2004-07-09

A Hydrothermal After-ripening Time Model of Seed Dormancy Loss in Bromus tectorum

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A HYDROTHERMAL AFTER-RIPENING TIME MODEL OF SEED DORMANCY LOSS IN *BROMUS TECTORUM*

by

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A thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Plant and Animal Sciences

Brigham Young University

August 2004

BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

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ABSTRACT

A HYDROTHERMAL AFTER-RIPENING TIME MODEL OF SEED DORMANCY LOSS IN *BROMUS TECTORUM*

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After-ripening, the process of seed dormancy loss in dry storage is associated with a decrease in the mean base water potential, one of the parameters of hydrothermal time. The rate of change of the mean base water potential is assumed to be a linear function of temperature above a specific base temperature and as a result can be described by a thermal after-ripening (TAR) time model, an extension of hydrothermal modelling. The thermal requirement for after-ripening is the thermal time necessary for the modelling base water potential of the seed to shift from its original value to its final value. In order to include the effects of water potential on the rate of dormancy loss, a hydrothermal after-ripening (HTAR) time model was developed. Laboratory and field studies were conducted using seeds of *Bromus tectorum*. These studies identified four important ranges of water potential that influence the rate of dormancy loss. The ranges are identified as follows: seeds experiencing soil water potentials <-400 MPa do not after-ripen, between -400 MPa and -150 MPa seeds after-ripen as a function of temperature (T) and water potential (ψ), seeds experiencing water potentials >-150 MPa after-ripen as a linear function of temperature, and somewhere above -40 MPa seeds are too wet to after-ripen. These ranges suggest that specific reaction thresholds associated with non-fully imbibed seeds also apply to the process of after-ripening. The HTAR model for *B. tectorum* seeds generally improved predictions of dormancy loss in the field under soil conditions that were too dry for TAR alone. Reduced after-ripening rate under extremely dry conditions is ecologically relevant in explaining how seeds may prolong dormancy under high soil temperature conditions.

ACKNOWLEDGEMENTS

I would like to express my gratitude to all the individuals that helped to accomplish this research. Without their help it would not have been completed. I appreciate the support and concern for success from my graduate advisor Dr. Phil Allen for his help and assistance in setting up, carrying out, and publishing this research. I thank Dr. Susan Meyer for her help and suggestions while analyzing and preparing the manuscript. I thank Dr. Bruce Roundy for being on my Graduate Committee and his input on my thesis. I appreciate the love and support of my family throughout all my education and for Kyle my husband. I would also like to thank the professors and secretary of the Plant and Animal Sciences for all their help throughout my years at Brigham Young University.

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A Hydrothermal After-ripening Time Model of Seed Dormancy Loss in *Bromus tectorum*
(prepared for submission to *Seed Science Research*)

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Abstract

After-ripening, the loss of seed dormancy during dry storage, is associated with a decrease in the mean base water potential for germination for the winter annual grass *Bromus tectorum*. This decrease is a linear function of temperature above a specific base temperature. Dormancy loss can therefore be described using a thermal after-ripening (TAR) model, an extension of hydrothermal modelling. In order to incorporate the influence of storage water potential (ψ) on TAR, i.e. create a hydrothermal after-ripening (HTAR) model of seed dormancy loss, two populations of recently harvested *B. tectorum* seeds were stored under controlled temperature (20º or 30ºC) and water potential (-400 MPa to -40 MPa) conditions. Sub-samples of seeds were periodically transferred to incubation in water or polyethylene glycol solutions (0 to -1.5 MPa) at temperatures of 15° or 25°C, and subsequent germination (radicle emergence) was recorded. Seeds stored at -400 MPa did not after-ripen. At ψs from -400 MPa to -150 MPa the rate of after-ripening increased approximately linearly with increasing ψ, while between -150 to -80 MPa there was no further increase in after-ripening rate. At -40 MPa, after-ripening rate could not be determined because seeds lost viability. These results suggest that specific reaction thresholds associated with non-fully imbibed seeds likely apply to the process of after-ripening. The HTAR model for *B. tectorum* seeds generally improved predictions of dormancy loss in the field under soil conditions that were too dry for TAR alone. Reduced after-ripening rate under extremely dry conditions is ecologically relevant in explaining how seeds may prolong dormancy under high soil temperature conditions.

Keywords: after-ripening, *Bromus tectorum*, dormancy loss, hydrothermal after-ripening time, hydrothermal time, modelling, water potential

Introduction

Germination is strongly influenced by temperature (T) and water potential (ψ) and can be described by models based on hydrothermal time. Hydrothermal concepts form the basis of many recent efforts to predict seed germination as well as dormancy loss (reviewed by Allen, 2003). A current application of hydrothermal concepts includes after-ripening of grasses such as *Bromus tectorum*, an introduced Eurasian winter annual that has invaded a wide variety of habitats in western North America within the past 100 years (Mack, 1981).

Hydrothermal time was first proposed by Gummerson (1986) and further developed by Bradford (1990, 1995). The hydrothermal time equation for germination of a given germination fraction (i.e. a single seed within the population) is:

$$\theta_{HT} = (\psi - \psi_b(g))(T-T_b)t_g \quad (1)$$

where θ_{HT} is the amount of hydrothermal time (i.e., MPa ° days) required for germination to occur, ψ is the water potential of the incubation medium, $\psi_b(g)$ is the base water potential below which germination will not occur for fraction g, T is the incubation temperature, T_b is the base (minimum) temperature for germination, and t_g is the actual time to germination for fraction g. In order to extend equation 1 to describe germination for a seed population, Gummerson assumed that the distribution of mean base water potentials within a population was normal. Probit transformation, which linearizes a cumulative normal distribution curve, could then be incorporated to predict germination for all seed fractions as follows:

$$\text{Probit}\,(g/g_m) = [\,\psi - \psi_b(50) - \theta_{HT}/((T-T_b)t_g)]/\sigma_{\psi b} \quad (2)$$

where g_m is the fraction of viable seeds in the population, $\psi_b(50)$ is the mean (median) base water potential of the population, and $\sigma_{\psi b}$ is the standard deviation of base water potentials within the population.

Hydrothermal models provide a quantitative description of seed germination rates based on the relationship with ψ and T conditions (Bradford, 1990; 2002). Treatments that lead to an upward shift in $\psi_b(50)$ (Bradford, 1990; 1995, Christensen, *et al.*, 1996) lead to a decreased germination rate and, if sufficient, a decrease in germination percentage as well. Similarly, germination rate and percentage are increased by more negative $\psi_b(50)$ values. Shifts in $\psi_b(50)$ above the ψ of the imbibition medium that prevent germination can explain germination behaviors such as dormancy cycling (Bradford, 2002) as well as the delay and inhibition of seed germination in the supra-optimal range of T (Bauer *et al.*, 1998; Shrestha *et al.*, 1999; Meyer *et al.*, 2000; Alvarada and Bradford, 2002; Rowse and Finch-Savage, 2003).

The discovery that after-ripening progresses as a linear function of T above a specific base T led to the use of hydrothermal models to describe dormancy loss (Christensen *et al.*, 1996). Dormancy loss in the field could then be simulated using a thermal after-ripening time (TAR) model. In order to predict germination in the field, Bauer *et al.* (1998) used the hydrothermal time parameter mean base water potential ($\psi_b(50)$) as an index of dormancy status. TAR models successfully predicted seed dormancy loss in *B. tectorum* in the field under soil ψ conditions that remained above -150 MPa. However, during an extremely dry year (soil ψ conditions were frequently <-150 MPa) the TAR model did not accurately predict dormancy loss, but instead predicted too rapid a rate of after-ripening for actual field data (S. Meyer and P. Allen unpublished data).

In earlier studies using seeds of wild oat and red rice, Foley (1994) and Leopold *et al.* (1988) reported that after-ripening could be slowed or prevented at very low seed water contents. Leopold and Vertucci (1989) reported that after-ripening would not occur at moisture contents below 0.05 g H_20 g^{-1} dw and occurred poorly at moisture above 0.15 g H_20 g^{-1}. Walters *et al.* (2001) suggested that specific reactions associated with water content thresholds in non-fully imbibed seeds could apply to a variety of developmental processes. For example, above -150

MPa catabolic activities via enzymes begin and below -150 MPa free radical production is increased (Leopold and Vertucci, 1989).

Inaccurate field prediction of seed dormancy loss could be associated with the influence of ψ, especially if the range of soil ψs included values above and below critical moisture thresholds for after-ripening. In order to incorporate the influence of storage ψ the TAR model needed to be expanded.

The objectives of this paper were (1) to develop a hydrothermal after-ripening (HTAR) model to describe seed dormancy loss and (2) to create a simulation model using HTAR concepts and measured soil ψ and T values to predict dormancy loss in the field.

Materials and Methods

Hydrothermal After-ripening Time Experiment with 1994 and 2002 Seeds

Mature florets (hereafter referred to as seeds) of *B. tectorum* were collected from two semi-arid Great Basin sites (Whiterocks, UT, a salt desert shrub site, and Hobblecreek Canyon, UT, a mountain brush site) during July of 1994. Seeds were air-dried to a water content of 8-10% (dry weight basis), cleaned by rubbing and fanning, and hand-examined to ensure fill.

Seeds were stored at Ts of 20 or 30°C and ψs of -300 or -150 MPa. Seeds were removed from storage at zero and 14 weeks and incubated at four ψs 0, -0.5, -1.0, or -1.5 MPa. Seeds stored from two to 14 weeks were incubated at 0 MPa for 28 days at two alternating Ts (10/20°C and 20/30°C, incubated for 12 hours at each T); all other steps for laboratory data are similar to those discussed below for seeds collected in 2002.

Mature seeds of *Bromus tectorum* were hand collected from the same two sites in June of 2002 and cleaned as described previously. Seeds were stored at Ts of 20°C or 30°C and ψs of -400, -350, -300, -200, -150, -80, or -40 MPa in the dark. Ψs were obtained by equilibrating seeds with the atmospheres above saturated salt solutions (Winston and Bates, 1960; Schneider and Schneider, 1972) or glycerol solutions (Forney and Brandl, 1992) in desiccators or containers. Seeds were placed on a porous material (i.e. plastic canvas) that was suspended above the saturated salt or glycerol solutions, and containers were sealed with high vacuum grease to prevent changes in ψ. Ψs of -400, -350, -300, -150, -80, and -40 MPa were created through use of the compounds $ZnCl_2$, KOH, LiCl, $MgCl_2$, $CaNO_3$, and NaCl, respectively. Glycerol solutions were used to obtain ψ values of -200 MPa and -150 MPa. A -200 MPa glycerol solution was used because of the difficulty in finding an appropriate saturated salt solution at this ψ and a -150 MPa glycerol solution was to verify that the glycerol and salt solutions produced similar results. Seed water content was determined from subsets of seeds equilibrated for eight weeks for all ψs and storage T treatments (Copeland and Miller, 2001).

