Stratification, freezing, and drying effects on germination and seedling growth of Altai wildrye

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Abstract

Altai wildrye (*Leymus angustus* (Trin.) Pilger) is recommended for late season forage and stabilization or improvement of salt affected land in the northern Great Plains. Establishment from seeding is erratic, perhaps due to environmental extremes that occur in the seedbed. Objectives of this study were to evaluate the effect of temperature and moisture variation on germination, solute leakage from seeds, and etiolated growth of seedlings of this perennial grass. Seeds were subjected to 5 preincubation treatments: stratification 

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**Materials and Methods**

'Prairieland' Altai wildrye seed was supplied by Dr. T. Lawrence of Agriculture Canada Research Station in Swift Current, Saskatchewan. At the time of this experiment seeds were approximately 10 months old. Throughout this study seeds were subjected to 5 preincubation treatments. Treatments included: 1. cool-moist (50 C) stratification for 48 hours (STR)—this treatment simulated cool-moist conditions in the seedbed; 2. cool-moist stratification for 48 hours followed by drying at 300 C for 8 hours (STR-D)—this treatment simulated cool-moist conditions of the seedbed followed by a short warming and drying period; 3. cool-dry (50 C) storage of seeds for 48 hours (COOL)—this simulated cool-dry conditions of a seedbed, and; 5. laboratory storage (LAB).

All pretreatments were applied to seeds in Petri dishes in which a #4 Whatman filter paper disc had been placed. For stratification treatments, 7 mL of distilled water was added to dishes, lids were replaced, and seeds were incubated at 50 C in darkness. After pretreatment, the moisture content of 4 samples of 25 seeds was determined after drying for 48 hours at 800 C (Table 1).

**Experiment 1—Temperature and Water Stress Effects on Germination**

Osmotic solutions were prepared by adding polyethylene glycol (PEG) (M.W. 20,000) to distilled water. Average osmotic poten-
Table 1. Mean moisture content expressed as percent of oven dry weight for Altai wildrye seeds following 5 pretreatments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Pretreatment</th>
<th>STR</th>
<th>STR-D</th>
<th>STR-FR</th>
<th>COOL</th>
<th>LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination</td>
<td></td>
<td>95.2</td>
<td>43.3</td>
<td>96.4</td>
<td>9.9</td>
<td>10.0</td>
</tr>
<tr>
<td>Solute leakage</td>
<td></td>
<td>90.4</td>
<td>38.5</td>
<td>98.8</td>
<td>9.9</td>
<td>10.0</td>
</tr>
<tr>
<td>Seeding growth</td>
<td></td>
<td>93.9</td>
<td>42.5</td>
<td>95.3</td>
<td>8.8</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Results

Germination

Germination was higher and more rapid, and occurred over a wider range of osmotic potentials, at 20°C than at 10°C (Figs. 1 and 2, Table 2). Within temperatures the interaction of pretreatment × osmotic potential significantly (P<0.05) influenced total germination and rate of germination. At both 10 and 20°C total germination and germination rate were lowest over the range of osmotic potentials for STR-FR.

