MODELING SEED GERMINATION AND SEEDLING EMERGENCE IN WINTERFAT (*Krascheninnikovia lanata* (Pursh) A.D.J. Meeuse & Smit): PHYSIOLOGICAL MECHANISMS AND ECOLOGICAL RELEVANCE

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By

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Abstract

Winterfat (*Krascheninnikovia lanata*) a native shrub has superior forage quality for livestock and wildlife, and is important in the structure and the function of the Northern Mixed Prairie of North America. Seedbeds in the Northern Mixed Prairie are characterized by high fluctuations in temperature and soil water, especially at the soil surface during the spring under unpredictable weather conditions. High seedling mortality is a major limitation for establishing winterfat from direct seeding. Objectives of this study were to: 1) quantify germination responses to temperature and water potential; 2) predict seed germination and seedling emergence using constructed threshold models; and 3) investigate physiological mechanisms and the ecological relevance of model parameters. The constructed thermal and hydrothermal time models predicted germination time in most controlled temperature and water potential regimes with the modification of model assumptions in winterfat. For the first time, it was proved that winterfat seeds have a subzero base temperatures (T_b) for germination, achieving 43 to 67% germination at – 3^{o}C. The estimated T_b was lower in the large seeds (-4.5^{o}C) than in the small seeds (-3.5^{o}C) and the difference between seed collections was also about 1^{o}C. Lower T_b favors large seeds to accumulate more thermal time at a given temperature, especially in early spring or fall when temperatures are low. Basic assumptions of hydrothermal time model, such as the constancy of model parameters, are invalid in winterfat. Model parameters varied with water potential, temperature and seed size within a seed collection. The predictability of constructed models is acceptable for seedling emergence only at optimal conditions in the field. Adverse seedbed conditions such as high soil temperatures (> 15^{o}C) and limited soil water (< -0.5 MPa) reduced predictability of seedling emergence with the hydrothermal time model. Pre- and post-germination events that affect seed deterioration, seedling mortality and seedling elongation may reduce the predictability of the hydrothermal time model. Small seeds required

approximately twice as long as large seeds to reach 50% germination at -1 to -3^{o}C. Greater cold tolerance in large seeds was correlated with greater membrane integrity, less cold imbibition damage, higher contents of soluble cryoprotective sugars, such as glucose, raffinose and sucrose during germination at low temperature. These sugars prevent from dysfunctions of cell membrane and enzymes at freezing temperatures.

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$\sigma_{\psi B}$: Standard deviation in ψ_b among seeds in a seed population

$\sigma_{\theta T:}$ Standard deviation in θ_T among seeds in a seed population

ψ: Water potential

$\psi_{B(G)}$: Base water potential for germination subpopulation (germination proportion)

ψ_B: Base water potential

θ_H: Hydro time

θ_{HT}: Hydrothermal time

$\theta_{T(G)}$: Thermal time to germination of percentage *g* in the seed population

$\theta_{T:}$ Thermal time

ABA: Abscisic acid

ACC: 1-aminocyclopropane-1-carboxylic acid

D_{50}: Days to achieve 50% germination

FPLSD: Fisher's protected least significant difference

GA: Gibberellic acid

$GR_{(G)}$: Germination rate, the reciprocal of germination time to achieve germination percentage *g*

LT_{50}: Lethal temperature resulting in 50% mortality

Probit (g): Probit transformation of percentage *g*

Rq: Metabolic heat production rate

Rco_2: CO_2 production rate from respiration

T: Temperature

T_b: Minimal or base temperature permitting germination

T_c: Maximal or ceiling temperature permitting germination

$T_{o:}$ Optimum temperature permitting germination

$T_{o(g):}$ Optimum temperature permitting germination for a given subpopulation or percentage *g*

1. Introduction

Winterfat (*Krascheninnikovia lanata* (Pursh) A.D.J. Meeuse & Smit, *Ceratoides lanata* (Pursh) Howell, *Eurotia lanata* (Pursh) Moq.) a long-lived native shrub, can live more than 120 years (Clarke and Tisdale, 1945) in the Great Plains of North America (Coupland, 1950). Winterfat belongs to the Goosefoot Family (*Chenopodiaceae*) and is widely distributed in the western United States from eastern Washington to western North Dakota, and south to Kansas, New Mexico and southern California. In Canada, it grows in the Mixed Prairie, mainly Saskatchewan and Alberta, and in the Yukon Territory (Neilson, 1968; Woodmansee and Potter, 1971). Winterfat is one of the dominant shrub species in the cold desert of the Intermountain West, often forming pure stands in valleys, and also occurs with sagebrush (*Artemisia* L.) and juniper (*Juniperus* L.) in the foothills in the Great Basin Desert (Kitchen, 2001). Winterfat is the most abundant shrub in the Mixed Prairie of Canada (Coupland, 1950) and may potentially be more abundant than previously recognized with proper grazing management (Romo et al., 1995).

