Predicting seed germination of slender wheatgrass
*Elymus trachycaulus* (Link) Gould subsp. *trachycaulus*
using thermal and hydro time models

M. P. Schellenberg¹, B. Biligetu¹, and Y. Wei²,³

¹Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, Swift Current, Saskatchewan, Canada S9H 3X2 (e-mail: mike.schellenberg@agr.gc.ca); ²Department of Plant Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, Canada S7N 5A8; and ³College of Life Sciences, Northwest A&F University, 22 Xinong Road, Yangling District, Shanxi Province, P. R. China, 712100. Received 28 January 2013, accepted 30 April 2013.

Schellenberg, M. P., Biligetu, B. and Wei, Y. 2013. Predicting seed germination of slender wheatgrass [*Elymus trachycaulus* (Link) Gould subsp. *trachycaulus*] using thermal and hydro time models. Can. J. Plant Sci. 93: 793–798. Slender wheatgrass [*Elymus trachycaulus* (Link) Gould subsp. *trachycaulus*] is a native caespitose grass used for forage production and reclamation. The objective of this study was to quantify seed germination requirements of slender wheatgrass using thermal and hydro time models. Slender wheatgrass, San Luis, had a base temperature (*T*ₐ) of 9.48 °C, and required 946.8 °C h to reach 50% of seed germination. Seed germination of San Luis occurred at a temperature range of 10–30 °C, with the highest germination rate being achieved at 20 °C, and the highest final germination percentage being achieved at 25 °C. At 20 and 25 °C, San Luis had a hydro time constant of 61 MPa h, and a median base water potential of approximately 1.0 MPa, but the germination had low uniformity in reduced water potentials. Final germination was reduced at or lower than −0.6 MPa. Compared with many other cool-season native grasses of Northern Great Plains, a relatively warm temperature would be necessary for uniform seedling establishment of this grass. In reclamation seeding, the seedling emergence could reach the highest level at a temperature of 25 °C.

**Key words:** Germination model, native wheatgrass, reclamation, forage

Predicting seed germination of slender wheatgrass [*Elymus trachycaulus* (Link) Gould subsp. *trachycaulus*] using thermal and hydro time models. Can. J. Plant Sci. 93: 793–798. Slender wheatgrass [*Elymus trachycaulus* (Link) Gould subsp. *trachycaulus*] is a native caespitose grass used for forage production and reclamation. The objective of this study was to quantify seed germination requirements of slender wheatgrass using thermal and hydro time models. Slender wheatgrass, San Luis, had a base temperature (*T*ₐ) of 9.48 °C, and required 946.8 °C h to reach 50% of seed germination. Seed germination of San Luis occurred at a temperature range of 10–30 °C, with the highest germination rate being achieved at 20 °C, and the highest final germination percentage being achieved at 25 °C. At 20 and 25 °C, San Luis had a hydro time constant of 61 MPa h, and a median base water potential of approximately 1.0 MPa, but the germination had low uniformity in reduced water potentials. Final germination was reduced at or lower than −0.6 MPa. Compared with many other cool-season native grasses of Northern Great Plains, a relatively warm temperature would be necessary for uniform seedling establishment of this grass. In reclamation seeding, the seedling emergence could reach the highest level at a temperature of 25 °C.