Seeds were removed from storage after intervals ranging from zero to 73 weeks and placed in germination treatments as described below. Seeds stored from two to 60 weeks were incubated at 0 MPa (i.e., in water) for 28 days. Seeds stored for zero or 73 weeks were incubated at ψs of 0, -0.5, -1.0, and -1.5 MPa. Four replications of 25 seeds were used for each incubation treatment. Seeds were placed at two constant Ts (15°C and 25°C) in 100 x 15 mm Petri dishes on two layers of blue blotter paper (Anchor Paper, St. Paul, Minnesota, USA). Blotters were saturated with water or solutions of polyethylene glycol (PEG) 8000 at the desired ψ. The PEG

was mixed according to Michel (1983). Dishes were stacked in clear plastic bags. A water-saturated paper towel was placed at the bottom of each bag to prevent excess evaporative loss. During incubation treatments, seeds received 12 hours of cool white fluorescent light and 12 hours of darkness per day. Germinated seeds (radicle emergence ≥ 1mm) were counted and removed on days 1, 2, 4, 7, 11, 14, 21, and 28.

The frequency of seed transfer from storage to germination conditions was determined based on how rapidly seeds lost dormancy (i.e., subsets of seeds were removed more frequently from treatments that resulted in more rapid dormancy loss). Germination percentage and rate indicated the level of dormancy loss associated with seeds stored under specific storage conditions. Seeds were stored at the lowest ψs for up to 73 weeks, while seeds at less negative ψs were stored for as little as 18 weeks.

Data from fully after-ripened seeds (stored for 73 weeks at -150 MPa) incubated at 0, -0.5, -1.0, and –1.5 MPa were used to determine hydrothermal time parameters for each seed population as described by Gummerson (1986); Bradford (1990, 1995); Christensen *et al*. (1996); and Bauer *et al.* (1998). Germination time courses for each incubation ψ (0, -0.5, -1.0, and -1.5 MPa) and T (15 and 25°C) were analyzed by repeated probit regression analysis. The probit analysis consisted of regressing probit(g) on $\psi_b(g)$, calculated as $[\psi - \theta_{HT}/((T- T_b)t_g)]$ (Bauer *et al.*, 1998), adjusting the value of θ_{HT} until the highest R^2 value for the regression was obtained. From the regression line with the best fit, probit $(g/g_m) = m(\psi_b(g)) + b$, a mean base water potential ($\psi_b(50)$) and standard deviation for base water potentials ($\sigma_{\psi b}$) was determined according to the relationships of $\psi_b(50) = -b/m$ and $\sigma_{\psi b} = 1/m$ (Christensen *et al.*, 1996).

Next, $\psi_b(50)$ values were calculated for all seed sets stored in the laboratory for durations ranging from two to 60 weeks and then incubated in water. Once the hydrothermal parameters θ_{HT} and $\sigma_{\psi b}$ were determined for a seed collection, the $\psi_b(50)$ characterizing a single germination curve was calculated from the relationship:

$$\psi_b(g) = -\theta_{HT}/(T\ (t_g)). \quad (3)$$

This equation can be derived from equation 1 by defining T_b=0 (base temperature) and ψ=0 MPa. Estimation of $\psi_b(50)$ for highly dormant seed lots (i.e., final germination <50%) is described in detail in Bauer *et al.* (1998).

Characterizing changes in $\psi_b(50)$ (i.e., dormancy status) through time at the two storage Ts for each storage ψ allowed us to determine the thermal time required for after-ripening. The TAR equation is:

$$\theta_{AT}=(T_s-T_l)t_{ar} \quad (4)$$

where θ_{AT} is the constant thermal time for after-ripening, T_s is the storage temperature, T_l is the base storage temperature (below which after-ripening does not occur), and t_{ar} is the actual time in storage required for completion of after-ripening (the time required for $\psi_b(50)$ to change from its starting value to its final value) (Bauer *et al*., 1998).

$\Psi_b(50)$ values for each collection X storage interval X storage T X storage ψ X incubation temperature combination were plotted against thermal after-ripening time. T_l for *B. tectorum* seeds was assumed to be 0°C based on Bauer *et al*. (1998). The linear equation is:

$$\psi_b(50)=m[(T_s-T_l)t_{ar})] +b \quad (5)$$

where b is the initial value of $\psi_b(50)$ before any thermal time is acquired and the slope (m) is the decrease in value in $\psi_b(50)$ per unit thermal time (i.e., $\psi_b(50)$/ °weeks). These slopes were then plotted against seed storage ψ to determine the influence of ψ on the rate of after-ripening.

Field Experiments with 1994 Seeds

The field retrieval study was conducted at Point of the Mountain, Utah, a sage-brush/grass site with sandy loam soil (66% sand, 18% silt, and 16% clay). Seeds were air-dried, placed inside nylon mesh bags, and buried approximately 5 mm below the soil surface. Each bag contained approximately 200 seeds as estimated by weighing. The bags were placed in four rows of 25 bags each and four bags of each collection (one bag per row) were retrieved weekly from the experimental site. Seeds were transported from the field to the laboratory (about 30 min transit time) in plastic bags to minimize changes in water content. Radicle emergence that occurred in the field was recorded, and the non-germinated seeds in each bag were divided into two approximately equal groups. The first group of seeds was incubated at 10/20°C and the second at 20/30°C. Seeds were incubated for 28 days and germination recorded as described previously.

T and water content of the seed zone (approximately the top 1 cm of soil) at the field site were measured using thermistor (Omnidata, Logan, UT, USA) and Aquatel (Automata Inc., Grass Valley, CA, USA) sensors, respectively. Measurements were recorded hourly, as an average of six 10 minute reads, using a data logger (Omnidata Easylogger 900, Logan, UT, USA). Aquatel sensors measure capacitance of the soil, which varies as a function of water content and soil characteristics. Laboratory calibrations were performed to determine water content values corresponding to Aquatel readings in soil. Corresponding ψ values were determined using a soil water release curve for this soil (Hanks, 1992).

A second method used to predict ψ in the field involved estimating seed zone ψ based upon measured T at the soil surface and relative humidity at 1 m above the surface, then solving for soil ψ according to the following equation:

$$\psi_a=(RT/Vm)(\ln e/e^o) \quad (6)$$

where ψ_a is the atmospheric water potential (in Megapascals, MPa), R is the gas constant (8.2 MPa $\cdot$ cm^3 $\cdot$ mol °K), T is the temperature of the soil in degrees Kelvin, Vm is the molar volume of water (18 cm^3 $\cdot$ mol), and ln e/e^o is the natural logarithm for actual water vapor pressure divided by the saturating vapor pressure for that temperature (ln e/e^o x 100 = relative humidity). This method assumes that the water vapor in the atmosphere and soil are the same, an assumption that is only valid in soils dry enough that the water content below the seed zone is negligible (i.e., no significant movement of water vapor to the seed zone from deeper soil depths).

A simulation model to predict changes in $\psi_b(50)$ in the field was created by using measured seed zone T and ψ values, thermal after-ripening time parameters, and initial and final $\psi_b(50)$ values from laboratory data (to know when to end the simulation). The model was created using Time-Zero software (Quaternary Software, Inc., Fort Collins, CO, USA) and is described in detail in Bauer *et al.*, (1998), but later run in Microsoft Excel (Microsoft Works,

Seattle, WA, USA). Predicted $\psi_b(50)$ values in the field at each incubation T for each seed collection were compared with observed values obtained from the weekly field retrievals.

Results

All seed populations were partially dormant when collected, as indicated by low initial germination percentages (Table 1). Seeds incubated at 25°C had germination percentages that were 13-50% lower than when seeds were incubated at 15°C. The values of θ_{HT} for the Hobblecreek collection were similar; however, θ_{HT} for the 1994 Whiterocks collection was nearly three times greater than the 2002 Whiterocks collection. This difference can possibly be explained by wide year to year variation resulting from natural selection of different genotypes within the population (Meyer and Allen, 1999a, 1999b). Values of θ_{HT} for Whiterocks seeds collected between 1992 and 1995 varied from 31 to 55 MPa-degree-days, at least double the value. The low θ_{HT} value for Whiterocks 2002 is offset by a high $\psi_b(50)$ value, which resulted in comparable germination rates in water (data not shown). The values of $\sigma_{\psi b,}$ which indicate uniformity of germination, were nearly double for Hobblecreek 2002 than for the other three collections. R^2 values from probit regressions ranged from 0.84 to 0.91, which were reasonably high and similar to those reported previously (Bauer *et al.* 1998).

Seeds after-ripened more quickly in 1994 when stored at -150 MPa than at -300 MPa (Figure 1). The influence of storage ψ was incorporated into a simulation model for field data (1994) but required several assumptions. First, seeds stored above -150 MPa were assumed to after-ripen independently of storage ψ (unpublished data). Second, in the absence of additional storage ψs below -150 MPa, the relationship between storage ψ and change in after-ripening rate was assumed to be linear between -150 and -300 MPa. Extrapolation from the different individual lines (Figures 1A and B) would predict different storage ψs where after-ripening is prevented. In the absence of additional data we chose to include all values in a single regression and extrapolate to predict where after-ripening was prevented completely (i.e., the storage ψ where change in $\psi_b(50)$ is zero). This led to a prediction that after-ripening ceases at around -375 MPa. Storage ψ was incorporated into the TAR model by adjusting TAR by the decrease in rate of change in $\psi_b(50)$ (i.e., slope in Figure 1C).

Field seedzone ψ for the week of July 7-14, 1994 showed wide diurnal fluctuation, with both methods estimating soil seed zone ψs between -200 to -800 MPa (Figure 2), during which time Ts fluctuated between 12 and 57°C (data not shown). Estimates based on Aquatel readings showed less overall fluctuation (-200 to -600 MPa) than did estimates based on relative humidity measurements (-200 to -800 MPa), but both were closely similar recording the same pattern of high to low fluctuations in soil ψs. Both methods showed that soil ψ values were very low (never reaching above -200 MPa), in the range where ψ potentially has a large slowing effect on after-ripening.

Thermal time (TAR) predictions of dormancy loss in the field were consistently the most rapid (Figure 3), nearly always faster than observed values for change in $\psi_b(50)$. For the two models based on HTAR, the model based on estimated soil ψ yielded the slowest predicted rate of after-ripening and the model based on capacitance sensors resulted in intermediate predictions. Three out of four observed plots of actual rates of change for $\psi_b(50)$ were at or between values predicted by one of the two HTAR approaches.

As expected, seeds stored at more negative ψs equilibrated at lower water contents (Figure 4). Under the range of storage conditions included in this study, water content values ranged from 2% to 19%. Water content values were very similar between the two accessions. The small increase in storage ψ for seeds stored over a given salt or glycerol solution at 20°C was a result of temperature being a variable in converting relative humidity to ψ. For example, $MgCl_2$ equilibrated a relative humidity of 32% at both storage Ts, but storage ψ at 20°C was slightly higher.

