Seed Treatments Improve the Germination and Seedling Emergence of Switchgrass (*Panicum virgatum* L.)

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Abstract. Switchgrass (*Panicum virgatum* L.) is one of the perennial, native, warm-season grasses recommended as a component of wildflower meadows. Seed treatments to alleviate low seed vigor and seed dormancy of switchgrass would enhance establishment of either plug transplants or seedlings after direct sowing into the meadow. "Heavy" seeds (45.5 mg/50 seeds) of open-pollinated switchgrass stored under cool and dry conditions (average 13 °C, 30% relative humidity) for 24 months had higher germination percentage than "light" seeds (26.0 mg per 50 seeds). In factorial combination, the heavy seeds were subjected to acid scarification (8 M H₂SO₄ for 5 min), sodium hypochlorite treatment (5.25% NaOCl for 15 min), and moist chilling (prechilling in 0.2% KNO₃ for 14 days). Acid scarification followed by NaOCl treatment additively increased germination, a response that was associated with marked corrosion of the lemma margin in the distal region of the caryopsis, as observed by scanning electron microscopy. Prechilling the seeds following acid scarification and NaOCl further increased germination. All three treatments combined (acid scarification, NaOCl, and prechilling) almost doubled the final emergence and greatly increased seedling shoot dry mass in both a warm and cool postsowing environment. However, the effectiveness of these seed treatments was lost after 32 months of dry storage.

Switchgrass, a perennial warm-season grass native to North America, is a dominant species of the tallgrass prairies. Switchgrass is an important forage crop (Vogel et al., 1991) and shows much potential as a sustainable herbaceous energy crop from which a renewable source of transportation fuel and/or biomass-generated electricity could be derived (Sanderson et al., 1996). Switchgrass is valued as a perennial ornamental, since it possesses attractive flowers and foliage, with newer vegetatively propagated cultivars having red or blue foliage (Still, 1994). *Panicum virgatum* is one of several "nonspreading" (intravaginal growth habit) grasses recommended for inclusion in seed mixtures for wildflower meadows (Martin, 1990). In such meadows, the switchgrass assists in stabilizing the soil and provides aesthetic interest.

Low seed vigor and seed dormancy, characteristics of many warm-season grass species that result in slow and inconsistent establishment (Beckman et al., 1993), have greatly limited switchgrass use (Panciera et al., 1987). Kneebone and Cremer (1955) found a positive relationship between switchgrass seed size and the percentage and rate of seedling emer-

gence. Sautter (1962) concluded that much of switchgrass seed dormancy was caused by the floret bracts. Such physical dormancy in switchgrass has been alleviated partially by a 15-sec abrasion of seeds with emery cloth (Jensen and Boe, 1991). Exposure of switchgrass seeds to 8 M H₂SO₄ for 5 min (Zarnstorff et al., 1994) or concentrated (18 M) H₂SO₄ for 10 min (Tischler et al., 1994) increased germination. Switchgrass germination also was increased by soaking the seeds in 5.25% sodium hypochlorite (NaOCl) for 15 min (Panciera et al., 1987). Since a 14-d moist chilling (prechilling) of switchgrass seeds prior to sowing also increased germination percentage (Hsu et al., 1985; Zarnstorff et al., 1994), seed dormancy in this species can be attributed to physiological as well as to physical factors.

Improved switchgrass establishment in the wildflower meadow, whether sown directly or transplanted as plugs, would benefit the landscape and nursery industries. Our objective was to examine the effects of seed size and a combination of seed treatments on the germination and emergence of switchgrass.

Materials and Methods

Seeds (fertile florets) of switchgrass were acquired from open-pollinated plants of the northern upland population grown in southern Minnesota in 1993 (Prairie Moon Co., Winona, Minn.). Following hand-harvesting in October 1993, the seeds were stored under cool and dry conditions [average 13 °C, 30% relative humidity (RH)] for 24 months before the present research was started (Sept. 1995). The seeds were separated into two mass classes using an air column (E.L. Erikson Products,

Brookings, S.D.). "Heavy" seeds averaging 45.5 mg (range = 43 to 48 mg) per 50 seeds (19% numerically of the seedlot) were selected for use in subsequent studies because "light" seeds averaging 26 mg (range = 23 to 29 mg) per 50 seeds (41% numerically of the seedlot) had <10% germination (data not shown).

