Germination Responses of Purpletop and Big Bluestem Caryopses Subjected to Distilled Water or Potassium Nitrate Prechilling, Sodium Hypochlorite, and Storage

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Abstract. Purpletop [Tridens flaves (L.) Hitchc.] and big bluestem (Andropogon gerardii Vitman) are incorporated into native grass seed mixes for use in ecological restoration. Alleviation of low seed vigor and poor stand establishment would benefit the restoration process by increasing the animal habitat in restored zones. This study determined the effectiveness of prechilling with distilled water (dH2O) versus potassium nitrate (KNO3), sodium hypochlorite (NaOCl) treatments, and short-term storage conditions on seed germination, seedling growth, and stand establishment of purpletop and big bluestem. Prechilling of ‘Niagara, NY Ecotype’ big bluestem for 7 days at 5 °C in dH2O increased final germination percentage (FGP) and germination rate provided caryopses were not dried before sowing. Optimal FGP, germination rate, and germination synchrony were obtained for ‘VA Ecotype’ purpletop after prechilling for 14 days at 5 °C in dH2O without subsequent caryopsis drying or at 0.2% KNO3 with or without subsequent caryopsis drying. Prechilling increased germination synchrony for purpletop but not for big bluestem. NaOCl treatments did not enhance germination or seedling vigor of purpletop or big bluestem. Using nonstored, prechilled caryopses resulted in greater root lengths than stored caryopses or nontreated controls, although optimal purpletop growth required that they be sowed moist, whereas optimal big bluestem growth required that they be dried-back before sowing. In greenhouse experiments, higher seeding rates were correlated with increased number of seedlings for nontreated or prechilled big bluestem and for prechilled purpletop, but not for nontreated purpletop, which had poor stand establishment at all seeding rates. The results indicate that prechilling of caryopses before incorporation into a warm-season grass seed mix increased seedling establishment of purpletop but not big bluestem.

Purpulop is a warm-season perennial grass native to the United States that grows in dry fields or along roadides in 36 states (Brown, 1979; USDA-NRCS, 2009b). It is able to grow within a variety of soil types and pH levels, including soils that are highly eroded (Foot and Jackobs, 1966). Removal of the palea and lemma of the caryopsis fruit (dehulling) and stratification for 14 d at 3 °C overcomes purpletop dormancy (USDA-NRCS, 2006); however, dehulling does not increase purpletop stand establishment (USDA-NRCS, 1996). Dormancy of purpletop is at least partly the result of seed-covering structures because their removal decreases time to germination, although morphological scarification did not increase germination percentage or germination uniformity (Olzewski et al., 2009). Publications regarding seed dormancy are lacking for many grass genera, including Tridens (Simpson, 1999). Big bluestem grows in dry open areas, along roadsides, or within fields in 43 states (Brown, 1979; USDA-NRCS, 2009a). In Pennsylvania, birds in warm-season grass fields containing big bluestem have greater nest success than those containing cool-season grasses (Giuliano and Daves, 2002). There are no specific competition effects among species of warm-season grasses during early establishment (Launtheraugh and Owensby, 1970); however, Robecker et al. (1953) determined that Canada wild rye (Elymus canadensis L.) grew more quickly than big bluestem. Among germinating members of orchardgrass (Dactylis glomerata L.), early-emerging individuals have competitive advantages over later-emerging individuals (Ross and Harper, 1972). Presowing wet chilling (prechilling) of big bluestem caryopses in 0.2% KNO3 for 14 d at 4 °C caused an increase of the temperature at which maximum germination percentage and germination rate occur (Hsu et al., 1985). Although big bluestem laboratory testing indicated that germination increased after solid matrix priming (~6 MPa for 2 d at 17 °C or 14 d at 4 °C), sowing nontreated caryopses resulted in higher field emergence than primed caryopses under dry environmental conditions (Beckman et al., 1993). Long-duration after-ripening and scarification of big bluestem caryopses were effective seed enhancement techniques and the mechanism of dormancy was hypothesized to result from seed-covering structures restricting gas exchange (Coukos, 1944). If seedling is delayed, then caryopses may have to be stored. Storage temperatures of 16 to 27 °C resulted in big bluestem germinating in higher percentages than those stored at cooler storage temperatures of 1 to 6 °C (Coukos, 1944). Because we know of no reports comparing H2O versus KNO3 during prechilling, the effects of posttreatment storage conditions on germination and seedling growth, and subsequent incorporation of prechilled caryopses into warm-season grass seed mixes, the following studies were undertaken using purpletop and big bluestem.