Winterfat plants have numerous annual branchlets, gray-green in color, and covered with fuzzy cottony hairs (Fig. 1.1). The leaves can remain on the plant for a longer period, making it a good winter forage for livestock and wildlife. The fruit of winterfat is a utricle enclosed by two bracts, forming a dispersal unit, called diaspore covered by dense tufts of white hair. Winterfat is highly palatable with a crude protein content of 10-22% throughout the year (Smoliak and Bezeau, 1967; Davis, 1979). Calcium and phosphorus content in winterfat range from 0.8-1.5% and 0.1-0.3%, respectively (Clarke and Tisdale, 1945; Smoliak and Bezeau, 1967). Winterfat is one of the most valuable rangeland forages for maintaining the weight of animals on winter grazing ranges. However, under high grazing pressure and improper range management, its population decreases rapidly (Romo et al., 1995). Winterfat also is an important component in the structure and the function of the Northern Mixed

Prairie of North America (Call and Roundy, 1991; Romo et al., 1995). In recent years, the interest in winterfat has increased considerably because the shrub is potentially useful for rangeland improvement and restoration, soil erosion control and land reclamation in dry areas across western North America (Wood et al., 1995). Several foundation and registered seeds of winterfat are available for restoration (US Department of Agriculture, http://plants.usda.gov/). The contribution of shrubs to species diversity on the Canadian Mixed Prairie has generally been overlooked and the importance of winterfat should be reevaluated (Romo et al., 1995).

High seedling mortality is a major limitation for establishing winterfat from direct seeding (Booth, 1987; Booth, 1992; Hou and Romo, 1997; Garvin et al., 2004). The timing of seed germination plays critical roles in the survival and persistence of plants in natural ecosystems. Since the seedling stage has the highest mortality rate (Fenner, 1987), mechanisms of delaying germination and spreading germination over a period of time can ensure better seedling survival and establishment (Probert, 1992). The ability to predict the timing of seedling emergence has been recognized as a critical component for integrated weed management (Swanton and Murphy, 1996; Roman et al., 1999), crop establishment and management options, thus affecting the yield and monetary value of crops (Benjimin, 1982; 1990; Finch-Savage et al., 1993). It also has valued applications in forage production, such as determing seeding date, field emergence patterns, stand establishment, and forage productivity.

Seedbeds in the Northern Mixed Prairie are characterized by high fluctuations in temperature and soil water, especially at the soil surface during the spring. Temperature and water mainly drive the rate of seed germination when aeration is not restrictive (Gummerson, 1986) and a hydrothermal time model is an effective method for quantifying germination response to temperature and water potential (Allen et al., 2000a; Bradford, 2002; Larsen et al., 2004). Population-based modeling approaches to predict germination as a function of temperature and/or water potential have been well developed in the last two decades as thermal time (θ_T) (Garcia-Huidobro et al., 1982) and hydrothermal time (θ_{HT}) concepts (Gummerson, 1986; Bradford, 1995; Bauer et al., 1998; Finch-Savage et al., 1998; Allen et al., 2000a). Thermal time (Degree-day or hour), the heat unit for plant development, is a firmly established

developmental principle for plants (Fry, 1983). Thermal time model has been implemented successfully in predicting agronomically important phenology for crops and weedy species (Alm et al., 1991), as well as seed germination under non water-limiting conditions (Garcia-Huidobro et al., 1982; Covell et al., 1986).

A hydrothermal time (MPa-degree-day or hour) model utilizes water potential and temperature for predicting seed germination and the model can be applied to a wider range of conditions. Because soil water is normally inadequate and greatly variable in the surface layer of soils where usually germination occurs, water stress often limits germination and affects the time of seedling emergence in the field (Benech-Arnold et al., 1995). A hydrothermal time model allows predicting germination under the combined conditions of temperature and water, and therefore, has been widely used under reduced water potential or field conditions in crops, such as lettuce (*Lactuca sativa* L.) (Bradford, 1990), tomato (*Lycopersicon esculentum* Mill.) (Dahal and Bradford, 1990) and carrot (*Daucus carota* L.) (Finch-Savage et al., 1998), and in weedy species (Christensen et al., 1996; Grundy et al., 1999; 2000; Kebreab and Murdoch, 1999; Roman et al., 1999; 2000; Meyer et al., 2000).

Most germination models, however, were developed and tested in controlled environments and relatively few reports were available describing direct application under field conditions (Finch-Savage et al., 1998; 2000; Hardegree and van Vactor, 2000). Seedling emergence under field conditions is determined by more complex interactions of weather conditions, soil, seed and seedling characteristics. Extreme seedbed conditions, such as soil impedance and reduced oxygen supply can override on seedling emergence. The timing of germination can account for much of the variation in the timing of onion seedling emergence (Finch-Savage and Phelps, 1993), while the post germination phase accounts for most pre-emergence seedling losses (Durrant, 1988) and the spread of seedling emergence over time (Finch-Savage et al., 1998). The predictability and contribution of germination models, therefore, may be species-specific and need to be further investigated under field conditions.