Figure 1. Response curves for total germination and coefficient of rate of germination (CRG) of Altai wildrye seeds subjected to 5 preincubation treatments and incubated at 10°C in a gradient of osmotic potentials.
Table 2. Regression equations for relationships between osmotic potential effects on total germination and germination rate of Altai wildrye following pretreatment.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Temperature (°C)</th>
<th>Regression equation</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>STR</td>
<td>10</td>
<td>Germination rate (%/day)</td>
<td>Y = 6.1 + 5.6X - 0.6X²</td>
</tr>
<tr>
<td>STR-D</td>
<td></td>
<td></td>
<td>Y = 7.2 + 9.2X - 2.8X²</td>
</tr>
<tr>
<td>STR-FR</td>
<td></td>
<td></td>
<td>Y = 4.2 + 3.8X</td>
</tr>
<tr>
<td>COOL</td>
<td></td>
<td></td>
<td>Y = 5.7 + 5.0X</td>
</tr>
<tr>
<td>LAB</td>
<td></td>
<td></td>
<td>Y = 5.6 + 4.9X</td>
</tr>
<tr>
<td>STR</td>
<td></td>
<td>Total germination (%)</td>
<td>Y = 77.1 - 39.0X - 81.4X²</td>
</tr>
<tr>
<td>STR-D</td>
<td></td>
<td></td>
<td>Y = 88.6 + 1.6X - 62.1X²</td>
</tr>
<tr>
<td>STR-FR</td>
<td></td>
<td></td>
<td>Y = 83.4 + 70.7X</td>
</tr>
<tr>
<td>COOL</td>
<td></td>
<td></td>
<td>Y = 76.1 - 43.1X - 93.9X²</td>
</tr>
<tr>
<td>LAB</td>
<td></td>
<td></td>
<td>Y = 82.1 - 20.5X - 78.7X²</td>
</tr>
</tbody>
</table>

1X is osmotic potential (-MPa).

2The same lower case letter within a parameter indicates non-significant (P≤0.05) differences between intercepts of regression equations.

3The same upper case letter within a parameter indicates non-significant (P≤0.05) differences between regression equations.

Table 3. Relative leakage ratio for Altai wildrye seeds subjected to 5 preincubation treatments.

| Osmotic potential (MPa) | STR | STR-D | STR-FR | COOL | LAB | SE
|-------------------------|-----|-------|--------|------|-----|----
| 0.00                    | 0.528 | 0.499 | 0.540 | 0.675 | 0.651 | SE
| -0.48                   | 0.870 | 0.861 | 0.868 | 0.868 | 0.866 | 0.028

1SE = Standard Error

Table 4. Mean root and shoot weights for Altai wildrye seedlings after seeds were subjected to 5 preincubation treatments and grown for 5 days at 15° C in darkness.

| Osmotic potential (MPa) | STR | STR-D | STR-FR | COOL | LAB | Root Weight (mg) | Shoot Weight (mg) | SE
|-------------------------|-----|-------|--------|------|-----|-----------------|-------------------|----
| 0.00                    | 18.4 | 18.1  | 21.4   | 21.8 | 21.1 | 16.3            | 24.6              | SE
| -0.48                   | 23.9 | 20.9  | 14.9   | 23.5 | 22.3 | 16.0            | 24.6              | 0.99
| 0.00                    | 23.3 | 25.4  | 26.4   | 26.6 | 25.1 | 16.3            | 26.3              | SE
| -0.48                   | 29.1 | 26.2  | 6.4    | 26.3 | 27.0 | 16.9            | 26.3              | 0.51

1SE = Standard Error

Solute Leakage From Seeds

The osmotic potential × pretreatment interaction significantly (P≤0.05) influenced the relative leakage ratio (RLR) for seeds (Table 3). Seeds that had been stratified had lower RLR's than unstratified seeds at 0.0 MPa, with STR-D being lowest. RLR was significantly (P≤0.05) higher at -0.48 MPa than at 0.0 MPa, but there were no differences between pretreatments.

Etiolated Seedling Growth

The interacting effects of osmotic potential and pretreatment significantly (P≤0.05) influenced etiolated root and shoot growth of seedlings (Table 4). Root weights were similar for all pretreatments at 0.0 MPa. However, root weight for STR-FR was lower than the other pretreatments at -0.48 MPa. Root weights following STR and STR-D were greater at -0.48 MPa than 0.0 MPa, but they were similar at 0.0 and -0.48 MPa for COOL and LAB pretreatments.

Shoot weights were similar between treatments at 0.0 MPa (Table 4). Shoot weight for STR-FR was 76% lower at -0.48 MPa than 0.0 MPa, and this weight was lower than the remaining pretreatments. In contrast shoot weight for STR was 25% higher at...
-0.48 MPa than at 0.0 MPa.