Materials and Methods

Single seedlots of ‘VA Ecotype’ purpletop, ‘Niagara, NY Ecotype’ big bluestem, ‘PA Ecotype’ Canadian wild rye (Elymus canadensis L.), ‘Rumsey’ Indian grass [Sorghastrum nutans (L.) Nash.], ‘Campes’ little bluestem [Schizachyrium scoparium (Michx.) Nash], and ‘Shelter’ switchgrass (Panicum virgatum L.) were purchased from Ernst Conservation Seeds (Meadville, PA) and used for the following experiments. After purchase of seed lots, the caryopses were stored under cool and dry conditions (7 °C and 32% relative humidity (RH)) for 7 (Expts. 1 and 2) or 12 (Expt. 3) months before experiments started. Little bluestem was used immediately after purchase and was not stored.

Expt. 1: Prechilling effects. Caryopses were subjected to prechilling by incubating 1.0 g caryopses between two layers of germination No. 385 blotter papers (Moisture Co., Chicago, IL) moistened with 20 mL of 0.2% (m/v; AOSA, 2007) KNO3 or dH2O contained in 125 × 80 × 20-mm transparent polystyrene boxes with light-tight lids for 7 or 14 d at 5 °C in darkness. Moisture lost as a result of evaporation from blotters was replaced with dH2O. After prechilling, one group of caryopses from each treatment was blotted with germination blotter and immediately sowed (moist caryopses). Another group of caryopses was subjected to drying at 23 °C and 48% RH for 7 d. Drying began in such a way that all experimental treatments ended simultaneously. Nontreated caryopses were included as a control. Caryopses were placed into polystyrene boxes containing two layers of germination blotter moistened with 20 mL of dH2O and incubated at 20/30 °C (14/10 h) with 70 μmol·m−2·s−1 photosynthetically active radiation (PAR) during the warmer period fulfilling germination requirements for big bluestem (AOSA, 2007) and...
purpletov (USDA-NRCS, 2006). Each polystyrene box contained 50 caryopses with four replications (boxes) per treatment arranged in a completely randomized block design. The number of seeds germinated (those having a visible coleorhiza/radicle) were counted daily and removed. From these counts, the angular transformation [arcsin √% transformation in degrees (deg.); Gomez and Gomez, 1984] of final germination percentage (FGP), days to 50% FGP (G50, an inverse measure of germination rate), and days between 10% and 90% FGP (G10–90, an inverse measure of germination synchrony) were subjected to analysis of variance (ANOVA) using PROC GLM (SAS 9.1; SAS Institute, Inc., Cary, NC).

**Expt. 2: NaOCl treatments and caryopsis storage.** To minimize seed deterioration by storage fungi, caryopses of purpletov and big bluestem receiving prechilling treatment (5 °C in 0.2% KNO3 for 14 d) were subjected to 10% sodium hypochlorite soaking (0.1% NaOCl) before storage. Caryopses (1.0 g) were enclosed in cheesecloth and soaked in NaOCl containing 0.015% (w/v) Tween-20 for 10 min (pretreatment with NaOCl) and then rinsed in running water for 10 min. One group of caryopses was not treated with NaOCl. After prechilling, caryopses that previously were treated with NaOCl were enclosed in cheesecloth and soaked in NaOCl containing 0.015% (w/v) Tween-20 for 5 min (posttreatment with NaOCl) and then rinsed in running water for 10 min. Caryopses then were blotted with germination blotter (moist caryopses) or dried as previously described in Expt. 1 before storage. Storage conditions were constant darkness at 5 or 21 °C and 50% RH, 12:12-h light-dark cycles, and reduced to 10% moisture for 10 min at 21 °C. Caryopses of big bluestem were nonstoring caryopses (eliminated in 27% C176·53°C in 0.2% KNO3 for purpletop and 7 d at 5 °C in distilled water for big bluestem) were selected for greenhouse experiments. Purpletov or big bluestem caryopses were nonstoring or prechilled followed by blotting with germination blotter immediately before sowing (moist-sowed caryopses). All warm-season grass seed mixes contained 20 seeds of Canadian wild rye, Indiangrass, little bluestem, and switchgrass. Mixtures also contained 10, 20, or 50 seeds of big bluestem and 10, 20, or 50 seeds of purpletov. Mixtures were sowed into moistened proprietary media (Redi- Earth Plug and Seedling Mix; SunGro Horticulture, Bellevue, WA) contained in 27 × 53 × 6-cm plastic trays. Seed mixtures were spread evenly over an area of 0.064 m2 and lightly covered with ∼2 mm of media. Media were irrigated as needed and arranged in a completely randomized block design with four replications in a greenhouse with filtered natural light (July/August; 187 μmol·m–2·s–1 PAR) at 18/29 °C (minimum/maximum). For identification, a key of vegetative characteristics was used (Phillips, 1962) as well as live references as shown in Figure 1. The number of emerged seedlings were counted 4 weeks after sowing and subjected to ANOVA on seedling rates using PROC GLM with linear regression.