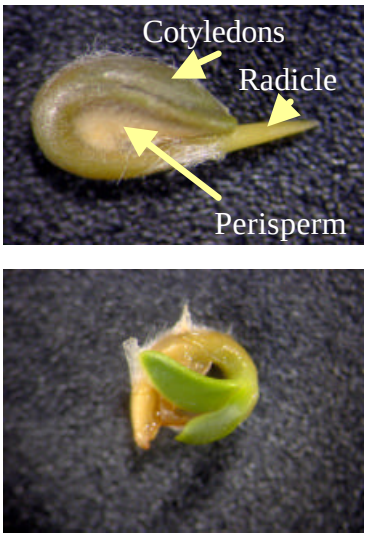
The objectives of this study are: 1) to construct thermal time (θ_T) and hydrothermal time (θ_{HT}) models for seed germination of winterfat and to determine the physiological and ecological meaning of model parameters for two seed

collections and two seed size groups; 2) to test assumptions of θ_T and θ_{HT} models with variation of seed collection and seed size; 3) to determine effects of seedbed conditions and the predictability of these models on seedling emergence under field conditions; and 4) to determine the physiological basis for variation in germination characteristics between seed size classes and correlate these with θ_T and θ_{HT} models. We hypothesized that: 1) parameters in θ_T and θ_{HT} models vary between seed size classes within a seed collection and between seed collections with different site characteristics; 2) the accuracy of θ_T and θ_{HT} models in predicting seed germination can be increased when some of the parameters are allowed to vary; 3) θ_T and θ_{HT} models can be used to predict seedling emergence of winterfat under field conditions; and 4) differences in germination characteristics between seed size classes as quantified by θ_T and θ_{HT} models have a physiological basis.

Natural population in mixed prairie
(Photo by Dr. J. T. Romo)

Individual plant
(Photo by Dr. J. T. Romo)

Twigs and blooming florescence
(Photo by Michael L. Charters)



Germinating seed
(Photo by Tim Dament)

Fig. 1.1. Natural population, plant morphology and seed structure of winterfat (*Krascheninnikovia lanata* (Pursh) A.D.J. Meeuse & Smit).

2. Literature Review

2.1 Environmental control of seed germination

Seed germination is an important stage in the life history of plant, affecting seedling development, survival, and population dynamics. Germination begins with seed water uptake and terminates with the elongation of the embryonic axis from the seed coat (Bewley and Black, 1994; Bewley, 1997). Germination events and subsequent establishment are controlled by nuclear and maternal genetics, and current and maternal environments (King and Bridgen, 1990; Platenkamp and Shaw, 1993; Cabin et al., 1997; Foley and Fennimore, 1998; Meyer and Pendleton, 2000; Baskin and Baskin, 2001). Genotypic inheritance can increase plant fitness to local habitats by adaptation, which enables seeds to germinate at the right time and the right place. Phenotypic variation, on the other hand, may increase the diversity of seed germination in time (Bradford, 1990; Gutterman, 2000; Baskin and Baskin, 2001), maintaining a soil seed bank. Environmental controls regulate seed dormancy release, seed germination rate and percentage, and seed deterioration and mortality. The microclimate that directly surrounds seeds in a seedbed determines the seed germination process; therefore, seedbed conditions affect seed germination timing and its landscape pattern, seedling establishment, and eventually population dynamics. The focus of this section will be on nondormant seeds.

2.1.1 Effects of environmental factors on germination

Environmental factors regulating germination include temperature, water, and oxygen for nondormant seeds, along with light and chemical environment for dormant seeds (Bewley and Black, 1994; Baskin and Baskin, 2001). Temperature and water mainly drive the rate of seed germination when aeration is not restrictive for non-dormant seeds (Garcia-Huidobro et al., 1982; Gummerson, 1986; Bradford,

1990; 2002; Probert, 1992). Temperature also plays a critical role in the elongation of the radicle and shoot, and thus, seedling emergence (Angus et al., 1981; Roman et al., 1999; 2000). For most species, temperature determines germination percentage and germination rate (GR) (Garcia-Huidobro et al., 1982; Ellis et al., 1986; Kebreab and Murdoch, 2000). Maximal germination can occur over a range of temperatures and germination declines sharply on either side of the range (e.g., Kebreab and Murdoch, 2000; Probert, 2000). Germination rate usually increases linearly with increasing temperature from a minimal or base temperature (T_b) up to an optimum and decreases linearly to a ceiling temperature (Garcia-Huidobro et al., 1982; Steinmaus et al., 2000; Bradford, 1990; 1995; 2002; Rowse and Finch-Savage, 2003). Cardinal temperatures, the minimum (T_b), the maximum (ceiling temperature, T_c) and optimum temperature (T_o), exist in most species and ecotypes (Bewley and Black, 1994) for seed germination. Thus, seeds germinate in a well-defined temperature range and germination rate depends on temperature. It is not surprising that germination response to temperature is related to ecological and geographical distribution of species and ecotypes (Probert, 2000; Baskin and Baskin, 2001), because germination is a critical stage of the life cycle reflecting adaptation to local habitats (Gutterman, 2000; Probert, 2000).