Seed germination. This 2 × 2 × 2 factorial study consisted of acid scarification, NaOCl treatment, and presowing wet chilling (prechilling) seed treatments. Acid scarification was accomplished by submersing seeds in 8 M H₂SO₄ (1000 seeds per 50 mL) for 5 min (Zarnstorff et al., 1994) with occasional stirring, then rinsing the seeds under running water for 5 min before air drying. Both scarified and nonscarified seeds were divided into two groups. One group received no treatment, while the other was submersed in 5.25% NaOCl (100% household bleach, 500 seeds per 25 mL) and stirred occasionally for 15 min according to Panciera et al. (1987). These seeds then were rinsed in running water for 20 min. One-half of the seeds in each of the now-four groups then were prechilled by placing them on two layers of germination paper (Seed Germination Blotters No. 385; Seedburo Co., Chicago) moistened to saturation with 0.2% KNO₃ (m/v; Association of Official Seed Analysts, 1993) contained in 125 × 80 × 20-mm transparent polystyrene boxes (100 seeds per box). The boxes were sealed with tight-fitting lids and placed in a 5 °C refrigerator for 2 weeks, after which the seeds were washed from the boxes and air dried.

Seeds from the eight groups were divided into four replications of 50 seeds and placed in 125 × 80 × 20-mm transparent polystyrene boxes containing two layers of germination paper moistened with 15 mL half-strength Hoagland solution (Hoagland and Arnon, 1950) to simulate the solute potential of soil solution (Poljakoff-Mayber, pers. comm.). Each treatment combination (box) was arranged in randomized blocks in an incubator maintained at 30/15 °C (16/8 h) in darkness, a temperature regime recommended for standard germination tests of switchgrass (Association of Official Seed Analysts, 1993; International Seed Testing Association, 1985). Seed germination (marked by radicle protrusion) was counted daily for 21 d, with germinated seeds being removed at the time of counting. From these data, the angular transformation of the final germination percentage (FGP), days to 50% of FGP (G₅₀, an inverse measure of germination rate), and days between 10% and 90% FGP (G₁₀₋₉₀, an inverse measure of germination synchrony) were calculated and subjected to analysis of variance (ANOVA).

Samples of untreated seeds and seeds that were treated with acid, NaOCl, or both (25 seeds minimum per sample) were glued onto aluminum sample holders and sputter-coated with gold-palladium under vacuum (Postek et al., 1980) in preparation for examination using a scanning electron microscope (model 301; Philips, Eindhoven, The Netherlands).

Seedling emergence. This experiment was a 4 (seed treatment) × 2 (postsowing environ-

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ment) factorial with four replications of 100 seeds each. Seeds for the treatments selected for this study (scarified; scarified, then prechilled; scarified, then treated with NaOCl, then prechilled; and untreated seeds) were prepared as described above. Seeds used in this study had been stored at 13 °C, 30% RH for 28 months. The seeds were sown into 17 × 12 × 6-cm plastic flats (inserts) filled with Pro-Mix BX (Premier Brands, New Rochelle, N.Y.). Each flat (replication) contained 100 seeds sown 0.5 cm deep in four rows. The flats were arranged in randomized blocks under two temperature regimes (warm = 35/23 °C and cool = 25/15 °C) in a greenhouse during February when natural irradiation was supplemented by high-pressure sodium lamps (14 h). The flats were provided water as needed.

Seedling emergence (coleoptile first visible) was counted daily for 21 d after planting. From these data, the angular transformation of the final emergence percentage (FEP), days to

50% of FEP (E_{50} , an inverse measure of emergence rate), and days between 10% and 90% FEP (E_{10-90} , an inverse measure of emergence synchrony) were calculated and subjected to ANOVA.