**Results.**

**Expt. 1: Prechilling effects.** All prechilling treatments of purpletov increased germination rate (lower G50 values) and synchrony (lower G10–90 values) except for 7 d prechilling in dH2O with or without subsequent air-drying, which did not increase synchrony (Table 1). Prechilling of purpletov caryopses for 14 d in dH2O, without air-drying, or for 14 d in KNO3, with or without air-drying, increased FGP compared with nontreated caryopses. Air-drying of purpletov caryopses prechilled increased FGP (group mean) from 82% to 91% after 7- versus 14-d treatment durations, respectively. Purpletov caryopses that were prechilled in KNO3 and air-dried before the germination assay had earlier germination than those prechilled in dH2O and air-dried. Prechilling of big bluestem caryopses for 7 d in dH2O or for 14 d in KNO3, without subsequent air-drying, increased FGP. The germination rate of big bluestem was increased by a single treatment (dH2O for 7 d at 5 °C, nondried caryopses); however, none of the experimental treatments increased germination synchrony. Storage of prechilled, dried-back purpletov caryopses at 5 or 21 °C for 10 d reduced FGP. Storage of prechilled, dried-back purpletov caryopses at 5 or 21 °C for 10 d reduced FGP and shoot lengths compared with prechilled, moist-stored caryopses.

**Expt. 3: Seed mixes with prechilled caryopses.** There were no slope differences of regressions for nontreated versus prechilled big bluestem, but seedling numbers were statistically different for nontreated versus prechilled purpletov (data not shown). According to the regression analysis, seedling number was positively correlated with higher seeding rate for nontreated or prechilled big bluestem (r2 = 0.95 and 0.03, respectively) and prechilled purpletov (r2 = 0.85; Fig. 2). However, there was no correlation (r2 = 0.22) between number of seedlings and seeding rates for nontreated purpletov caryopses.

**Discussion.** Although certified germination was 77% and 96% for big bluestem and purpletov, respectively (Ernst Conservation Seeds, Meadville, PA), FGP of nontreated seed was 46% and 75%, respectively (Table 1). The shortest duration of dH2O or KNO3 prechilling necessary to increase FGP of purpletov was 14 d. Germination of purpletov was 91% for moist-sowed caryopses prechilled in dH2O drying before sowing (Table 2). Pre- and posttreatments with NaOCl did not enhance germination or seedling vigor of purpletov or big bluestem; however, precocious germination occurred during 21 °C moist storage with NaOCl for big bluestem and purpletov (8%) and 10% germination, respectively; data not shown). Precocious germination also occurred for purpletov and big bluestem (both less than 1% germination; data not shown) during 21 °C moist storage with no NaOCl treatment. Storage of prechilled and NaOCl-treated big bluestem caryopses in a moist condition at 21 °C for 10 d reduced FGP. Storage of prechilled, dried-back purpletov caryopses at 5 or 21 °C for 10 d reduced FGP and shoot lengths compared with prechilled, moist-stored caryopses.
Table 1. Final germination percentage (FGP) and its angular transformation (deg.), days to 50% of FGP (G50), and days between 10% and 90% of FGP (G10–90) of purpletop and big bluestem caryopses at alternating 20/30 °C (14/10 h, dark/light).^x