Temperature as a kinetic requirement for biochemical processes to proceed and heat for plant development is a firmly established developmental principle for plants (Angus et al., 1981; Fry, 1983). Germination rate increases linearly with temperature at the sub-optimal temperature range (temperatures between T_b and T_o), which is a consequence of thermal dynamics. Reasons for the decrease of GR at the supra-optimal temperature range (temperatures between T_o and T_c) has been proposed as thermal denaturation of proteins, membrane dysfunction, and interactions with water (reviewed by Bradford, 2002). Other possible mechanisms for the decrease of GR may be associated with the decrease of metabolic efficiency above the optimal temperature (Cridle et al., 1997; Thygerson, 2002).

Water is essential for seed germination. Generally, GR increases linearly with water availability (Gummerson, 1986; Bradford, 1990; Dahal and Bradford, 1990) and germination percentage is reduced at reduced water potential (ψ) (Shrestha et al.,

1999; Grundy et al., 2000; Kebreab and Murdoch, 2000). Meanwhile, water has more complicated effects on germination than temperature, especially at low ψ. When ψ is lower than -0.5 MPa, physiological adjustment occurs (Ni and Bradford, 1992; Bradford, 1995). When ψ is below the threshold (base water potential, ψ_b) for radicle emergence, a metabolic advancement or priming effect takes place (e.g., Hardegree et al., 2000; Kaur et al., 2002). Final germination percentage remains constant after approaching a lower level at reduced ψ, rather than gradually increasing over time as it does at low temperatures (Bradford, 1990). The minimal water potential for seed germination, ψ_b, shifts with seed physiological status (Ni and Bradford, 1992), dormancy (Meyer et al., 2000), and imbibition environmental conditions (Dahal and Bradford, 1994; Alvarado and Bradford, 2002; Rowse and Finch-Savage, 2003).

The sensitivity of seeds to water availability can change during the germination process. The initiation of radicle emergence is often more sensitive to water availability than subsequent seedling growth (Allen et al., 2000b). This sensitivity may be under physiological control (Dahal and Bradford, 1990; Welbaum and Bradford, 1991), and seeds remain desiccation tolerant only before embryo growth is initiated. Some physiological and biochemical activities associated with germination indicate a threshold-type behavior at low ψ (Chen and Bradford, 2000; Bradford, 2002). For example, endosperm weakening in tomato (*Lycopersicon esculentum* Mill.) is the primary determinant at reduced ψ during germination (Foolad and Jones, 1991).

Environmental factors often interact in regulating seed germination. For examples, seeds are capable of germinating at higher levels of water stress at optimum temperatures (Kebreab and Murdoch, 1999). Meanwhile, water requirements for seed germination, i.e., ψ_b, increase with increasing temperature at supra-optimal temperature range in potato (*Solanum tuberosum* L.) seed (Alvarado and Bradford, 2002), carrot (*Daucus carota* L.) and onion (*Allium cepa* L.) (Rowse and Finch-Savage, 2003). The relationship between GR and temperature can be modified by water availability (Kebreab and Murdoch, 1999).

2.1.2 Effects of environmental factors on seedling emergence in the field

Seedling emergence in the field involves two biological processes: seed germination (radicle protrusion through the seed coat), and radicle and shoot elongation. Each process may require different environmental conditions to proceed (Fyfield and Gregory, 1989; Roman et al., 2000). Unlike seed germination, which is driven by temperature and water potential (Gummerson, 1986), radicle and shoot elongation are mainly driven by temperature (Fyfield and Gregory, 1989; Wheeler and Ellis, 1992; Dahal and Bradford, 1994). The relationship between environmental factors and seed response can be different before and after radicle emergence. For example, seedling growth rate is more likely to have an exponential relation to temperature during post-germination rather than a simple linear relation during pre-germination (Whalley et al., 1999; Finch-Savage et al., 2001). Meanwhile, the linearity of post-germination growth usually occurs within a limited temperature range, which varies with species and seed physiological status (Finch-Savage et al., 2001; and references therein). Once embryo growth is initiated, seeds or seedlings lose their desiccation tolerance, thus dehydration could cause high seedling mortality (Hou et al., 1999).

Seedbed conditions, other than soil temperature and soil water, such as soil mechanical impedance, burial depth and reduced oxygen supply can also affect seedling emergence (Whalley et al., 1999; Kolb et al., 2002). The depth of seed burial influences seedling mortality, seedling emergence percentage, emergence rate, and emergence time (Prostko et al., 1998; Boyd and Acker, 2003; Grundy et al., 2003). As a general rule, species with small seeds emerge better from shallow soil depths than large seeded species. In fact, small seeded species often have a maximum emergence on the soil surface (Grundy et al., 1996; 2003). The response of seedling emergence to burial depth can be well-described by a quadratic function model, and species-specific optimum depth for emergence also depends on the interaction of seed mass and seed shape (Grundy et al., 2003).