**Mots clés:** Modèle de germination, élme indigène, restauration, fourrage

Slender wheatgrass [*Elymus trachycaulus* (Link) Gould subsp. *trachycaulus*] (Barkworth et al. 2007) is native to the plains, boreal, and sub-arctic areas of North America (Pringle et al. 1975). It is among the first native grasses to colonize disturbed sites (Patton and Parkinson 1982), and also provides excellent nesting habitat for many birds (Native Seed Network 2012). Slender wheatgrass can form an association with vesicular-arbuscular mycorrhizae in soil, which allows successful re-colonization and growth of herbaceous plants on disturbed soils (Zak and Parkinson 1982), and also provides excellent nesting habitat for many birds (Native Seed Network 2012). Slender wheatgrass is also a preferred forage grass, and contains less lignin than crested wheatgrass [*Agropyron cristatum* (L.) Gaertner] and smooth brome grass [*Bromus inermis* L.] at various growth stages (Patton and Gieseker 1942). Lignin, in forage plants, is practically unavailable to the ruminant (Traxler et al. 1998). Even though slender wheatgrass is considered an
important grass both for forage production and reclamation, limited information is available on its water and temperature requirements for seed germination. To increase seedling establishment success under a range of growth conditions, it is necessary to understand seed germination requirements of this species.

Seed germination is a critical step in the process of seedling recruitment of a population (Baskin and Baskin 1998), which is mainly controlled by temperature and water (Gummerson 1986; Dahal and Bradford 1994). The ability to predict the timing of seedling emergence under a range of temperature and water regimes is useful for determining seeding date and subsequent seedling establishment success (Benjamin 1990; Finch-Savage and Phelps 1993). Various germination models have been used to describe seed germination, specifically, the thermal-time model has been used extensively to quantify the temperature dependence of seed germination (Probert 1992; Bradford 2002). The hydro time model describes seed germination responses to water potential changes (Gummerson 1986; Bradford 1990). The parameters of the models have proven to have a physiological basis and an ecological significance (Allen et al. 2000; Bradford 2002); therefore, these modelling approaches have been successful in prediction of field seedling emergence of forages (Hardegree and Van Vactor 1999) and weed species (Roman et al. 2000).

In western Canada, seed production of slender wheatgrass was low in dry years. In addition, slender wheatgrass seeded for reclamation at higher elevations produces a limited amount of mature seeds (Darroch and Acharya 1995). Slender wheatgrass cultivars derived from southern ecotypes produce more seeds than northern ecotypes from Canada (Pringle et al. 1975). The seeds of cultivars from southern ecotypes are distributed for forage and reclamation use in western Canada by seed companies. We hypothesized that the southern ecotype of slender wheatgrass would have a high base temperature for germination and could germinate at a low water potential. The objective of this study was to predict seed germination of slender wheatgrass San Luis under various temperatures and water potential using population-based germination models.

**MATERIALS AND METHODS**

**Seed Source and Germination Test**

Certified seeds of San Luis slender wheatgrass were purchased from Ag-Vision Seed (Carrot River, SK). San Luis was originally collected in the San Luis Valley, Rio Grande County, Colorado (USDA Natural Resource Conservation Service). It was selected for its good seedling establishment and longevity of stand. Seed viability, for San Luis, was 94% based on tetratolium chloride test (Grabe 1970). Germination tests were conducted under 12/12 h light/darkness at designated constant temperatures of 5, 10, 12.5, 15, 17.5, 20, 25, 30 and 35°C for the construction of the thermal time model. A randomized complete block design with four replicates with 50 seeds each was used, and the replicates were placed into growth chambers (Conviron Model CMP 3244, Winnipeg, MB) at 30-d intervals. Designated temperatures were randomly allocated to each growth chamber. Seeds were imbibed in 9-cm-diameter sterilized plastic petri dishes on top of two layers of filter paper (Whatman 597) moistened by 5 mL of distilled water. The petri dishes were enclosed and sealed in polyethylene bags to prevent desiccation. Germination counts were made daily with germinated seeds being removed. In our study, we did not see emergence of seed coyledon, so seeds with a radicle greater than 2 mm were considered germinated. Measured actual temperatures of growth chambers were 4.9, 9.4, 12.4, 15.2, 17.3, 20.2, 24.8, 29.8 and 35.2°C, respectively, which were used in thermal time model. Germination tests were terminated after more than 90% germination or no germination occurred for 7 consecutive days.