| Solution (dH2O or KNO3) | Duration (d) | Caryopsis drying | FGP % (deg.) | G50 (d) | G10–90 (d) | FGP % (deg.) | G50 (d) | G10–90 (d) |
|-------------------------|--------------|------------------|--------------|---------|------------|--------------|---------|------------|
| dH2O                    | —            | —                | 75 (60)      | 31.3    | 18.7       | 46 (42)      | 7.8     | 9.4        |
| dH2O                    | 7            | No               | 81 (64)      | 18.4    | 17.0       | 54 (47)      | 5.3     | 9.8        |
| dH2O                    | 14           | No               | 91 (74)      | 12.5    | 9.1        | 44 (41)      | 6.9     | 11.3       |
| dH2O                    | 14           | Yes              | 85 (66)      | 16.6    | 9.8        | 49 (44)      | 8.7     | 10.9       |
| KNO3                    | 7            | No               | 82 (65)      | 20.3    | 13.2       | 49 (44)      | 7.5     | 10.7       |
| KNO3                    | 7            | Yes              | 81 (64)      | 19.1    | 10.4       | 50 (44)      | 9.3     | 12.7       |
| KNO3                    | 14           | No               | 95 (76)      | 12.9    | 6.9        | 54 (47)      | 8.0     | 14.1       |
| KNO3                    | 14           | Yes              | 93 (76)      | 14.6    | 8.2        | 51 (45)      | 8.9     | 9.8        |

| LSD0.05             | (10)        | 2.6              | 3.8         |         |            | (4)         | 1.9     | 4.3        |

Significance

Prechilling treatment NS NS ** NS NS NS NS
Caryopsis drying (CD) ** *** *** NS NS NS NS
Prechilling duration (PTD) NS NS NS NS NS NS NS
PT × CD NS NS NS * NS NS NS
PT × PTD × CD NS NS NS NS NS NS NS

Table 2. Final germination percentage (FGP) and its angular transformation (deg.), shoot length (mm), and root length (mm) of purpletop and big bluestem 14 d after planting caryopses onto a slant test apparatus at alternating 20/30 °C (14/10 h, dark/light).^x

| Caryopsis storage | Purpletop | Big bluestem |
|-------------------|-----------|--------------|
| Temperature (°C)  | Condition | NaOCl treatment | FGP % (deg.) | Shoot length (mm) | Root length (mm) | FGP % (deg.) | Shoot length (mm) | Root length (mm) |
| Nonstored (dry/moist) | Dry*       | —              | 0 (2)        | 0.0             | 0.0             | 29 (32)       | 5.1             | 2.4             |
| Nonstored (moist)  | Moist      | —              | 81 (65)      | 9.0             | 7.6             | 32 (34)       | 8.3             | 1.9             |
| 5                  | Dry        | No             | 46 (42)      | 4.7             | 1.9             | 30 (33)       | 8.6             | 2.1             |
| 5                  | Dry        | Yes            | 16 (21)      | 2.1             | 0.7             | 29 (32)       | 8.7             | 2.0             |
| 5                  | Moist      | No             | 72 (59)      | 6.5             | 1.6             | 25 (29)       | 8.0             | 2.0             |
| 5                  | Moist      | Yes            | 70 (58)      | 6.4             | 1.3             | 29 (32)       | 8.7             | 1.9             |
| 21                 | Dry        | No             | 30 (31)      | 2.5             | 1.3             | 32 (34)       | 7.1             | 2.3             |
| 21                 | Moist      | No             | 64 (53)      | 7.8             | 2.0             | 22 (28)       | 4.3             | 1.8             |
| 21                 | Moist      | Yes            | 52 (46)      | 6.6             | 1.2             | 11 (17)       | 2.8             | 1.5             |

| LSD0.05             | (12)       | 2.0             | 1.5           |         |            | (10)         | 3.6             | 1.2             |

Significance

Storage temperature (ST) NS NS NS (NS) NS ** NS
Storage condition (SC) **** *** NS (**) NS NS NS
Sodium hypochlorite (SH) NS NS NS (NS) NS NS NS
ST × SC NS NS NS NS NS NS NS
SC × SH NS NS NS NS NS NS NS
ST × SC × SH NS NS NS NS NS NS NS