At 21 d after planting, shoots of ten randomly chosen seedlings from each flat were cut from the root system just below the coleoptilar node and placed in a drying oven (65 °C). Seedling emergence variables and shoot dry mass were subjected to ANOVA.

Results

Seed germination. Acid scarification, NaOCl treatment, and prechilling each increased FGP; however, these treatments interacted in affecting FGP (Table 1). All seed treatments resulted in 60% to 79% FGP compared with 46% FGP for untreated seeds. The FGP of seeds that were only scarified (60%) was similar to that of seeds that received only

NaOCl (61%) or prechilling (62%). Seeds that received acid scarification and/or NaOCl treatment before prechilling had a greater FGP than seeds that received only prechilling. FGP was greater when seeds received both acid scarification and NaOCl treatment than when they received only one of these treatments, irrespective of prechilling. For seeds that received both NaOCl treatment and prechilling, acid scarification failed to increase FGP further, but increased germination synchrony (lower G_{10-90}).

Prechilled seeds germinated more rapidly (lower G_{50}), irrespective of scarification or NaOCl treatments (Table 1), whereas those treated only with NaOCl germinated more slowly (higher G_{50}) than untreated seeds. Synchrony of germination was greater (lower G_{10-90}) than that of untreated seeds only in seeds that received all three treatments.

Seedling emergence. Irrespective of post-sowing temperature regime, all treatments increased FEP but did not affect rate or synchrony of emergence (Table 2). The FEP and seedling shoot dry mass of scarified seeds were increased when the scarification was followed by both NaOCl and prechilling, but not when followed by prechilling alone. Providing seeds with all three treatments (scarification, NaOCl, and prechilling) resulted in FEP values that were 1.8- and 1.9-fold those of untreated seeds in the cool and warm environments, respectively. Seedling shoot dry masses from seeds given all three treatments were 1.7- and 1.3-fold those of untreated seeds in these respective postsowing environments.

Scanning electron micrographs of switchgrass seeds (Fig. 1) revealed that acid or NaOCl treatments caused corrosion of the distal margin of the lemma, the corrosion being more severe when these treatments were combined.

Discussion

Germination of "light" seeds was 7% vs. 45% for "heavy" seeds (26.0 and 45.5 mg per 50 seeds, respectively) when germinated at 30/15 °C (16/8 h) in 0.2% KNO_3 (m/v) with no presowing seed treatments (data not shown). Kneebone and Cremer (1955) also found a positive relationship between switchgrass seed size and percentage seedling emergence. Removal of lighter, possibly less mature seeds from the seedlot thus improved germination. Delaying harvest of seeds to increase the proportion of heavier, more mature seeds likewise may improve seedlot percentage germination.

Acid scarification, NaOCl treatment, and prechilling acted additively in increasing FGP (Table 1). Although acid scarification failed to increase the FGP of seeds that had received NaOCl and prechilling, all three treatments increased the rate (lower G_{50}) and synchrony (lower G_{10-90}) of germination.

Seed prechilling had a greater effect in increasing FGP than did acid scarification and NaOCl treatment (Table 1). Such a promotive effect of prechilling on switchgrass germination was noted by others (Hsu et al., 1985; Panciera et al., 1987; Zarnstorff et al., 1994).

Table 1. Final germination percentage (FGP), days to 50% of FGP (G_{50}) and days between 10% and 90% of FGP (G_{10-90}) of *Panicum virgatum* seeds at 30/15 °C (16/8 h) following acid scarification, sodium hypochlorite treatment, prechilling, or combinations thereof.