Seedling elongation can also be affected by mechanical impedance and air quality of the soil. Compacted soil increases impedance for seedling penetration and seedling elongation is reduced (Whalley et al., 1999). Air in soil consists of three

main biologically active gases: oxygen, carbon dioxide, and water vapor. When soil is flooded, the ratio of carbon dioxide to oxygen typically increases and seed germination and seedling emergence are reduced due to energy shortages and metabolic and biochemical dysfunction (Kolb et al., 2002). Anaerobic soil conditions affect seed viability if seed water is greater than 15% (Roberts and Ellis, 1989; Forcella et al., 2000) and the relative emergence decreases exponentially with increasing anaerobic duration in susceptible species (Khosravi and Anderson, 1990). The sensitivity of seedling emergence to reduced oxygen is species-specific and is related to their evolution or habitat. For example, oxygen has relatively little effect on seed germination and seedling emergence in winterfat (Booth, 1992) but a greater influence on melon (*Cucumis melo* L.) (Edelstein et al., 2001). A recent study showed that vapor transport between a seed and the soil is a dominant mechanism for seed imbibition (Wuest, 2002) and seed-soil contact may make little contribution to imbibition rather than that previously recognized (Bewley and Black, 1994).

Physical conditions of soil including temperature, ψ and air quality also affect pathogenic activity, which, in turn, influences seed germination, especially seed and seedling mortality. Garvin et al. (2004) concluded that the regeneration of shrubs from the chenopod family in the Great Basin, including winterfat, was affected largely by pathogenic activities in the soil. Soil-borne damping off pathogens can infect multiple host species and the infection is associated with pathogen virulence, the epidemic history of the site, environmental condition, and the host vigor (Kitajima and Fenner, 2000).

Environmental conditions that directly surround seeds determine germination success and subsequent seedling emergence and establishment (Harper, 1977). Under field conditions, these environmental factors interact and associate with each other. For example, soil texture, influences soil temperature, soil water and air quantity profiles; higher temperature is often associated with more rapid drying of the soil surface layer. These environmental factors can also be modulated by litter residues on the soil surface and by management practices (Vleeshouwers and Kropff, 2000; Romo, 2004). In cultivated fields, tillage regimes affect soil temperatures and soil water, and consequently, the emergence of weedy seedlings (Cussans et al., 1996;

Spandl et al., 1998). Interactions among climatic conditions, soil, seeds and seedling characteristics on seedling emergence are complex under field conditions (Whalley et al., 1999; Vleeshouwers and Kropff, 2000).

2.2 Modeling seed germination and seedling emergence

Two approaches, an empirical model and a mechanism model, are usually used to model seed germination and seedling emergence. Empirical models in various levels of empiricism can do an excellent job of matching individual data of germination overtime, but models may need more empirical variables (Brown and Mayer, 1988). The empirical approach may be useful for a specific purpose, but it is difficult to interpret biological significance for derived model parameters (Bradford, 1990). Mechanical models, on the other hand, are based on known and experimentally quantify environmental effects on seed germination and seedling emergence. It has the greatest chance to be successful in the long term (Bradford, 1990; Forcella et al., 2000). Mechanical threshold models for seed germination and seedling emergence have achieved some success (Forcella, 1993; Benech-Arnold and Sánchez, 1995; Allen et al., 2000a; Roman et al., 2000; Bradford, 2002; Rowse and Finch-Savage, 2003).

Temperature and water mainly drive the rate of seed germination when aeration is not restrictive (Gummerson, 1986). The hydrothermal time model is an effective method for quantifying germination response to temperature and water potential (Allen et al., 2000a; Bradford, 2002). Population-based modeling approaches to predict germination as a function of temperature and/or water potential have been well developed in the last two decades as thermal time (θ_T) (Garcia-Huidobro et al., 1982) and hydrothermal time (θ_{HT}) concepts (Gummerson, 1986; Bradford, 1995; Bauer et al., 1998; Finch-Savage et al., 1998; Allen et al., 2000a).

2.2.1 Thermal time model

Thermal time (Degree-day or hour), the heat unit for plant development, is a firmly established developmental principle for plants (Fry, 1983), including seed

germination (Garcia-Huidobro et al., 1982; Ellis et al., 1986). According to the model, the thermal time for the percentage germination *g*, $\theta_{T(g)}$, is:

$$\theta_{T(g)} = (T-T_b)\, t_{(g)} \qquad \text{(Eq. 2.1)}$$

where T is the actual temperature, T_b is the base temperature for germination, and $t_{(g)}$ is the time to germination of *g*. Since GR is defined as the inverse of the time to radicle emergence of a specific percentage of the population, the Eq. 2.1 can be rewritten as:

$$GR_{(g)} = 1 / t_{(g)} = (T-T_b) / \theta_{T(g)} \qquad \text{(Eq. 2.2)}$$

where germination rate ($GR_{(g)}$) is the reciprocal of germination time ($t_{(g)}$) to a given germination percentile (g). Germination rate is linearly related to temperature within the range of sub-optimum temperature for non-dormant seeds. The linear relationship between $GR_{(g)}$ and T varies among subpopulations in the slope of the linear regression lines, which is equal to the reciprocal of thermal time ($\theta_{T(g)}$).