After-ripening, as indicated by a decrease in $\psi_b(50)$ over time, was essentially prohibited at the most negative ψ (-400 MPa; Figure 5). To verify that seeds had not been killed by this treatment, subsets of seeds stored at -400 MPa for 73 weeks were transferred to storage at 20 or 30°C and -150 MPa for eight weeks. Seeds were then incubated at 15 or 25°C and 0 MPa for 28 days and averaged 85% germination after four days and 91% germination after 28 days incubation. In contrast, seeds stored at -40 MPa began to lose viability after only eight weeks of storage, which led to spurious $\psi_b(50)$ values (data not shown). Storing seeds from -350 to -150 MPa resulted in progressively increased rates of after-ripening.

Seeds stored at ψ conditions ranging from -150 MPa to -80 MPa after-ripened quicker than at other storage ψs (Figure 5). At storage ψs between -200 and -80 MPa after-ripening occurred more rapidly during the first 240 degree-weeks. After 240 degree-weeks the rate of after-ripening generally slowed as seeds neared completion of after-ripening. At -350 MPa, the decrease in $\psi_b(50)$ appeared linear. Seeds stored from -400 to -200 MPa did not complete after-ripening before the experiment was terminated. To calculate the after-ripening slopes (i.e., $\psi_b(50)$/thermal time) needed to adjust TAR for the effects of ψ, $\psi_b(50)$ values from zero to 240 degree-weeks were used. The decision to omit later values was based primarily on the observation that most after-ripening had occurred by this time, the response was nearly linear over this range, and a simple linear slope for each storage ψ was easier to fit into the hydrothermal after-ripening model. The rate of after-ripening for seeds stored at -40 MPa was initially rapid, but seeds soon began losing the ability to germinate and eventually deteriorated, as a result these values were not included in Figure 6.

The influence of storage ψ on the after-ripening slope was similar for both collections and incubation temperatures (Figure 6). At -400 MPa or above -150 MPa there was generally no change in the rate of after-ripening as influenced by storage ψ. Between -150 and -350 MPa the rate of after-ripening progressively increased with less negative storage ψ. At both storage Ts the influence of ψ on rate of after-ripening was very similar, suggesting that thermal after-ripening time is a valid model to describe seed dormancy loss in *B. tectorum*. Inherent in the thermal time concept is the mathematical relationship wherein the same amount of after-ripening can be achieved by several different combinations of T and duration. For example, seeds stored at 20°C required longer storage to fully after-ripen, but produced similar changes in the rate of after-ripening for seeds stored at 30°C.

Seeds from both collections required approximately the same amount of time for after-ripening to occur. Hobblecreek seeds reached a much lower $\psi_b(50)$ value than did Whiterocks seeds (Table 1), which is also indicated by a much more rapid decrease in $\psi_b(50)$ for Hobblecreek seeds (Figures 5 and 6).

Discussion

The results of this study led to a proposed conceptual framework to account for the influence of ψ on after-ripening in *B. tectorum* seeds (Figure 7). The diagram includes four important ranges of storage ψ with associated thresholds that determine which model (TAR vs HTAR vs no after-ripening) best predicts dormancy loss. These ranges are as follows: 1) seeds stored below about -375 MPa do not after-ripen; 2) between -375 and -150 MPa seeds after-ripen as a function of both T and ψ and dormancy loss can be explained by a hydrothermal after-ripening model (HTAR); 3) seeds stored at or above -150 MPa experience after-ripening as a linear function of T alone (TAR); and 4) seeds stored above -40 MPa are too wet for after-ripening to occur (they deteriorate, slowly progress toward germination, or remain imbibed but dormant). In the widely fluctuating T and ψ environment that characterizes the soil seed-zone, seeds likely pass through many of these ranges repeatedly. The ability to successfully predict the rate of dormancy loss in the field suggests that seeds indeed progress toward after-ripening as a cumulative summation of T and ψ. Additional evidence for the HTAR model is provided by similarly successful field predictions of after-ripening for seeds of the perennial bunchgrass *Elymus elymoides* (Bair, 2004; Appendix A).

Seeds stored at very low water contents have previously been shown to experience negligible after-ripening (Steadman *et al.*, 2003; Foley, 1994; Leopold *et al.*, 1988), but this finding for *B. tectorum* provides an ecologically relevant explanation for how seeds can be prevented from losing dormancy too soon. *B. tectorum* seeds mature in early to mid summer, and typically experience weeks to months of hot, dry conditions that are not conducive to successful seedling establishment. If after-ripening was solely based upon thermal time, seeds would after-ripen very quickly and precocious germination (e.g. following summer thunderstorms) could result, reducing the probability of seedling survival. Seeds are prevented from losing dormancy at these times by an inhibited rate of after-ripening at very low ψs.

While long term storage at very low ψs could damage seeds (Walters, 1998), we have never observed this to occur in the field for wild plants that are adapted to semi-arid habitats (unpublished data). Low water content probably does not affect viability of seeds in the field, possibly because fluctuating soil ψs either prevent the accumulation of reactions that favor seed aging and subsequent loss of viability, or they allow for repair processes to occur. When seeds of *B. tectorum* and *Elymus elymoides* were buried in field soils for several months, they did not lose viability even though soil ψ was regularly <-400 MPa (data not shown).

Seed water content is often associated with regions of differing water-binding characteristics as seen in Figure 4. The data is consistent with earlier results and the implications are discussed in detail by Vertucci and Leopold (1984). The concept that there are levels of physiological activity associated with water binding regions where different reactions occur appears to be applicable to the process of after-ripening (Leopold and Vertucci, 1989) and is supported in data reported herein.

The second range of storage ψs suggested in Figure 7 predicts dormancy loss according to a hydrothermal after-ripening time model. Model development often requires many simplifying assumptions. One decision made in the development of TAR, (Bauer *et al.*, 1998), was to ignore the influence of soil ψ. The assumption was based on the fact that on average soil ψ values were above -150 MPa during the year of study (1995). However in 1994, a particularly dry year, the TAR model consistently over-estimated the rate of after-ripening for field data. By

including the combined influence of storage ψ and T on the rate of after-ripening, the HTAR model more accurately predicted dormancy loss than TAR three out of four times for field data (Figure 3). We believe that simulation modelling of dormancy loss in *B. tectorum* is limited by the ability to accurately estimate seed ψ in the field. Seed water content does not necessarily reflect soil ψ values under wide diurnal fluctuations. When *B. tectorum* seeds were alternated between -150 and -300 MPa, seeds water content on average was much closer to the equilibrium value for -150 than for -300 MPa (unpublished data).

After-ripening of seeds stored between -150 and -375 MPa was approximately linear initially, followed by a progressively slower rate that often led to an overall curvilinear response. In some storage treatments, seeds failed to complete dormancy loss, which is partially a result of the experiment being terminated before a fully after-ripened state was attained. In Figure 5 seeds stored at -350, -300, and -200 MPa after-ripened successively slower and even began to level off at less negative $\psi_b(50)$ values, this is particularly evident in plots C, D, G, and H. By viewing after-ripening as a complex process with multiple reactions occurring at different rates, some reactions might be slowed or prevented at water potentials above -400 MPa (Vertucci and Leopold, 1989). In addition, if a dominant reaction controls the rate of initial after-ripening and a secondary reaction (i.e., with a slower rate) takes longer to complete, the combined result would be a curvilinear response. Gianinetti and Cohn (unpublished data) used a log transformation to linearize the curvilinear relationship (the change in $\psi_b(50)$ as a function of thermal time) rather than just using the linear initial phase as the measure of dormancy loss in red rice seeds. For the purpose of predicting dormancy loss in the field, using the steep initial portion appears to be sufficient and simplified model development.

When soil ψs are above -150 MPa (the third region identified in Figure 7), thermal after-ripening time is sufficient to predict after-ripening (Bauer *et al.*, 1998). For the TAR portion of the model we assumed that the relationship between storage T and after-ripening rate was the same for both incubation Ts. This implies that after-ripening status would be approximately equal for all incubation Ts, with only initial and possibly final $\psi_b(50)$ values varying with incubation T (Meyer *et al.*, 2000). This assumption is not supported by the limited data in Figure 1. However, the much larger data set used to generate Figure 6 generally supports the argument that incubation T produces roughly similar relationships between storage ψ and the after-ripening slopes. Failure to include this assumption would require a separate model for each incubation T and collection combination.

Results from -40 MPa storage treatments were difficult to interpret due to rapid loss of viability. As seeds age they lose vigor, take longer to germinate (Ellis and Roberts, 1980; 1981) and eventually lose the ability to germinate (Walters, 1998). The loss of viability at -40 MPa storage ψ is not significant in the field probably due to fluctuating ψs. Although seeds regularly experienced ψs greater than -40 MPa in the field in 1995 (Bauer *et al*., 1998), it was generally for just a few hours before seeds were either close to 0 MPa or much drier. As with seeds frequently exposed to very dry soil conditions, seeds of *B. tectorum* that encountered moist soil conditions did not lose viability in the field.

The fourth range, which probably includes most of the range above -40 MPa, is too wet for after-ripening to occur. At these storage ψs, seeds deteriorate, accumulate progress toward germination, or remain imbibed but dormant. Storage ψs >-40 MPa present experimental difficulties that prevent meaningful interpretation of after-ripening results.

In this paper, we propose a model that defines four ranges of ψ that influence the rate of after-ripening. The first range involves seeds that are experiencing very dry soil ψ conditions where negligible after-ripening occurs. In the intermediate range, decreasing ψ has a progressively inhibiting influence on the rate of after-ripening. The third range can be explained by thermal after-ripening time alone, and the wettest range fails to promote after-ripening (i.e., "dry after-ripening" does not occur in wet seeds). Incorporating ψ into models that predict dormancy loss through after-ripening provides more accurate field predictions of dormancy loss than thermal after-ripening time alone. The model is also supported by a body of empirical and theoretical literature on the physiology of dry seeds, which suggests that the hydrothermal after-ripening time provides a valid explanation of after-ripening rates. The model has potential for making better predictions of dormancy loss under the widely fluctuating soil ψ conditions that occur in the field. Linking the hydrothermal after-ripening time with hydrothermal time for germination will be an important step in creating a combined model to account for both dormancy and germination under fluctuating ψ and T conditions.

Acknowledgements

Funding for this research is from Brigham Young University Department of Plant and Animal Sciences. Technical work was provided by Brian Anderson, Jennifer Baker-Xanthos, Julie Beckstead, Heather Beecher, Eric Castle, Maren Bauer, Becky Peterson, and Amanda Silvester.

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Table 1

Initial and final percent germination and hydrothermal time parameters for four fully after-ripened *Bromus tectorum* seed collections. Seeds were collected during June or July of 2002 and 1994.