In addition, seeds were germinated in separate experimental units at 20 and 25°C (actual measured temperatures of 20.2 and 24.8°C) at water potentials of 0, −0.2, −0.4, −0.6, and −0.8 MPa using randomized complete block design to construct a hydro time model. These two temperatures were chosen due to high germination rate and total germination percentages. Polyethylene glycol (PEG8000) was dissolved in distilled water to make solutions with designated water potentials using the method described by Michel (1983). Distilled water was used as the control (0.0 MPa). Germination conditions were the same as the germination experiment for the thermal time model, and counts were made daily with germinated seeds being removed.

**Statistical Analysis and Model Construction**

Cumulative germination of distilled water was calculated for each temperature. The seed viability percentage based on the tetrazolium chloride test was used as a scaling factor (Ellis et al. 1986; Hardegree and Van Vactor 1999). The scaling factor adjusted the germination percentage by taking into account the actual seed viability. Seed populations were considered to be composed of subpopulations (10, 20, 30, 40, 50, 60, 70, 80, and 90%) based on relative germination rate to facilitate modeling purposes (Garcia-Huidobro et al. 1982). These percentile rankings were assumed to represent subpopulations that would germinate in the same relative order regardless of thermal conditions (Hardegree and Van Vactor 1999). For thermal time model construction, germination time course of four replicates within each temperature was modeled using the Chapman 3-parameter function in SigmaPlot 12.0, and the sigmoidal equation obtained was then used to calculate the germination time course (t) for the 10% to the 90% subpopulations at each temperature (Qiu et al. 2006). The germination rate of subpopulations \( GR(g) = 1/t_g \), where \( GR(g) \) is the reciprocal of germination time \( t_g \) to a given germination percentile \( g \) was then
calculated as the reciprocal of the germination time (Garcia-Huidobro et al. 1982). Germination rate for each subpopulation was plotted against temperature using non-linear PROC NLIN procedure with a statement option of same base temperature ($T_b$) in SAS 9.1 (2003) (Littell et al. 1996) to estimate the $T_b$. PROC PROBIT procedure in SAS 9.1 (2003) was used to calculate thermal time for 50% germination and its standard deviation. An adjusted $R^2$ value (Pseudo $R^2$ value) was used to measure variance explained by the thermal model. The thermal time model describes seed germination responses to temperature using the following function:

$$ O_{T(g)} = (T - T_b)tg $$

where $O_{T(g)}$ is thermal-time (°C h) for germinating a given germination percentile (g) in the population, $T$ is incubation temperature (°C), $T_b$ is constant base temperature of the population, and $t_g$ (d) is the time to germinate a “g” percentile of seeds. $O_T$ is assumed to vary normally, while $T_b$ is assumed to remain constant, which means the threshold to get germination (Bradford 2002).

At 20 and 25°C, cumulative germination was calculated at each water potential and adjusted using a scaling factor. The hydro time model was calculated at each temperature using the equation described by Bradford (1990). The hydro time model describes seed germination responses to water potential ($\Psi$) using the following function:

$$ \theta_H = (\Psi - \Psi_{bg})tg $$

where $\theta_H$ is the hydro time (MPa h) required for germination, $\Psi$ is the actual water potential of the germination medium (MPa), $\Psi_{bg}$ is the theoretical threshold or base water potential that will just prevent germination of fraction g, and $t_g$ is the germination time (h) of the corresponding fraction g. The model assumes that $\Psi_{bg}$ varies among fractions of a seed population, but $\theta_H$ is constant for a seed population (Bradford 1990). PROC PROBIT procedure in SAS 9.1 (2003) was used to calculate base water potential ($\Psi_{bg50}$) for 50% germination and its standard deviation. Adjusted $R^2$ value was estimated for the hydro time model.

A one-way analysis of variance (ANOVA) was conducted for final germination at different water potentials at 20 and 25°C using the PROC MIXED procedure of SAS 9.1, and the means were separated using least square means comparisons at the 0.05 level of significance.