Seed treatments ^a			FGP		G_{50}	G_{10-90}
Scarification	NaOCl	Prechilling	(%)	[deg.]	(d)	(d)
—	—	—	46	[43]	3.1	4.3
+	—	—	60	[51]	3.3	3.9
—	+	—	61	[51]	4.1	6.5
—	—	+	62	[52]	1.6	2.6
+	+	—	67	[55]	2.9	3.3
+	—	+	70	[57]	1.9	2.2
—	+	+	73	[60]	2.5	5.9
+	+	+	79	[62]	2.2	2.0
LSD _{0.05}			[3]		0.4	2.2
Significance						
Scarification (S)			**		**	**
NaOCl (N)			**		***	*
S × N			NS		***	**
Prechill (P)			***		***	*
S × P			*		*	NS
N × P			NS		NS	NS
S × N × P			*		*	NS

Scarification = 5-min exposure to 8 M H_2SO_4 ; NaOCl = 15-min exposure to 5.25% sodium hypochlorite; prechilling = 2-week exposure to 0.2% KNO_3 at 5 °C.

NS, *, **, ***Nonsignificant or significant at $P < 0.05$, 0.01, 0.001, respectively.

Table 2. Final emergence percentage (FEP), days to 50% of FEP (E_{50}), days between 10% and 90% of FEP (E_{10-90}) and seedling shoot dry mass at 21 d after planting of *Panicum virgatum* seeds subjected to acid scarification, sodium hypochlorite treatment, prechilling, and postsowing temperature regimes.

Seed treatment ^a	Postsowing temp regime ^b	FEP		E_{50}	E_{10-90}	Shoot dry mass (mg/shoot)
		(%)	[deg.]	(d)	(d)	
None	Cool	34	[36]	6.7	3.2	0.6
Scarified	Cool	45	[42]	7.3	5.1	0.6
Scarified + prechilled	Cool	47	[43]	6.2	3.3	0.7
Scarified + NaOCl + prechilled	Cool	61	[51]	6.3	3.1	1.0
None	Warm	27	[31]	5.3	2.3	1.8
Scarified	Warm	38	[38]	6.1	3.2	1.7
Scarified + prechilled	Warm	45	[42]	5.4	4.0	2.0
Scarified + NaOCl + prechilled	Warm	51	[45]	4.7	2.7	2.3
LSD _{0.05}		[4]		0.6	1.3	0.3
Significance						
Seed treatment (ST)		***		***	*	***
Postsowing temperature (PT)		**		***	NS	***
ST × PT		NS		NS	*	NS

^aScarified = 5-min exposure to 8 M H_2SO_4 ; NaOCl = 15-min exposure to 5.25% NaOCl; prechilled = 2-week exposure to 0.2% KNO_3 at 5 °C.

^bWarm = 35/23 °C and cool = 15/12 °C, (14/10 h).

NS, *, **, ***Nonsignificant or significant at $P < 0.05$, 0.01, 0.001, respectively.

The Association of Official Seed Analysts (1993) rules for testing seeds require that "fresh" switchgrass seeds be prechilled to estimate potential seed germination. If prechilling of "fresh" switchgrass seeds were not a realistic option, either acid scarification or NaOCl treatment would increase FGP, but providing both these treatments sequentially would increase FGP further (Table 1).

Sautter (1962) showed that the hard lemma and palea of switchgrass inhibited germination. He obtained 84% germination when these structures were removed vs. 0% germination of intact seeds. Increased FGP following acid scarification (Table 1) was reported by Jensen and Boe (1991) and Zarnstorff et al. (1994), and the promotive effect of NaOCl on germination (Table 1) by Panciera et al. (1987). The corrosive effect of H_2SO_4 (Fig. 1B) or NaOCl (Fig. 1C) was most evident on the distal margins of the lemma, with treatments applied sequentially appearing to have an additive effect (Fig. 1D). Increased gas exchange and water uptake might be expected from the scarification effects of both these oxidants. Increased germination of stinkweed and wild mustard (Hsiao, 1980), wild oats (Hsiao and Quick, 1984), and green needlegrass (Frank

and Larson, 1970) seed was attributed to the scarification effect of NaOCl in enhancing oxygen supply to the seed or embryo. Hsiao and Quick (1984) suggested that the promotive effect of NaOCl on germination of wild oats may have resulted from increased sensitivity of the embryo to endogenous gibberellins, or the destruction of germination inhibitors. Increased FGP when NaOCl treatment followed acid scarification, irrespective of prechilling (Table 1), may indicate either an additive corrosive effect on the floral bracts or an increased stimulatory effect of NaOCl on pregerminative physiology as a result of enhanced NaOCl uptake into the seeds.