		Initial		Final					
		15°C	25°C	15°C	25°C	θ_{HT}	$\psi_b(50)$	$\sigma_{\psi b}$	
	Year	—(%)———		—(%)———		(MPa°day)	(MPa)	(MPa)	R^2
Whiterocks	1994	69	56	97	95	42	-1.22	0.306	.89
Whiterocks	2002	32	15	95	98	16	-0.80	0.24	.84
Hobblecreek	1994	83	32	100	99	37	-1.17	.311	.91
Hobblecreek	2002	31	18	92	94	31	-1.22	0.56	.85

Figure 1.

After-ripening of 1994 *B. tectorum* seeds as indicated by change in $\psi_b(50)$ per unit thermal time: (A) Whiterocks, (B) Hobblecreek, and (C) best fit regression for all values extrapolated to predict where after-ripening is completely inhibited.

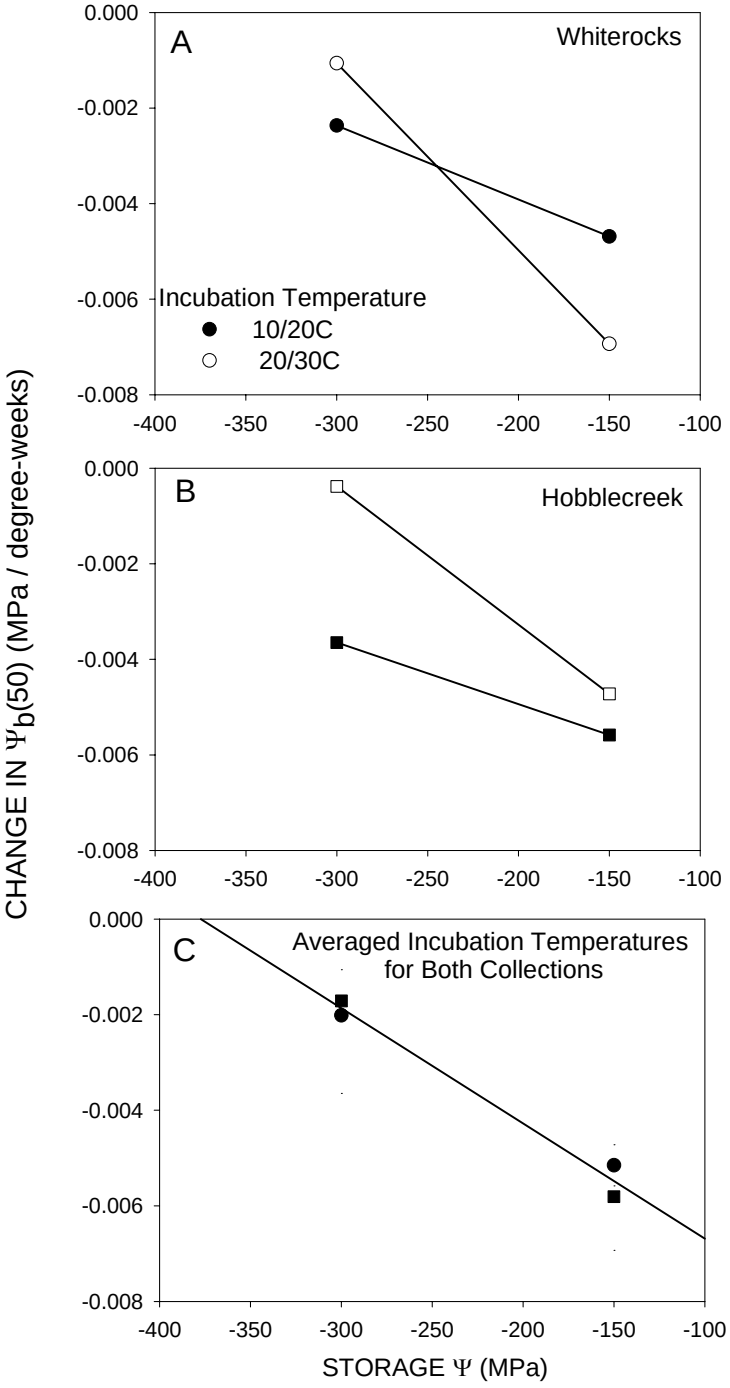


Figure 2.

Field seed-zone water potential for the week of July 7-14, 1994 at Point of the Mountain, Utah: (A) water potential estimates based on capacitance readings which were converted to water potential as described in the text and (B) water potential estimates based on measured atmospheric temperature and humidity, corrected for seed-zone temperature as described in the text. The solid lines are at -150 MPa, below which after-ripening is progressively reduced. The dotted lines are at -375 MPa, where after-ripening is completely halted. These two ψs are threshold values in the HTAR model as illustrated in Figure 7.

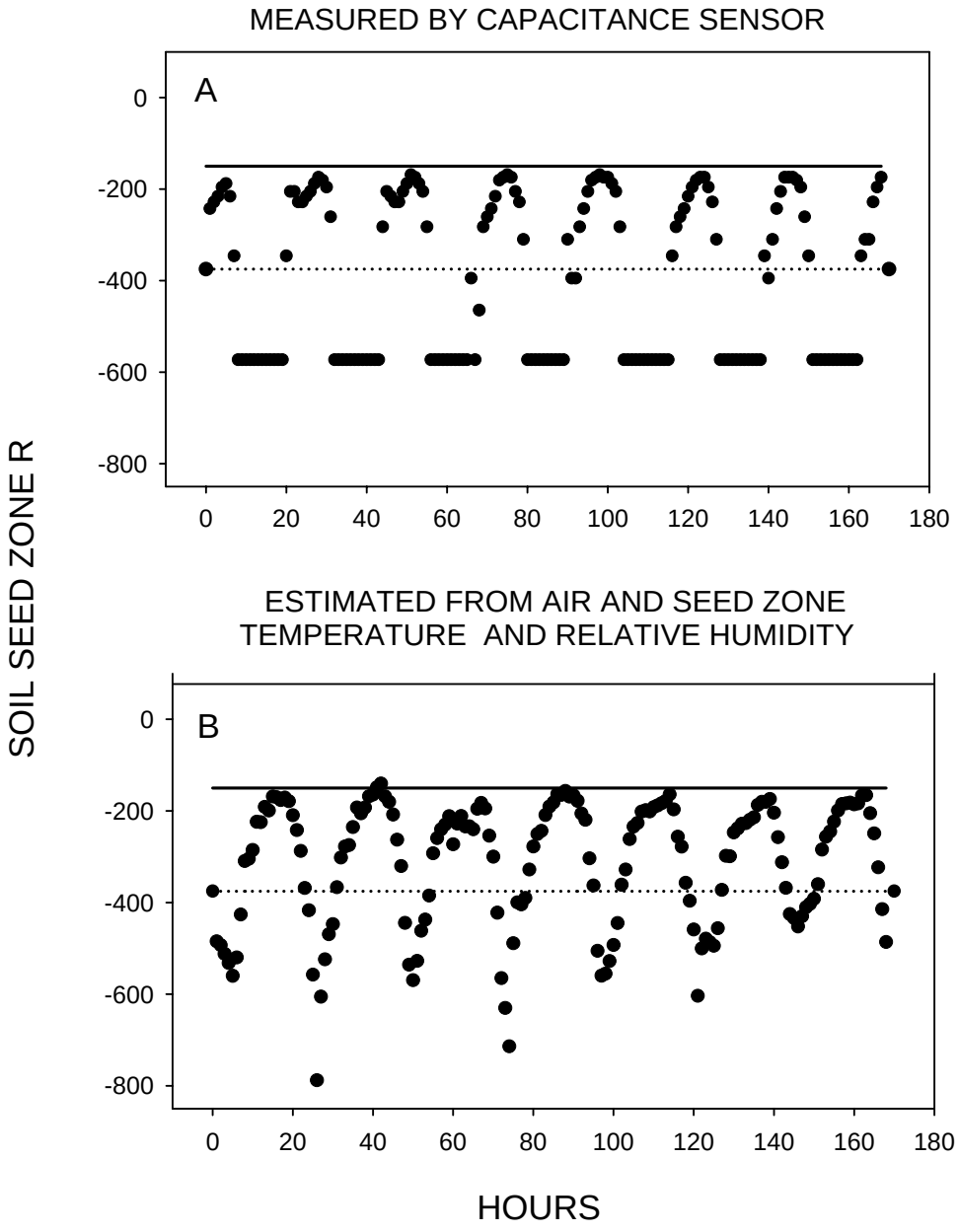


Figure 3.

Predicted and observed changes in $\psi_b(50)$ during field after-ripening at Point of the Mountain, Utah for 1994 *Bromus tectorum* seeds.

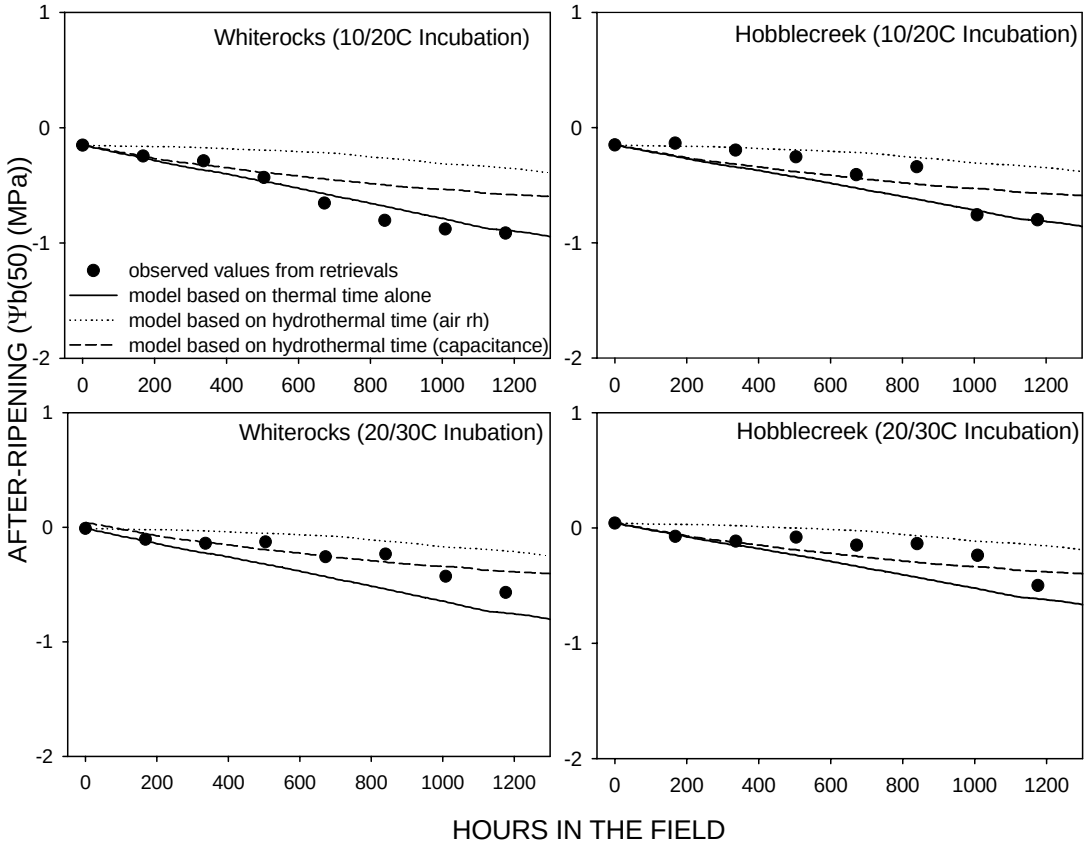


Figure 4.