**RESULTS**

**Germination as Affected by Temperature**

The dynamics of seed germination of slender wheatgrass in response to temperature was well described by the thermal-time model ($R^2 = 0.94$, $P < 0.001$). San Luis slender wheatgrass required a base temperature ($T_b$) of 9.48°C for germination based on the model (Fig. 1). Based on the thermal time model, San Luis required 946.8°C h to reach 50% germination with a standard deviation of 37.3°C h among subpopulations (data not shown).

Maximum germination of San Luis under favorable conditions can reach the estimated seed viability of 94%, but it also showed a conditional dormancy at low and high temperatures. Final germination percentage was the highest at 25 and 30°C, followed by 17.5 and 20°C, and lowest at 12.5°C (Fig. 2). No germination occurred after 49 d in temperatures of 5, 10, and 35°C (Fig. 2). Based on germination rate, the majority of subpopulations had higher germination rate at 20°C (20–80% of subpopulations), but the first 10% of subpopulation showed higher germination rate at 30°C.

**Germination as Affected by Water Potential**

Seed germination of San Luis is relatively tolerant of low water potential under favorable temperatures (Table 1). At 20 and 25°C, final germination percentage after 21 d was similar at water potentials of 0, −0.2, and −0.4 MPa, reaching more than 80% (Table 1), but seed germination rate of subpopulations increased at −0.2 and −0.4 MPa than at 0 MPa (Figs. 3 and 4). The germination percentage was reduced at −0.6 MPa and thereafter (Figs. 3 and 4). Final germination at various water potentials after 21 d was higher at 25°C than 20°C (Table 1), but no germination occurred for −1.0 MPa for both temperatures (data not shown).

The fitted hydro time model provided a good description of seed germination in response to water availability for slender wheatgrass ($R^2 = 92$ and 89% at 20 and 25°C, respectively, $P < 0.001$) (Table 2). Hydro
time ($\theta_t$) for San Luis was similar between temperatures of 20 and 25°C, which was 61 MPa h (Table 2). San Luis had a median base water potential ($\Psi_{b(50)}$) of 1.02 and 1.03 MPa at 20 and 25°C, with a standard deviation ($\sigma_{\Psi_b}$) of 1.15 and 1.18, respectively (Table 2). The uniformity of germination (described by $\sigma_{\Psi_b}$) was relatively low in reduced water potentials. The base water potentials for all subpopulations slightly shifted toward a negative value when the temperature increased from 20 to 25°C (data not shown).

**DISCUSSION**

San Luis slender wheatgrass had a high final germination (94%) under favorable conditions, implying a potential of a high seedling emergence under suitable growth conditions. It also showed a relatively high base temperature ($T_b$) requirement and a relatively broader range of optimum germination temperature for germination. The unique base temperature requirement for seed germination is likely a reflection of San Luis being collected and selected from a warmer climate. In other studies, a number of native prairie species adapted to the northern edge of Northern Great Plains have been reported to have $T_b$ at or below freezing point (Kitchen and Monsen 1994; Wang et al. 2004; Wei et al. 2009). The low temperature requirement for seed germination of Northern Great Plain species has a number of advantages, such as allowing the seeds to accumulate heat when temperature is low, and complete germination and seedling recruitment using early spring soil moisture resulting from snowmelt (Wang et al. 2004). The high base temperature of San Luis for seed germination would be a limitation to seeding in early spring, which is a common practice for forage seeding in the Canadian prairies. This may also suggest more work needs to be done on northern germplasm collections of slender wheatgrass and its genetic enhancement for regional use. San Luis also exhibited a conditional dormancy at