**Discussion**

An apparent reduction in vigor, caused by stratifying seeds and subjecting them to -10°C, was expressed in reduced germination and seedling growth. Crocker and Barton (1957) concluded that low vigor seeds may need more water to germinate than high vigor seeds, and Hegarty (1978) contended that loss of vigor precedes loss of viability. Furthermore, seeds with reduced vigor may not germinate over as broad a range of conditions as high vigor seeds (Abdul-Baki and Anderson 1972). Under conditions of increasing moisture stress, the negative effects of STR-FR became more pronounced as indicated by reduced germination and seedling growth under water stress. On the other hand, stratifying seeds or stratification and dehydration either improved or had no effect on germination and seedling growth.

Wilson et al. (1974) reported that exposing seeds of several rangeland grasses to winter conditions increased the rate of germination of seeds when they were incubated in the laboratory. However, germination time increased when the soil temperatures were coldest (approximately -5°C) (Wilson et al. 1974). This same reaction to freezing temperatures was found in the present experiment.

Hydration and dehydration treatments increased germination of crested wheatgrass (*Agropyron desertorum*) (Maynard and Gates 1963) and Lehmann lovegrass (*Eragrostis lehmanniana*) (Haferkamp and Jordan 1977). Stratifying seeds and stratification followed by drying also improved the rate of germination for Altai wildrye. It is proposed that the ATP synthesized during stratification was not degraded by dehydration and additional ATP was formed upon rehydration (Haferkamp et al. 1977).

Absorbance at 280 nm gives an indication of the leakage of UV-absorbing cytoplasmic compounds from the cells (Morris 1987). Leakage is rapid during the early stages of imbibition until the phospholipid membranes become intact (Simon 1984). Thus, the lower RLR at 0.0 MPa for stratified seeds is attributed to membrane repair that occurred during stratification. In contrast, the unstratified seeds (COOL and LAB) were probably undergoing earlier stages of the membrane repair processes and leakage was higher (Hegarty 1978, Simon 1984). Leakage of UV-absorbing compounds from the seed appendages may have also contributed to the higher RLR for unstratified seeds. Higher RLR at -0.48 MPa than 0.0 MPa is ascribed to less water availability, reduced rate of hydration and more solute leakage due to a reduction in the extent and rate of membrane repair. Seeds that hydrate slowly may deteriorate rather than progressing through the activation and repair phases (Hegarty 1978).

It is proposed that STR-FR damaged seeds through the mechanical effects caused by ice crystal formation and damage to membranes (Grout and Morris 1987); however, these damaging effects were not substantiated by solute leakage from seeds. This determination actually indicated an equal or better physiological condition than seeds subjected to the other pretreatments. These results underscore the need to document seedling growth in addition to germination.

Data from the present study and others (Lawrence and Kilcher 1972, McElgunn 1974, Young and Evans 1982) suggests that low temperatures play a role in the poor germination, growth, and establishment of Altai wildrye. When this grass is seeded it appears that precautions must be taken to minimize temperature extremes of the seedbed to maximize germination and growth. This study supports Lawrence and Kilcher’s (1972) tenet that the slow emergence of Altai wildrye at low temperatures indicates that this species may not be expected to establish early in the spring like other grasses that are commonly seeded on rangeland. They suggested that delaying seeding until soils warm may be advantageous for stand establishment. Delaying seeding until warmer temperatures prevail may not only enhance the rate of germination, emergence, and seedling growth (Lawrence and Kilcher 1972, McElgunn 1974), but it may also limit the potential reduction in germination caused by freezing temperatures.

Delaying seeding may also be disadvantageous because it can shorten the potential period of growth caused by temperature or moisture limitations. Therefore cultural practices are paramount for germination and establishment of Altai wildrye. Seeding into cool seedbeds is probably the preferred alternative provided attempts are made to minimize temperature and moisture extremes. Creating rough seedbeds or seeding into grain stubble may be the preferred options because temperature fluctuations are likely to be less than on smooth seedbeds or sites devoid of vegetation (Evans and Young 1970, 1972; Hull 1970; Fowler 1983).

**Literature Cited**


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