Water content for Whiterocks and Hobblecreek *Bromus tectorum* seeds stored at 20 or 30°C at different water potentials. Standard error bars are all smaller than symbols.

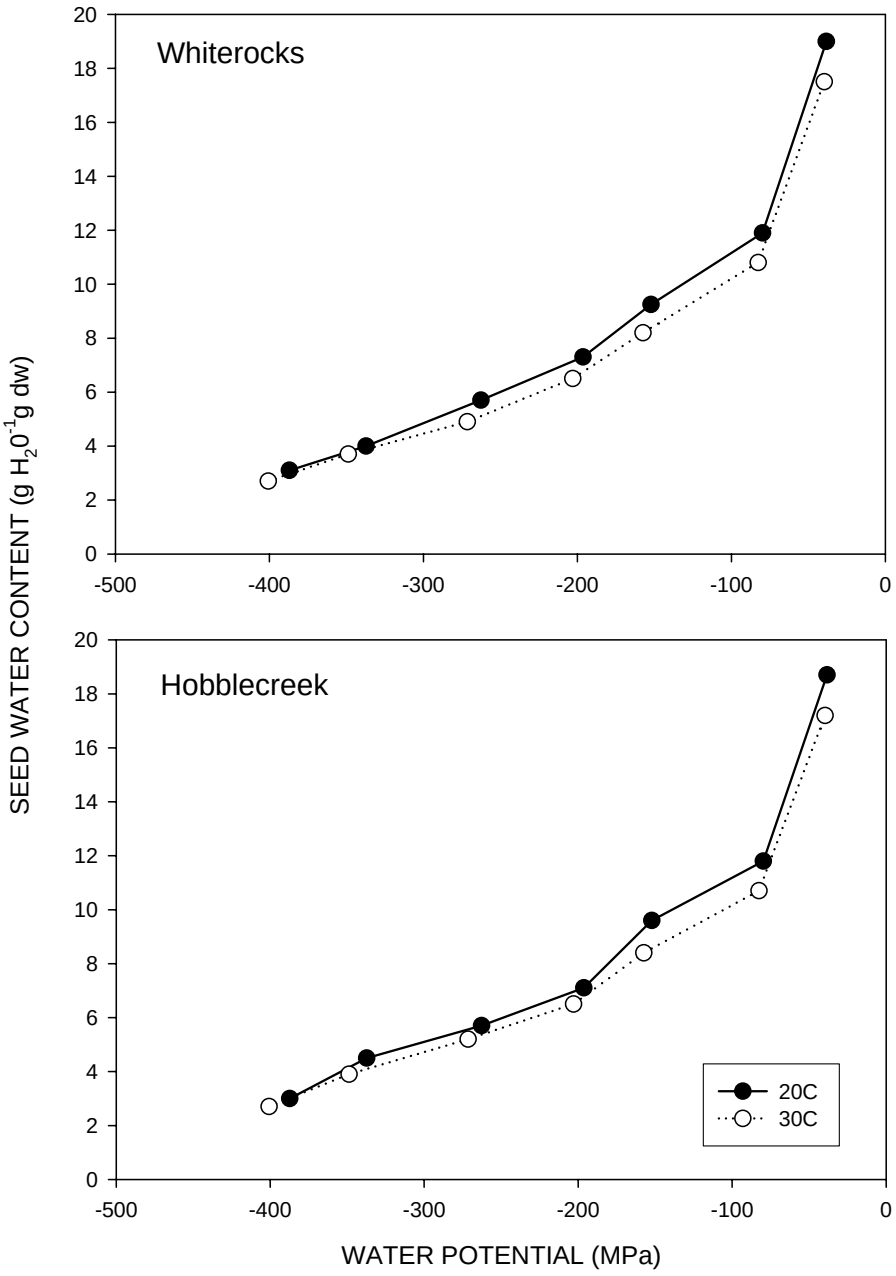


Figure 5.

After-ripening (as measured by the change in $\psi_b(50)$) in *B. tectorum* seeds as influenced by storage water potential, storage temperature, and incubation temperature for two seed collections (2002). * indicate seeds were equilibrated above glycerol solutions; all other water potentials were achieved above saturated salt solutions.

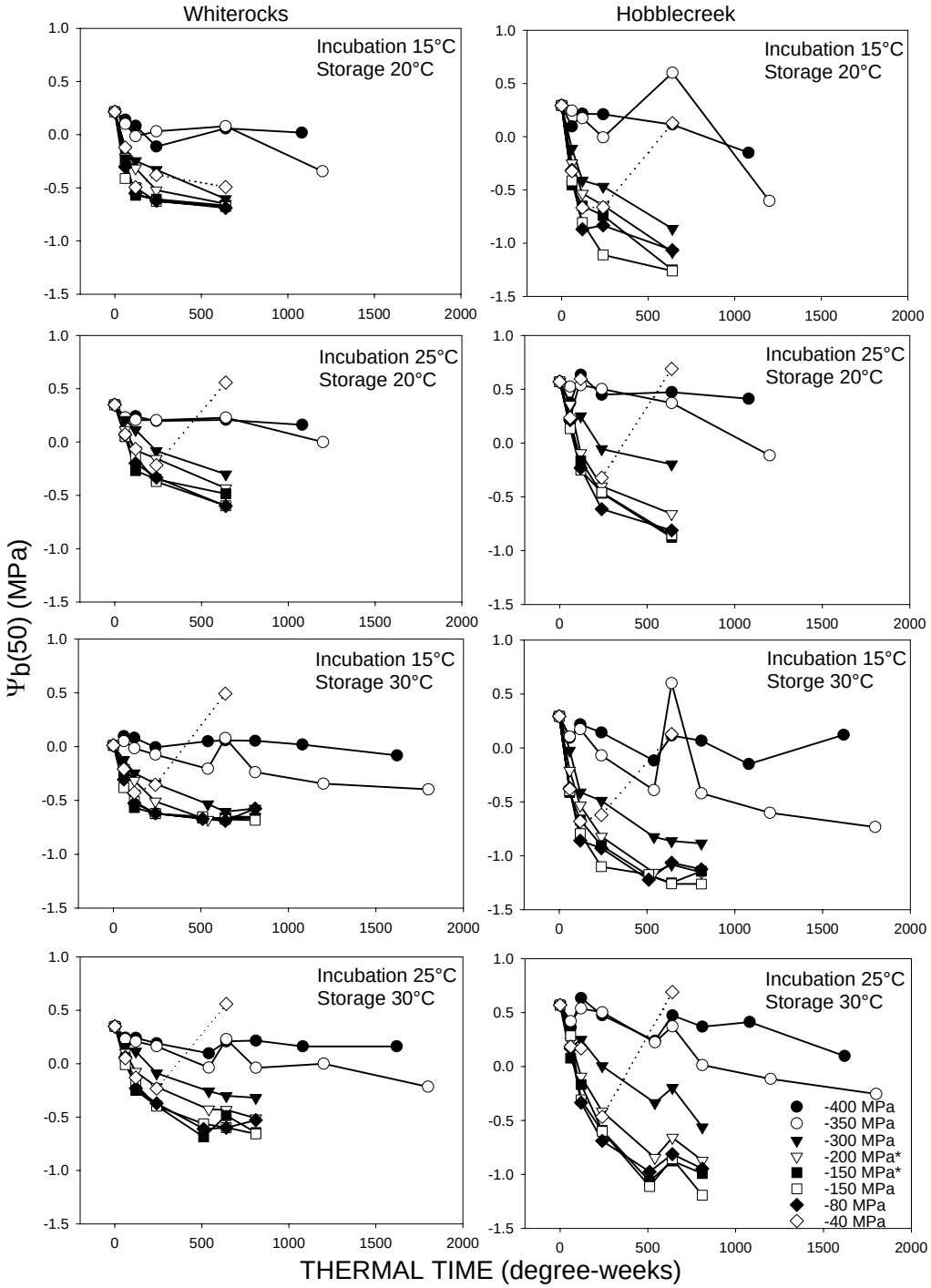


Figure 6.

After-ripening of 2002 *B. tectorum* sees as indicated by change in $\psi_b(50)$ per unit thermal time: (A) Whiterocks and (B) Hobblecreek. Zero to 240 degree-weeks were used to calculate the after-ripening slope in the regressions. There is no value for -40 MPa due to rapid seed deterioration at this water potential.