If the variation of $\theta_{T(g)}$ within a seed population follows a normal distribution (Ellis et al., 1986; Covell et al., 1986), then the germination time course in terms of thermal time can be described using repeated probit analysis:

$$\text{Probit (g)} = [(T-T_b)\, t_{(g)} - \theta_{T(50)}] / \sigma_{\theta T} \qquad \text{(Eq. 2.3)}$$

where probit (g) is the probit transformation of the cumulative germination percentage g and $\theta_{T(50)}$ is the thermal time of 50% germination, or median thermal time to germination, and $\sigma_{\theta T}$ is the standard deviation of θ_T among individual seeds in the population. Once T_b is estimated, thermal time to germination $t_{(g)}$ can be normalized on a thermal time scale by multiplying the factor $(T-T_b)$.

The thermal time model has been used successfully to predict phenological development in crops and weedy species (Alm et al., 1991), as well as seed germination under non water-limiting conditions (Garcia-Huidobro et al., 1982; Covell et al., 1986). A thermal time model was used to predict optimal planting dates for corn (*Zea mays* L.) at water potential above –0.5 MPa (Gupta, 1985; Gupta et al., 1988), onion seedling emergence above a critical water potential (Finch-Savage and

Phelps, 1993), and several other species (Washitani and Takenaka, 1984; Benech-Arnold et al., 1990). However, the use of the thermal time model for predicting seedling emergence is limited in practice due to the variation of soil water in the field. Thermal time accounted for about 80% of the variation in the cumulative percentage of seed germination at non-limiting water conditions and only accounted for 55% at limiting water in an annual forb, Powell's amaranth (*Amaranthus powellii* S. Wats.) (Oryokot et al., 1997).

2.2.2 Hydrothermal time model

Hydrotime (MPa-day or hour) is proposed to describe the germination response to reduced ψ (Gummerson, 1986; Bradford, 1990; 1995). Analogous to the thermal time scale as used for temperature response, hydrotime constant, θ_H, is calculated as:

$$\theta_H = (\psi - \psi_{b(g)})\, t_g \qquad \text{(Eq. 2.4)}$$

where ψ is the actual water potential, and $\psi_{b(g)}$ is the minimal water potential or base water potential that prevents germination of percentage *g*. Assuming that the variation in ψ_b within a seed lot follows a normal distribution, the probit analysis can be used to estimate parameters in the hydrotime model for the whole seed population (Bradford, 1990) as:

$$\text{Probit}\,(g) = [\psi - (\theta_H / t_g) - \psi_{b(50)}] / \sigma_{\psi b} \qquad \text{(Eq. 2.5)}$$

where $\psi_{b(50)}$ is the median ψ_b, and $\sigma_{\psi b}$ is the standard deviation in ψ_b among subpopulations within a seed population.

Hydrothermal time (MPa-degree-day or hour) is a combination of thermal time and hydrotime, namely, the combination of water potential above a base water potential and temperature above a base temperature (Gummerson, 1986; Bradford, 1990; 1995). Hydrothermal time, θ_{HT}, is then:

$$\theta_{HT} = (T - T_b)\,(\psi - \psi_{b(g)})\, t_g \qquad \text{(Eq. 2.6)}$$

The parameters in the hydrothermal time model can be estimated using repeated probit analysis, according to the equation derived from Eqs. 2.5 and 2.6:

$$\text{Probit}\,(g) = [\psi - (\theta_{HT} / (T-T_b)\, t_g) - \psi_{b(50)}] / \sigma_{\psi b} \qquad \text{(Eq. 2.7)}$$

Since germination rates using θ_T instead of real time in hydrothermal time model are proportional to water potential (Gummerson, 1986), the common θ_{HT} for the whole seed population can be estimated as the inverse slope of the linear regression of $1/\theta_{T(g)}$ on ψ.

$$1/\theta_{T(g)} = (\psi - \psi_{b(g)}) / \theta_{HT} \quad \text{(Eq. 2.8)}$$

The hydrothermal time model has many advantages for quantifying and analyzing temperature response and water relations for seed germination. It is based on variation of θ_T and sensitivities of seeds to ψ within a seed population. The model provides insights into how physiological and environmental factors interact to regulate the germination behavior of seed populations (Bradford, 1995; 2002). The model also uses the simplest way, the timing of germination, to detect physiological adjustments that occurred during seed incubation (Bradford, 1990). Parameter shifting of the hydrothermal time model, e.g., $\psi_{b(g)}$, reflects dormancy status and hormone regulations (Ni and Bradford, 1992; Meyer et al., 2000; Alvarado and Bradford, 2002). However, the assumptions of the generalized model may not be applicable for all species or variable conditions (Bradford, 1990; Kebreab and Murdoch, 1999).

2.2.3 Application of hydrothermal time model

Because water stress often limits germination under field conditions (Benech-Arnold et al., 1995) and soil water is normally inadequate and greatly varies in the soil surface where germination occurs, water potential can often have an overriding importance in the time of seedling emergence. Hydrothermal time allows the prediction of germination in combinations of temperature and water, thus has the potential for predicting seedling emergence under field conditions. In fact, θ_{HT} has been widely applied for predicting germination under reduced water potentials and/or field conditions in crops, such as lettuce (Bradford, 1990), tomato (Dahal and Bradford, 1990) and carrot (Finch-Savage et al., 1998), and in weedy species (Christensen et al., 1996; Grundy et al., 1999; 2000; Kebreab and Murdoch, 1999; Roman et al., 1999; 2000; Meyer et al., 2000).