The germination lid (Rost and Simper, 1975) is a hinged structure with a slightly indented outline on the proximal surface of the lemma (Fig. 2A and B). During germination, the coleorhiza forces open the lid, which is a site of low resistance in the hard, silicated lemma, through which the coleorhiza and radical emerge (Fig. 2C). The coleoptile emerges by forcing open the lemma and palea at the distal end of the floret (Fig. 2C). Although increased gas exchange and water uptake into the seeds through the corroded distal margin of the lemma (Fig. 1) as a result of acid and

NaOCl treatment may have been sufficient to promote switchgrass germination, these oxidants also may have weakened the lemma in the region of the germination lid.

Irrespective of postsowing temperature regime in the greenhouse, FEP and seedling shoot dry mass of scarified seeds increased only when the scarification was followed by both NaOCl treatment and prechilling, rather than prechilling alone (Table 2). Thus, seedling emergence and growth were greatest when all three treatments (scarification, NaOCl, prechilling) were provided. Combining all three treatments increased seedling shoot dry mass in both postsowing environments, but E_{50} values were unaffected. This greater shoot dry mass thus would appear to reflect increased seedling growth rate but not increased emergence rate.

While the combined effects of acid scarification, NaOCl treatment, and prechilling increased FGP 1.7-fold (Table 1), this effect was not observed when an identical study was conducted with the same seedlot stored for 32 months at 13 °C and 30% RH (nontreated = 71% FGP, data not shown). Robocker et al. (1953) reported an increase in germination from 43% in 150-day-old seed to 69% in

Fig. 1. Scanning electron micrographs of switchgrass seeds (fertile florets), which average 2.3 mm in length. (A) Untreated seeds, (B) Acid-scarified seeds (8 M H_2SO_4 , 5 min), (C) Sodium hypochlorite-treated seeds (5.25% NaOCl, 15 min), (D) Acid-scarification followed by NaOCl treatment. Seeds treated with acid or NaOCl exhibited corrosion of the distal margin of the lemma, the corrosion being more severe when these treatments were combined.

A **B** **C**

Fig. 2. Scanning electron micrograph showing base of the lemma (arrow) of a fertile floret of switchgrass (A) marking the location of the "germination lid" (B), a hinged structure with a slightly indented outline. Light micrograph (C) showing the coleorhiza (CR) protruding through the "germination lid," the radical (RA) protruding through the coleorhiza, and the emerged coleoptile (CP) that had forced open the lemma and palea at the distal end of the floret (bar = 1 mm).

switchgrass seeds that had been stored for 1 year at 23 °C. Jensen (1985) found higher germination when switchgrass seeds were stored for 4 months at room temperature rather than at 7 °C. Zarnstorff et al. (1994) showed that 90 d storage at 23 °C overcame most of the short-term switchgrass seed dormancy that also was broken by prechilling. Thus, the benefit of acid scarification, NaOCl treatment, and prechilling on seed germination (Table 1) and seedling emergence (Table 2) apply only to seeds that have experienced little afterripening (dry, warm storage).

Our studies have shown that, since heavier seeds have a higher percentage germination than lighter seeds within a seedlot, removal of lighter seeds improves seedlot germination. Acid scarification (exposure to 8 M H₂SO₄ for 5 min) followed by a 15-min exposure to 5.25% NaOCl additively increased germination, a response that was associated with marked corrosion of the lemma margin in the distal region of the caryopsis. Prechilling the seeds [14-d exposure to 0.2% (m/v) KNO₃ at 5 °C] following their exposure to H₂SO₄ and NaOCl further increased germination. All three treatments combined (acid scarification, NaOCl, and prechilling) almost doubled final emergence and greatly increased seedling shoot dry mass in both a warm and cool postsowing environment. The effectiveness of these treatments was not observed in seeds of the same seedlot that had experienced 32 months of afterripening (storage at 13 °C, 30% RH) prior to treatment.

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