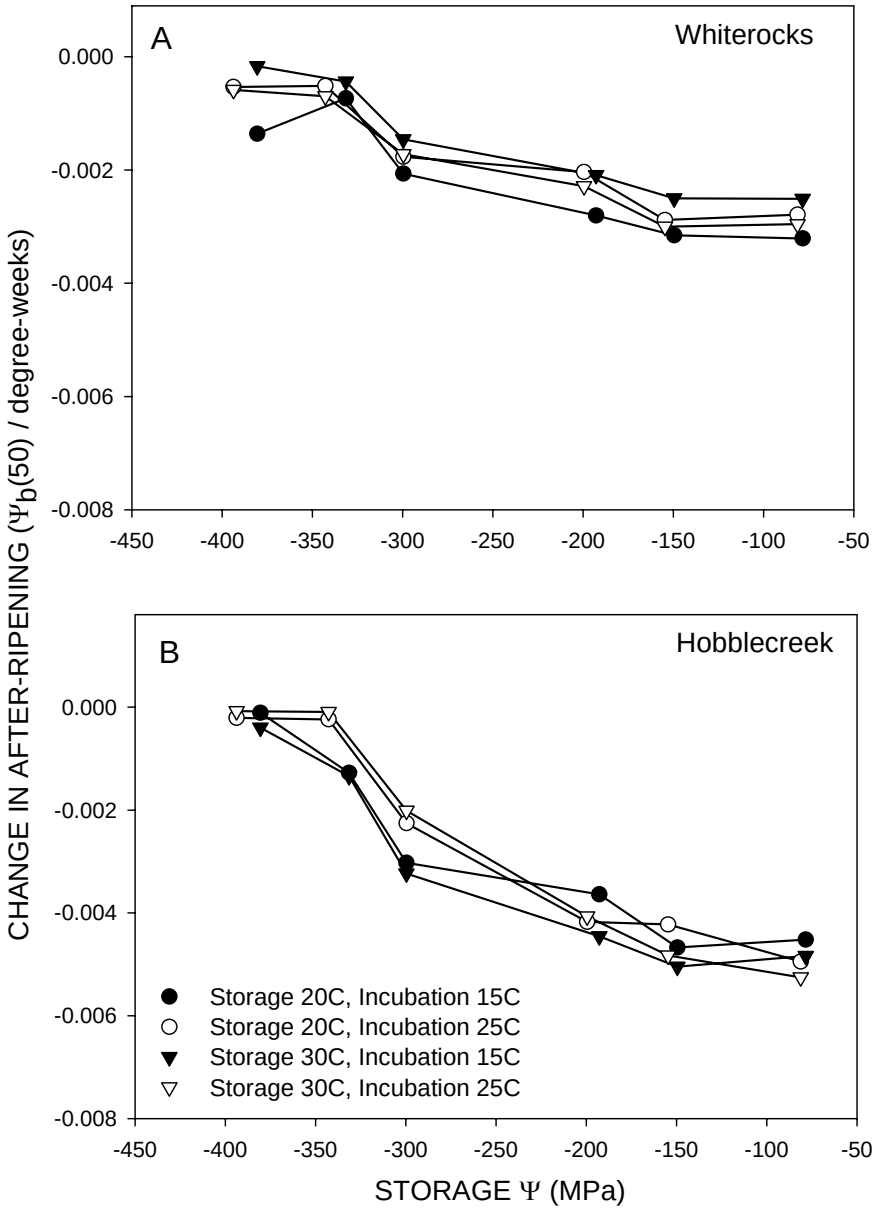
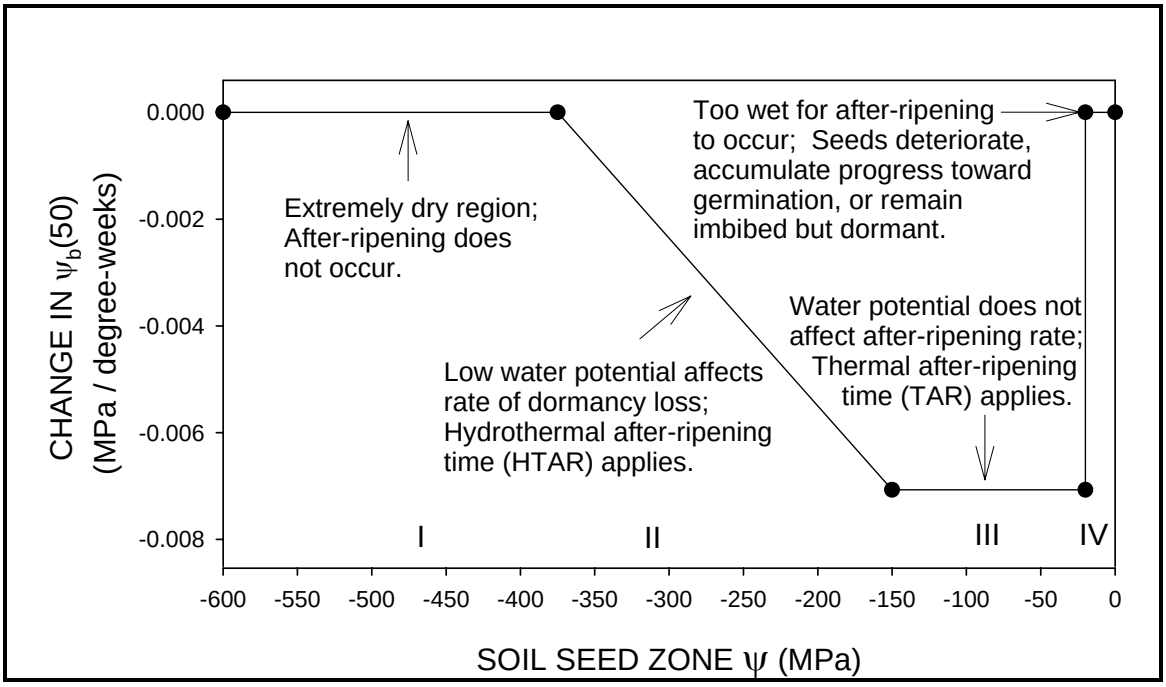


Figure 7.

Conceptual diagram of how storage storage water potential influences after-ripening in *B. tectorum*:



Literature Review

Introduction

Seed germination is often defined as radicle emergence from the seed coat; it begins with imbibition and ends with elongation of the embryonic axis. Seed germination includes many processes and reactions involved in radicle growth (i.e., protein hydration, respiration, macromolecular synthesis, and cell elongation). A seed that is resting, where none of the germination processes are taking place, is said to be quiescent. Seeds can remain and survive in the quiescent state for years and then resume high metabolic activity when the seed experiences conditions such as an appropriate temperature, oxygen, and hydration levels that encourage germination. Sometimes seeds experience conditions favorable for germination but do not achieve radicle emergence, these seeds are expressing dormancy (Bewley and Black, 1994). Dormancy is a state of inhibited germination and dormant seeds require that specific conditions be met before germination can occur.

Seed dormancy allows seeds to time germination to occur during conditions that are favorable for seedling establishment. A precise definition of dormancy has been argued among researchers, but Benech-Arnold *et al*. (2000) concluded that an accurate definition would read, "Dormancy is an internal condition of the seed that impedes its germination under otherwise adequate hydric, thermal and gaseous conditions."

Seed dormancy has two functions. First, dormancy restricts germination to seasons when soil conditions are favorable for establishment, thereby increasing the probability of seedling survival. Second, dormancy allows seeds of some species to remain viable in the soil for one or more years. Seeds express dormancy due to different genetic and environmental components that can be categorically defined.

Categories of Dormancy

Dormancy can be divided into two categories based on when the dormant status is attained: primary and secondary. Primary dormancy is imposed during seed development. Primary dormancy in grasses has a strong genetic component and is selected for phenotypically (Meyer and Allen, 1999; Simpson, 1990; Foley, 1994). Development of dormancy can also be affected by environmental conditions such as temperature and moisture levels that influence which genotypes are selected from the population. Seeds of *Chrysothamnus nauseosus* (rabbit brush), *Penstemon* spp (penstemons), and *Linnum perenne* (blue flax) showed variation in dormancy status associated with habitat conditions (Allen and Meyer, 1998; Dunbabin and Cocks, 1999).

Secondary dormancy normally occurs in seeds that do not possess dormancy or have lost primary dormancy and is also associated with environmental conditions such as water, nitrate, and temperature (Dunbabin and Cocks, 1999). For some species, buried seeds are known to enter secondary dormancy due to the lack of water availability and warm temperatures. Fluctuations of environmental conditions can lead to dormancy cycling wherein seeds can reversibly switch from being non-dormant to dormant (Baskin and Baskin, 1985; Dunbabin and Cocks, 1999; Benech-Arnold *et al*., 2000).

Through primary and secondary dormancy, species are able to increase the probability of seedling survival through variation of germination timing. Seedling survival depends largely on timing of germination, especially under unfavorable environmental conditions, to avoid precocious germination (Allen and Meyer, 1998). Variation in the timing of dormancy release within a population can result in several flushes of germination. Environmental stresses following a germination flush might result in seedling death (i.e. young seedlings could experience drought conditions), but because of variation in timing of dormancy release a second flush of seedlings may be exposed to environmental conditions more favorable to seedling survival (Voigt and Tischler, 1997).

Dormancy Loss

Several endogenous and environmental factors affect the loss of seed dormancy: plant growth substances, temperature, oxygen, and seed coat layers are examples. Abscisic acid (ABA) prevents precocious germination during seed maturation (Romagosa *et al*., 2001) and the ratio of gibberellic acid to abscisic acid influences the level of dormancy (Bewley and Black, 1985). In two alpine grasses an increase in temperature positively correlated with an increase in total germination percentage due to dormancy loss (Acharya, 1989). In *Rumex obtusifolius*, temperatures above 30°C and below 15°C inhibited germination (Benvenutie *et al.*, 2001). Research on rice, *Oryza sativa* L., showed that dormancy loss could be increased by the fermentation products formed under anaerobic conditions (Frantz and Bugbee, 2002). Martinez-Gomez and Dicenta (2001) found that the seed coat in *Prunus persica* (L.) inhibited germination. Removal of the seed coat or cool, moist stratification followed by removal of the endocarp resulted in dormancy loss.

Dormancy loss often involves a complex interaction of exogenous and/or endogenous factors. Light and temperature interact to influence dormancy loss of lettuce seeds (Fielding *et al*., 1992). Lettuce seeds do not germinate in the dark at high temperatures and the upper temperature limit appears to be governed by phytochrome (Pfr). Factors that influence the alteration of the upper temperature limit, above which seeds do not germinate, probably do this by changing temperature dependence of Pfr action (Fielding *et al*., 1992). Light acts through the red:far-red reversible phytochrome system. Phytochromes are proteins that are sensitive to red light and trigger responses based upon the ratio of red light to far red light (Bradbeer, 1988).

In some species germination may be inhibited by a hard seed coat wherein the testa inhibits water uptake, gas exchange, or mechanically prevents growth (Plummer *et al*., 1995). It is only after the removal or scarification of the seed coat, to weaken the testa that dormancy loss can occur. Removal of this hard outer coat often causes leaching of inhibitory chemicals, which results in dormancy loss. In seeds of *Lomandra sonderi* (Dasypogonaceae) germination is enhanced by removal of the pericarp in addition to chemical stimulation, including the application of hormones such as GA_3 or zeatin to the embryo to increase cell division (Plummer *et al*., 1995). In Woolly Cupgrass (*Eriochloa villosa* [Thunb.] Kunth.) the seed coat appears to inhibit germination by limiting the oxygen available to the embryo (Hatterman-Valenti *et al*., 1996).

The main thrust of this thesis research involves the category of seed dormancy that is lost during dry storage. This phenomenon is termed after-ripening and is primarily associated with graminaceous species. Efforts to predict dormancy loss through after-ripening require an

understanding of how environmental factors during storage, especially temperature and water potential, influence after-ripening.

After-ripening

After-ripening is a poorly understood process through which seeds stored in dry conditions lose dormancy. Loss of dormancy is strongly influenced by storage conditions, specifically temperature and water potential. Under appropriate temperature and water potential conditions, after-ripening will occur in the field or the laboratory. Seeds that after-ripen do so during the summer and early fall, depending upon the species and their genetic requirements for dormancy loss (Dunbabin and Cocks, 1999). Once the seed has fully after-ripened and dormancy is lost, there is an increased probability of germination following a precipitation event. The weedy Eurasion winter annual *Bromus tectorum* (cheat grass) after-ripens in the late summer and germinates in the fall when moisture is available. *B. tectorum* is a very successful weed, within the last 100 years it has invaded a wide variety of habitats in western North America (Mack, 1981). Understanding how and when seeds of *B. tectorum* after-ripen could lead to improved weed control strategies for this species.