<table>
<thead>
<tr>
<th>Water potential (MPa)</th>
<th>20°C</th>
<th>25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>$81.0 \pm 1.0a^a$</td>
<td>$95.5 \pm 1.0a$</td>
</tr>
<tr>
<td>−0.2</td>
<td>$82.0 \pm 2.0a$</td>
<td>$95.5 \pm 1.3a$</td>
</tr>
<tr>
<td>−0.4</td>
<td>$83.5 \pm 2.2a$</td>
<td>$96.5 \pm 2.1a$</td>
</tr>
<tr>
<td>−0.6</td>
<td>$61.0 \pm 2.4b$</td>
<td>$88.5 \pm 4.9b$</td>
</tr>
<tr>
<td>−0.8</td>
<td>$24.5 \pm 2.1c$</td>
<td>$27.0 \pm 10.4c$</td>
</tr>
</tbody>
</table>

Values are means ± standard error.

a–c Means within the column with the same lower case letters are not significantly different ($P > 0.05$).
higher than 30°C in our study, which may be due to thermo-inhibition effect reported for other species (Bradford and Somasco 1994). Based on seed germination models, the value of $\Psi_{b(g)}$ threshold has been reported to be at a minimum at the optimum temperature, and shift towards a more positive value (or requiring more water for seed germination) at supra-optimal temperature (Bradford and Somasco 1994; Kebreab and Murdoch 1999; Alvarado and Bradford 2002). Seeds with a minimum value of $\Psi_{b(g)}$ can germinate under low water availability without conditional dormancy. An increase in temperature above 30°C in our study may have shifted the $\Psi_{b(g)}$ threshold towards a high value causing conditional dormancy.

The base water potentials for slender wheatgrass San Luis were relatively low under favorable temperature, indicating that the seeds are capable of germinating under relatively high levels of water stress. Moreover, seed germination rate increased in slightly lower water potential than distilled water. Even though increased germination under slightly lower water potential (as compared with distilled water) is not common, it has been reported previously (Welbaum et al. 1990; Dodd and Donovan 1999). We suggest three possible causes for this phenomenon. First, slender wheatgrass is a drought- and salinity-tolerant grass, and may be physiologically adapted to germinate under low osmotic potential, as proposed for other halophytes (Ungar 1991). Second, imbibition in PEG solution could increase O$_2$ availability for seed germination even though it is a poorly understood phenomenon (Welbaum et al. 1990). Finally, a priming effect has occurred from the slight change in water potential. Priming can modify the base temperature and thermal time requirement during seed germination (Hardegree et al. 2002). The effect of priming on seed is the physiological advancement for seed germination, which is related to endosperm weakening (Bradford 2002) and mobilization of storage protein and synthesis of new proteins (Gallardo et al. 2001). In our study, reduced water potential (below −0.6 MPa) lowered germination rate, final germination percentage, and decreased germination uniformity, which is similar to other species (Dahal and Bradford 1990; Shrestha et al. 1999).

In conclusion, thermal and hydro time models are suited to predicting seed germination of slender wheatgrass. We accepted our hypothesis that the southern ecotype of slender wheatgrass would have a high base temperature for germination and could germinate at a lower water potential than many other native cool-season grasses. Based on our results, close attention should be paid to temperature requirements for seed germination of San Luis slender wheatgrass in reclamation or early spring seeding in western Canada.

**ACKNOWLEDGEMENTS**

We thank M. Kehler of the plant ecology laboratory, Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Centre, for technical assistance. We also thank Agriculture and Agri-Food Canada Growing Forward Initiative and the Beef Science Cluster for funding support.

**References**


<table>
<thead>
<tr>
<th>Temperature</th>
<th>Median base water potential $\Psi_b (50)$ (MPa)</th>
<th>Standard deviation of base water potential $\sigma_{\Psi_b}$ (MPa)</th>
<th>Hydro time $\theta_H$ (MPa h)</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>1.02</td>
<td>1.15</td>
<td>61.68</td>
<td>0.92</td>
</tr>
<tr>
<td>25°C</td>
<td>1.03</td>
<td>1.18</td>
<td>61.20</td>
<td>0.89</td>
</tr>
</tbody>
</table>

$r^2$ is coefficient of determination for models fitted to germination data.