The accuracy of the θ_{HT} model, however, depends on the ψ range to which seeds are exposed, and the assumptions of the θ_{HT} model are not always valid. There is less fit to the model when seeds germinate at the lower range of ψ (Bradford, 1990; 1995; Ni and Bradford, 1992). Kebreab and Murdoch (1999) indicated that two crucial assumptions, constancy of θ_{HT} and independence of ψ_b on T and ψ in a given seed lot, are invalid in Egyptian broomrape (*Orobanche aegyptiaca* Pers.). Finch-Savage et al. (2000) pointed out that it is not always true in θ_{HT} model that parameters such as T_b, $\psi_{b\,(g)}$, θ_{HT} and $\sigma_{\psi b}$ are intrinsic properties and treated as constants for a given seed population. And the changes of seed water status are considered as rapid and to the same extent as the surrounding soil.

Most germination models are developed and tested in a controlled environment and relatively few reports are available in direct applications under field conditions (Finch-Savage et al., 1998; 2000; Hardegree and Van Vactor, 2000; Allen, 2003; Hardegree et al., 2003). Germination models can be used to quantify the temperature dependence of seed germination and seedling emergence (Probert, 1992). However, seedling emergence under field conditions is determined by more complex interactions of weather conditions, soil, and seed and seedling characteristics. Extreme seedbed conditions, such as soil impedance and reduced oxygen supply can have considerable effect on seedling emergence (Whalley et al., 1999; Kolb et al., 2002). The timing of germination can account for much of the variation in the timing of onion seedling emergence (Finch-Savage and Phelps, 1993), while the post-germination phase accounts for most pre-emergence seedling losses (Durrant, 1988) and the spread of seedling emergence time (Finch-Savage et al., 1998).

2.3 Physiological mechanisms of germination at low temperature

2.3.1 Cold and freezing tolerance

Physiological mechanisms related to plant cold or freezing tolerance include: 1) accumulation of compatible solutes, i.e., sugars and proline (Hoekstra et al., 2001, and references therein; Gusta et al., 2004); 2) protection of dysfunction or the damage

of cell membranes and quantitative and qualitative changes in membrane fluidity and integrity (Leborgne et al., 1992; Sharom et al., 1994; Saltveit, 2002; Wisniewski et al., 2003; Gusta et al., 2004); 3) higher metabolic rates under stressed temperatures (Massardo et al., 2000); 4) synthesis of cold induced protein and peptides, such as heat shock proteins (HSPs) and chaperones, late embryogenesis abundant (LEA) proteins (Bray et al., 2000) and synthesis of anti-freezing proteins (Griffith and Yaish, 2004); and 5) less oxidative injury using a more efficient antioxidant mechanism (Bohnert and Sheveleva, 1998; Massardo et al., 2000; Rentel and Knight, 2004). Not all these physiological and biochemical mechanisms are present in every species, and they are commonly triggered by cold and their relative dominance may vary with development stages and species (Massardo et al., 2000).

Abiotic stresses are interconnected and may induce similar cellular damage in plants. Freezing stress is often associated with desiccation stress due to intercellular ice formation (Wisniewski et al., 2003), and oxidative stress (Wang et al., 2003). Therefore, diverse environmental stresses often activate similar cell signaling pathways (Knight and Knight, 2001; Zhu, 2002) and cellular responses (Wang et al., 2003). Upon water loss, the accumulation of compatible solutes plays an important role in reducing molecular interactions that can cause protein denaturation and membrane fusion (Hoekstra et al., 2001). Oligosaccharides, such as sucrose and raffinose, can stabilize proteins by preferential exclusion and by forming a glassy matrix (Hoekstra et al., 2001; Peterbauer and Richter, 2001). Oligosaccharide concentration can be increased during natural and artificial acclimation of cold stress (Bachmann et al., 1994; Gusta et al., 2004).

2.3.2 Germination at low temperature

Cold or freezing tolerance in temperate plants is a prerequisite for surviving winter and for resuming growth and development in the spring. Low temperature tolerance in seeds represents the ability to germinate early in the spring, which is particularly important for regions with a short growing season. Early emerging seedlings also have competitive advantages in plant communities (Hendrix, 1984; Hou and Romo, 1998) and a higher chance of establishment because they can capture

the earliest opportunity in a growing season. Low temperatures during germination can reduce the percentage and timing of embryo protrusion in several species (Roberts, 1988; Zheng et al., 1994; Massardo et al., 2000; Fellner and Sawhney, 2001). Relative to adult plants and seedlings, mechanisms of cold and freezing tolerance are poorly understood for early developmental stages of plants including seed germination. In many cases, cold tolerance during vegetative growth was independent of cold tolerance during seed germination in tomato (*Lycopersicon esculentum* Mill.) germplasm evaluation (Foolad and Lin, 2000). Cold tolerance may then vary with different development stages. Knowledge of freezing tolerance in hydrated seeds is also limited to a few species (Bai et al., 1998).