Little is known about the molecular basis of dormancy modulation during after-ripening. Leubner-Metger and Meins (2001) found that after-ripening is mediated in the species *Nicotiana tabacum* L. cv. Havana 425, in part at least by β-1,3-glucanase. This occurs either through weakening the cell wall directly or by indirectly releasing elicitor-active β-1,3-glucanase oligosaccharides that serve also as signaling molecules during plant-pathogen interactions.

The physiological basis of after-ripening is also not fully understood but can be mathematically described. As seeds after-ripen, they germinate more rapidly and completely under a wider range of temperatures and water potentials (Foley, 1994; Allen *et al*., 1995; Allen and Meyer, 2002). The process of after-ripening is cumulative and can be modeled which has the potential of predicting dormancy loss in the field (Allen and Meyer, 1998).

Model Development

Models are tools used by scientists to represent, predict and help further understand specific phenomena. Many types of models are used in all aspects of research; however the discussion here in will be restricted to models that predict and describe germination and dormancy loss. Discussed below are four important models that are the basis for the development of the fifth model which is proposed through this thesis research.

Thermal time can be used to incorporate temperature effects into the timing of various biological processes such as seed germination (Gummerson, 1986). Thermal time can be applied to germination as described by the following equation:

$$\theta_T = (T-T_b)t_g \qquad (1)$$

where θ_T is the constant thermal time required for germination (e.g. degree days), T is the storage temperature, T_b is the base temperature below which germination will not occur, and t_g is the actual time to germination for fraction *g*. Thermal time assumes that the rate of germination

increases linearly as a function of temperature above a minimum threshold temperature (Garcia-Huidobro, 1982).

Hydrotime is analogous to thermal time and accounts for the reduced rate of a process at decreasing water potentials. Hydrotime is highly useful in describing seed germination. As water potentials increase above a threshold water potential, the rate of germination increases and vice versa. Hydrotime can be expressed through the equation:

$$\theta_H = (\psi - \psi_b(g))\ t_g \qquad (2)$$

where θ_H is the constant hydro time required for germination, ψ is the water potential of the medium, $\psi_b(g)$ is the base water potential below which germination does not occur, and t_g is the time to germination (Gummerson, 1986).

Hydrothermal time is the combination of thermal and hydro time and can also be used to describe germination through modelling (Gummerson, 1986; Bradford, 1990; 1995; 1996). Hydrothermal models have been shown to effectively predict germination (Dahal *et al.*, 1994; Christensen *et al.*, 1996; Bauer *et al.*, 1998; Vleeshouwers and Kropff, 1999; Roman *et al.*, 1999; Shrestha *et al.*, 1999; Meyer *et al.*, 2000; Grundy *et al.*, 2000). Of particular interest to this thesis research are hydrothermal models used to describe germination through dormancy loss in response to dry storage conditions (Christensen *et al.*, 1996; Bauer *et al.*, 1998; Meyer *et al.*, 2000).

The use of hydrothermal models to predict germination as influenced by water potential and temperature was proposed by Gummerson (1986) and further developed by Bradford (1990; 1995; 1996). The hydrothermal model unifies and describes possible germination of seed populations (Bradford, 2002). An important aspect of hydrothermal models is that they consider both germination rate and percentage simultaneously.

The hydrothermal time equation for a single seed is:

$$\theta_{HT} = (\psi - \psi_b(g))\ (T - T_b)\ t_g \qquad (3)$$

where θ_{HT} is the constant hydrothermal time required for germination (e.g. MPa°days), $\psi_b(g)$ is the base water potential below which seeds will not germinate ($\psi_b(g)$ varies among seeds in the population) and all other terms are as previously defined (Gummerson, 1986).

In order to extend equation 3, which describes germination of an individual seed to an equation applicable to an entire seed population Gummerson assumed that the distribution of mean base water potentials is normally distributed within a population. Probit transformation, which linearizes a cumulative normal distribution can be used to predict germination of an entire population and is described in the equation below:

$$\text{Probit}\ (g/g_m) = [\psi - \psi_b(50) - \theta_{HT}/((T - T_b)t_g)]/\sigma_{\psi b} \qquad (4)$$

where g_m is the fraction of viable seeds in the population, $\psi_b(50)$ is the mean base water potential of the population, and $\sigma_{\psi b}$ is the standard deviation of base water potentials within the population (Bradford, 1990).

Not all seeds possess the same base water potential, which prevents seeds within the population from all germinating at the same time. The distribution of base water potentials is represented by the standard deviation of base water potentials (Bradford, 1990). The standard

deviation of base water potentials determines the spread of germination times. Seeds with base water potentials above the water potential of the incubation medium will not germinate. Base water potentials greater than zero indicate that seeds will not germinate in free water, the seeds are dormant. High mean base water potentials are indicative of greater dormancy within a population (Bradford 1995; 1996).

Hydrothermal models predict germination through several important features. First, germination rate and percentage is increased by a more negative $\psi_b(50)$ value. If an upward shift of this $\psi_b(50)$ value occurs, changing the fraction of the seed population that is at or below $\psi_b(50)$ germination percentage will decrease. This upward shift in $\psi_b(50)$ above the ψ of the current medium will prevent germination of the seed, this can explain germination behaviors such as dormancy cycling (Bradford, 2002). Also, the upward shift in $\psi_b(50)$ could be responsible for the delay and inhibition of seed germination in the supra-optimal range of T (Bauer *et al.*, 1998; Shrestha *et al.*, 1999; Alvarada and Bradford, 2002; Rowse and Finch-Savage, 2003). Second, base water potential and germination rate are inversely proportional and because of this factors such as hormones, priming, and after-ripening that lower base water potential also increase germination rate and vice versa (Bradford, 1990; 1995; 2002; Hardegree and Emmerich, 1992; Foley, 1994; Meyer *et al*., 2000). Third, radicle emergence within a population is assumed to be normally distributed over time. Probit regression, which linearizes the normal distribution of radicle emergence within a seed population, is valuable because it provides a slope and intercept that can be used to identify the hydrothermal parameters for the seed population.

Hydrothermal models have accurately predicted germination in the laboratory for several species (Christensen *et al.*, 1996, Bauer *et al.*, 1998; Meyer *et al.*, 2000; Grundy *et al.*, 2000; Roman *et al.*, 1999; Shrestha *et al.*, 1999; Dahal *et al.*, 1994, Bradford, 1990; 1996; Gummerson, 1986) and a current focus of hydrothermal modelling involves application of these models to field situations (Bauer *et al.*, 1998). In the species *Stellaria media* a hydrothermal model accurately fit the data set under conditions normally encountered in horticultural seed beds and the model was found to be suitable for modelling germination under field conditions for this species (Grundy *et al.*, 2000). Simulation modelling efforts to predict germination have found varying degrees of success in the field and are extensively reviewed in Forcella *et al.* (2000).

Application of hydrothermal principles in relation to dormancy loss through after-ripening was first addressed by Christensen *et al.* (1996). Variations in germination time course curves during after-ripening and at different incubation temperatures and water potentials can largely be explained by changes in the parameter $\psi_b(50)$, which is highly associated with the mean base water potential of the population. The ability of seeds to shift base water potentials in response to environmental signals helps to explain dormancy loss (Christensen *et al.*, 1996; Bauer *et al.*, 1998; Meyer *et al.*, 2000). As after-ripening progresses, mean base water potential decreases thereby increasing the probability of germination after a precipitation event. Germination is linked with current water potential conditions at all stages of dormancy loss.

Christensen *et al.* (1996) allowed $\psi_b(50)$ to vary while all other parameters were held constant, the incubation water potential and temperature values were known, and t_g was determined by germination treatments. These researchers were then able to incorporate known values into equation 4 and determine changes in $\psi_b(50)$, which was used as an index of dormancy status. Changes in $\psi_b(50)$ explained the increase or decrease in the rate of after-ripening in the species *Bromus tectorum*.

Efforts to predict the rate of after-ripening under field conditions necessitated the need to account for fluctuating temperature conditions in the field. This led Bauer *et al.* (1998) to develop a thermal after-ripening time model:

$$\theta_{AT}=(T_s-T_l)t_{ar} \quad (5)$$

where θ_{AT} is the thermal time (i.e. ° weeks) requirement for after-ripening, T_s is the storage temperature, T_l is the lowest or base temperature (at or below which after-ripening does not occur), and t_{ar} is the time necessary for after-ripening to occur (the time required for $\psi_b(50)$ to change from its initial value to its final value) (Bauer *et al*., 1998). This model assumes that the rate of change of $\psi_b(50)$ is a linear function of temperature above a base temperature.

A thermal after-ripening (TAR) model for *B. tectorum* seeds accurately explained dormancy loss when soil conditions were dry (above -150 MPa) (Bauer *et al.*, 1998). However, when soil conditions were extremely dry (below -150 MPa) TAR consistently over-estimated the rate of after-ripening (Meyer and Allen, unpublished data). In earlier studies Foley (1994) and Leopold *et al.* (1988) in wild oat and red rice (respectively) showed that after-ripening could be slowed or prevented at low seed moisture content. Walters *et al.* (2001) found that reaction thresholds associated with non-fully imbibed seeds could also apply to the process of dormancy loss. Inhibition of after-ripening at low water contents, the idea of specific reaction thresholds associated with dormancy loss, and field data from 1994 inspired the proposal of a hydrothermal after-ripening (HTAR) time model that incorporates the influence of storage ψ on TAR. The HTAR model is conceptually presented in Figure 1 and the purpose of this thesis research is to provide supporting data for a HTAR model.

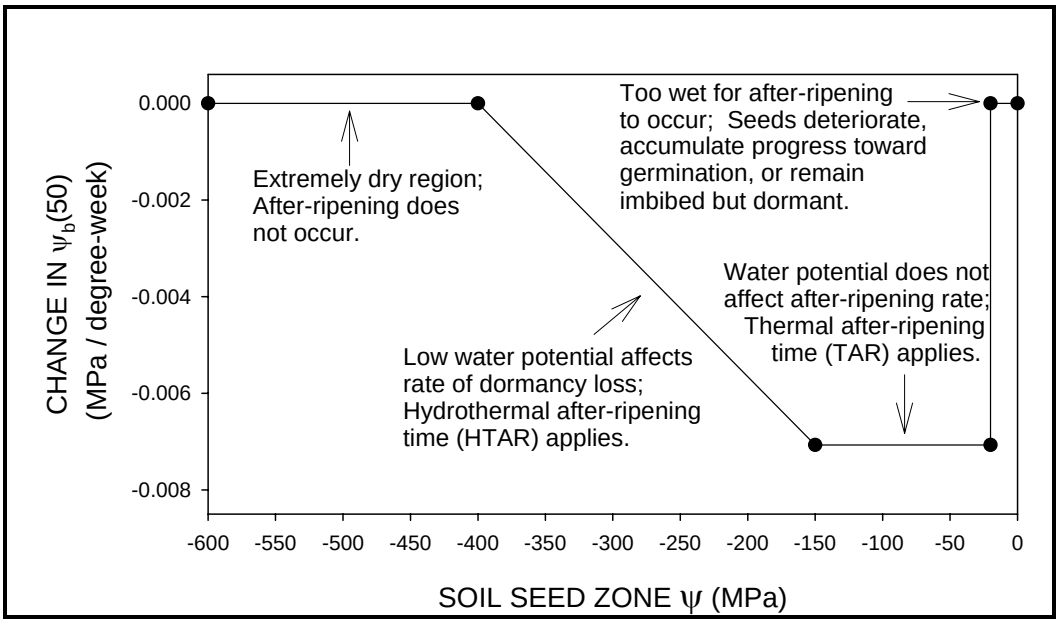


Figure 1. Conceptual diagram of the influence of hypothesized storage water potential on TAR and after-ripening. Specific thresholds on the water potential axis are conjectural at this point.

