germination of Texas persimmon seed. J. Range Manage. 37:189-192.
 Fulbright, T.E., E.F. Redente, and A.M. Wilson. 1983. Germination requirements of green needlegrass (*Stipa viridula* Trin.). J. Range Manage. 36:390-394.
 Kisko, D.C., and M.R. Haferkamp. 1983. Presowing seed treatment effects on germination of *Engelmannia pinnatifida* and *Indigofera mini-ata* var. *leptosepala*. J. Range Manage. 36:94-97.
 Lehmann, V.W. 1984. Bobwhites in the Rio Grande Plain of Texas. Texas A&M Univ. Press, College Station.
 Maguire, J.D. 1962. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 2:176-177.
 Mayer, A.M., and A. Poljakoff-Mayber. 1975. The germination of seeds. Pergamon Press, Oxford.
 Scifres, C.J. 1974. Salient aspects of huisache seed germination. Southw. Natur. 18:383-392.
 Scifres, C.J. 1980. Brush management-principles and practices for Texas and the southwest. Texas A&M Univ. Press, College Station.
 Scifres, C.J., and J.H. Brock. 1969. Moisture-temperature interrelations in germination and early seedling development of honey mesquite. J. Range Manage. 22:334-337.
 Snedecor, G.W., and W.G. Cochran. 1967. Statistical methods, 6th edition. The Iowa State University Press, Ames.
 Stidham, N.D., R.M. Ahrling, J. Powell, and P.L. Claypool. 1980. Chemical scarification, moist prechilling, and thiourea effects on germination of 18 shrub species. J. Range Manage. 33:115-118.
 Thornburg, A.A. 1982. Plant materials for use on surface-mined lands in arid and semi-arid regions. USDA-SCS, SCS-TP-157.
 Vines, R.A. 1960. Trees, shrubs and woody vines of the southwest. University of Texas Press, Austin.
 Warnock, B.H. 1977. Wildflowers of the Davis Mountains and Marathon Basin, Texas. Sul Ross State University, Alpine, Texas.
 Young, J.A., R.A. Evans, B.L. Kay, R.E. Owen, and J. Budy. 1981. Collecting, processing, and germinating seeds of western wildland plants. USDA-SEA, ARS-W-3.

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