Air-drying of primed caryopses for 7 d reduced FGP of bottlebrush squirreltail [Sitanion hystrix (Nutt.) J.G. Smith]; storage (up to 70 d) of primed and air-dried caryopses resulted in loss of germination benefits of canby bluegrass (Poa canbyi Scribn; Hardegree, 1994). Thus, any benefits imparted by grass seed hydration treatments may be lost after storage or drying procedures. Drying prechilled big bluestem caryopses followed by immediate sowing resulted

for 14 d. Purpletop germination also was improved (higher FGP, lower G50, and lower G10–90) after a prechilling treatment in KNO3 for 14 d. The most significant benefit of using KNO3 versus dH2O was increased germination synchrony for purpletop. The effect of nitrate ions on grass seed germination has been reviewed elsewhere (Simpson, 1990); however, exact reasons for germination stimulation remain elusive. Beckman et al. (1993) showed that seed hydration treatments (priming) for 2 d at 17 °C or for 14 d at 4 °C increased big bluestem emergence. In the current study, big bluestem FGP was increased by prechilling at 5 °C in dH2O for 7 d or at 5 °C in KNO3 for 14 d provided that caryopses were not dried before sowing. Either the degree of dormancy or the type of dormancy is different from purpletop because prechilling treatments that alleviated purpletop dormancy were not as effective for big bluestem.
in increased root length; however, this effect on big bluestem was lost on storage (Table 2). Restriction of oxygen diffusion was suggested as a possible mechanism of warm-season grass seed dormancy (Coukos, 1944), and changes in the gaseous environment have been associated with loss of dormancy in grass seed [review by Simpson (1990)]. It is possible that drying of prechilled big bluestem caryopses allowed for more oxygen diffusion into the seed than did nondried, prechilled (moist-sowed) caryopses placed directly on the slant testing apparatus, resulting in longer root lengths. For purpletop, however, dried-back prechilled caryopses subjected to 5 or 21 °C storage reduced FGP and seedling shoot length, whereas nonstored, moist-sowed prechilled caryopses were most beneficial for purpletop germination and seedling growth.

Short-duration treatments of NaOCl were effective at controlling fungal proliferation during priming of corn caryopses (Parera and Cantiliffe, 1991). Although it was hypothesized that NaOCl treatments would be beneficial to caryopses by disinfecting surfaces, the main effects of pre- and posttreatments of purpletop with NaOCl was decreased FGP (Table 2). The inhibitory effect of NaOCl on purpletop germination was not observed for big bluestem, but the highest amount of precocious big bluestem germination was observed during moist storage at 21 °C with NaOCl treatments, suggesting a slight stimulatory effect. Enhanced germination after NaOCl treatments of caryopses previously was reported (Emal and Conrad, 1973; Hsiao and Quick, 1985). The results of our study, however, show that there was no benefit of using 0.6% NaOCl to increase germination or seedling vigor of purpletop or big bluestem.

Incorporating prechilled big bluestem caryopses into seed mixes does not result in greater seeding establishment (Fig. 2), although germination testing indicated that FGP and germination rate increased after prechilling (Table 1). Our results are in agreement with Laun¬chbaugh and Owensby (1970) who noted that big bluestem stand establishment is correlated linearly with seeding rate. Maximum big bluestem germination is short-lived during storage (Coukos, 1944). Future dormancy research should include testing oxygen diffusion as a limiting factor for big bluestem germination. Prechilling procedures increased purpletop early seedling establishment, whereas, regardless of application rate, few nontreated purpletop caryopses emerged 4 weeks after sowing.

The results of this study show that germination of purpletop and big bluestem was improved by prechilling but that these changes may or may not be related to seedling establishment dependent on grass species. Increased seedling root length occurred if nonstored, prechilled caryopses were used rather than stored, prechilled caryopses; thus, prechilling treatments must be applied immediately before sowing. Seedling emergence of purpletop was enhanced by prechilling and our results indicate an improvement of purpletop establishment after incorporation of prechilled caryopses into a warm-season grass seed mix.

Fig. 2. Regressions of the number of seedlings emerged on seeding rate 4 weeks after planting warm-season grass seed mixes containing nontreated (•) or prechilled (□) caryopses. (A) Nontreated or prechilling (14 d at 5 °C in 0.2% KNO3) effects on purpletop emergence and (B) nontreated or prechilling (7 d at 5 °C in dH2O) effects on big bluestem emergence. Seeding rates are actual number of seeds per treatment plot (0.064 m2).
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