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APPENDIX A
Report of data not included in manuscript

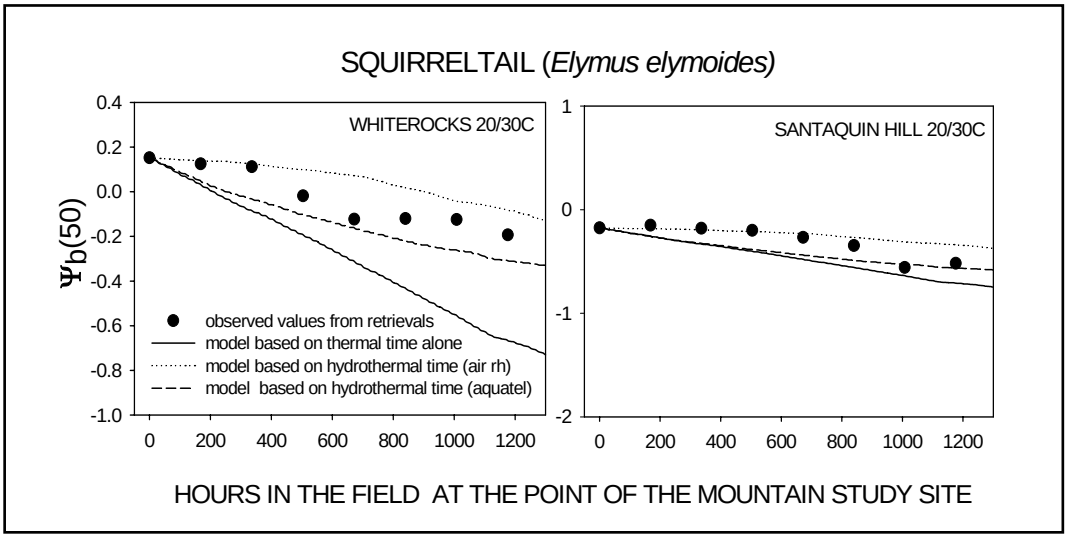
Table 1.

Germination of *Bromus tectorum* seeds removed from -400 MPa storage after 73 weeks at 20 or 30°C and then placed in storage treatments of -150 MPa and 20 or 30°C for eight weeks. Rapid after-ripening led to the conclusion that seeds had been dormant but still viable. Germination treatments began October 24, 2003 and ended November 11, 2003.

Collection	Incubation Temperature	Storage Temperature	Total % Germination
Whiterocks	15C	20C	91.8
Whiterocks	15C	30C	92.0
Whiterocks	25C	20C	92.9
Whiterocks	25C	30C	87.9
Hobblecreek	15C	20C	94.0
Hobblecreek	15C	30C	88.8
Hobblecreek	25C	20C	87.4
Hobblecreek	25C	30C	91.8

Figure 1.

Predicted and observed changes in $\psi_b(50)$ during field after-ripening for squirreltail seeds, measured at an alternating 20/30ºC incubation temperature regime. Seeds were non-dormant at 10/20ºC incubation.



APPENDIX B
SAS programs required to develop a hydrothermal after-ripening time model for seed dormancy loss

Explanatory note

SAS is a statistical software program used to develop models to analyze data. Below are the written programs used in this data analysis to determine hydrothermal and hydrothermal after-ripening parameters:

SAS programs

a. To determine germination fraction run the proceeding SAS statements. The output will be used in the next procedure. Place data in an excel file and import it into SAS as a 'text delimited prn' file. With output create a new excel file and prepare it to be imported into the SAS file that determines the parameters for hydrothermal time.

DATA Necia;

INFILE 'a:/NECIAHTTPARAMETERS.PRN';

INPUT ACC $ STEMP SDUR SHUM ITEMP PEGPOT REP D1 D2 D4 D7 D11 D14 D21 D28 TV;

G1=D1/(D1+D2+D4+D7+D11+D14+D21+D28+TV);

G2=(D1+D2)/(D1+D2+D4+D7+D11+D14+D21+D28+TV);

G4=(D1+D2+D4)/(D1+D2+D4+D7+D11+D14+D21+D28+TV);

G7=(D1+D2+D4+D7)/(D1+D2+D4+D7+D11+D14+D21+D28+TV);

G11=(D1+D2+D4+D7+D11)/(D1+D2+D4+D7+D11+D14+D21+D28+TV);

G14=(D1+D2+D4+D7+D11+D14)/(D1+D2+D4+D7+D11+D14+D21+D28+TV);

G21=(D1+D2+D4+D7+D11+D14+D21)/(D1+D2+D4+D7+D11+D14+D21+D28+TV);

G28=(D1+D2+D4+D7+D11+D14+D21+D28)/(D1+D2+D4+D7+D11+D14+D21+D28+TV);

PROC SORT; BY ACC ITEMP;

PROC SUMMARY; BY ACC ITEMP;

CLASS PEGPOT;

```
VAR G1 G2 G4 G7 G11 G14 G21 G28;
OUTPUT OUT=SUM MEAN= ;

PROC PRINT DATA=SUM;
RUN;
```

b. To determine the hydrothermal parameters for these populations use the data from the previous SAS program. Create an excel file with five headings: accession, temperature, water potential, time to germination, and germination fraction (these last two should come from the output of the first SAS program).

First begin by changing θ_{HT} (thetaht) (the constant hydrothermal value for hydrothermal time for that population) until the highest possible R^2 value is obtained, then toggle the base temperature until the R^2 value does not increase. Adjust θ_{HT} again to see if the R^2 value will increase at all. Continue adjusting and repeating the regression until the highest possible R^2 value is identified. From the equation of the line it is possible to determine hydrothermal parameters as discussed in Christensen *et al*. (1996).

```
DATA necia probfrac;
INFILE 'A:/NEW HTTDAT.prn';
INPUT ACC $ TEMP PSI GTIME GERMFRAC;

data B15;
set necia probfrac;
if acc='B15';

IF (GERMFRAC>0.05) AND (GERMFRAC<0.95) THEN
PROBFRAC=PROBIT(GERMFRAC);
THETAHT=15;
TBASE=2;
TTIME=GTIME*(TEMP-TBASE);

INDEP=PSI-(THETAHT/TTIME);

PROC REG data=B15;
```

```
MODEL PROBFRAC=INDEP;
OUTPUT OUT=PRED P=PREDPF;
RUN;
PROC PLOT DATA=PRED;
PLOT PROBFRAC*INDEP=temp PREDPF*INDEP='*' /OVERLAY VAXIS=-2 TO 2 BY 0.5
 HAXIS=-4 TO 3 BY 1;
RUN;
```

c. The third program involves determining thermal time for all seed collection, incubation temperature, storage temperature, and storage water potential combinations. The output of this program gives thermal time in degree weeks. From this output the data can be graphed in order to obtain the slope of each line. The slope determines the rate of change for each $\psi_b(50)$ (from it's initial to final value).

```
data mbwp;
infile "A:\mbwpfinalcorrected.PRN";
INPUT ACC $ ITEMP STEMP SHUM SDUR MBWP;
TBASE=0;

IF ITEMP=2 THEN INCTEMP=15;
ELSE IF ITEMP=4 THEN INCTEMP=25;

IF STEMP=20 THEN  TEMP=20;
ELSE IF STEMP=30 THEN TEMP=30;

IF SDUR=1 THEN TIME=4;
ELSE IF SDUR=2 THEN TIME=8;
ELSE IF SDUR=3 THEN TIME=12;
ELSE IF SDUR=4 THEN TIME=17;
ELSE IF SDUR=5 THEN TIME=27;
ELSE IF SDUR=6 THEN TIME=2;
ELSE IF SDUR=7 THEN TIME=3;
```

```
ELSE IF SDUR=9 THEN TIME=6;
ELSE IF SDUR=18 THEN TIME=18;
ELSE IF SDUR=44 THEN TIME=32;
ELSE IF SDUR=54 THEN TIME=54;
ELSE IF SDUR=60 THEN TIME=60;

TTIME=TIME*(TEMP-TBASE);

PROC SORT; BY ACC SHUM ITEMP;

PROC SUMMARY; BY ACC SHUM ITEMP;
CLASS  TTIME;
VAR  MBWP;
OUTPUT OUT=SUM MEAN= ;

PROC PRINT DATA=SUM;
RUN;
```

d. The last SAS program used in this research was to look at all unplanned comparisons and see what interactions are significant and which ones are not. The procedure is ANOVA.

```
data mbwp;
infile "A:\MBWPFINAL.PRN";
INPUT ACC $ ITEMP STEMP SHUM SDUR MBWP;
TBASE=0;

IF ITEMP=2 THEN INCTEMP=15;
ELSE IF ITEMP=4 THEN INCTEMP=25;

IF STEMP=20 THEN  TEMP=20;
ELSE IF STEMP=30 THEN TEMP=30;
```

```
IF SDUR=1 THEN TIME=4;
ELSE IF SDUR=2 THEN TIME=8;
ELSE IF SDUR=3 THEN TIME=12;
ELSE IF SDUR=4 THEN TIME=17;
ELSE IF SDUR=5 THEN TIME=27;
ELSE IF SDUR=6 THEN TIME=2;
ELSE IF SDUR=7 THEN TIME=3;
ELSE IF SDUR=9 THEN TIME=6;
ELSE IF SDUR=18 THEN TIME=18;
ELSE IF SDUR=44 THEN TIME=32;
ELSE IF SDUR=54 THEN TIME=54;
ELSE IF SDUR=60 THEN TIME=60;

TTIME=TIME*(TEMP-TBASE);

PROC SORT; BY ACC;

PROC GLM; BY ACC;
CLASS INCTEMP SHUM;
MODEL MBWP=TTIME|INCTEMP|SHUM;
RUN;
```