Selection for rapid germination under stress is effective and significantly improves progeny seed germination rate under cold stress (Foolad et al., 2003), other than using common methods of stress evaluation for later development stages, such as LT_{50} or LD_{50} (Lethal Temperature or Lethal Duration for 50% mortality under stress). Low temperature tolerance during germination was studied relatively more for tomato than other species and the germination of tomato under cold stress was demonstrated to be under genetic control (Foolad and Jones, 1991). Fellner and Sawhney (2001) found that the ability of tomato seeds to germinate at low temperature is associated with the regulation of endogenous ABA levels. Over-expressed dehydrins in response to chilling stress act as a radical-scavenging protein to protect membranes in transgenic tobacco (*Nicotiana tabacum* L.) and to improve germination at lower temperature (Hara et al., 2003). The ability to germinate at low temperature is also associated with the maintenance of higher metabolic activities under the stress (Massardo et al., 2000; Edelstein et al., 2001).

2.4 Seed water relations

2.4.1 Cellular activities related to water uptake phases

Germination is initiated by the imbibition of water in quiescent dry seeds and the seed germination process can be characterized by tri-phasic water uptake (Bewley and Black, 1994). Changes in physiological and biochemical activities during seed

germination have been correlated with these phases. Upon imbibition of water by dry seeds, metabolic activities soon resume and a burst of respiration activities and their related enzymes can be detected within several hours (Bewley and Black, 1994). Protein synthesis-related activities start rapidly after rehydration, such as polysome assembling and new ribosome synthesis (Dommes and Van der Walle, 1990). The repair of cell membranes and DNA, and protein synthesis using extant mRNAs occur in the physical water uptake phase (Phase I). How repairs to desiccation- and rehydration-induced damage to membranes and organelles are achieved remains unknown (Bewley, 1997). The activation of metabolic activities and biochemical processes, i.e., mitochondria synthesis and protein synthesis using new mRNAs commence in Phase II. Seed water content rises very gradually or not at all in phase II depending on water availability, seed composition, and seed dormant status (Bradford, 1990). The length of the water uptake plateau is sensitive to factors that influence the timing and extent of germination. Mobilization of seed reserves and the resumption of growth, cell elongation and division occur in Phase III (Bewley, 1997).

The influx of water entering cells of dry seeds temporarily perturbed the structural of membranes, which in turn leads to an immediate and rapid leakage of solutes and low molecular weight metabolites from seeds into the surrounding imbibition solution (Bewley and Black, 1994). The initial leakage is a symptom of the transition of membrane phospholipid components from the gel phase achieved during maturation drying to the normally hydrated liquid-crystalline state (Crowe and Crowe, 1992). Shortly after rehydration, the membranes return to their more stable configuration, at which time solute leakage is reduced. The initial imbibition rate is important because rapidly imbibition of dry seeds can cause greater damage, particularly at low temperatures (e.g., Ismail and Hall, 2002). On the other hand, priming effects can occur under controlled hydration when seeds experience a ψ lower than ψ_b or with the cycles of hydration and dehydration before embryo growth, advancing toward germination. Biochemical changes take place in seeds during the priming, for example, decreased electrolyte leakage (Zheng et al., 1994), ACC and ethylene production, activity of ACC oxidase (Khan et al., 1995), and total dehydrogenase activities (Kepczynska et al., 2003). Seed germination and seedling

emergence are also improved, especially at low temperatures (Zheng et al., 1994; Hardegree and Van Vactor, 2000).

2.4.2 Water relations and critical water content

Generally, seeds imbibed at low ψ have low water content, a long water uptake plateau, low germination percentage, and slow germination rates (Bradford, 1990). Water flowing across tissues has three routes: through cell walls (apoplastic path), from cell to cell across either the plasmodesmata (symplastic path), or traversing the cell membranes (transcellular path) through aquaporins, a water selective channel (Preston et al., 1992; Agre et al., 1998). Embryo cell growth is determined by turgor pressure, cell wall expansion ability, and the presence and strength of any external tissues that impede embryo expansion (Bradford, 1990; Ni and Bradford, 1992). Water relations can be important in regulating embryonic growth during germination because cell growth is driven by water uptake into expanding cells. Water redistribution that is induced by ethylene within the embryo permits radicle growth during precocious germination (Fountain et al., 1998). Isolated radicles elongate immediately upon access to free water (Fountain et al., 1990). Initial embryo growth can occur only when the increased turgor pressure due to water uptake exceeds the mechanical restraints from the cell wall and external tissues (Welbaum et al., 1998). Therefore, increased turgor pressure and reduced mechanical restraints both can initiate embryonic elongation. Cell wall modifications during germination are biochemical processes related to hormones, such as GA (Chen et al., 2002), cell wall expansin (Chen and Bradford, 2000), and cell wall hydrolases (Chen et al., 2002; Mo and Bewley, 2003). These processes may also be adjusted according to water availability during seed imbibition (Dahal and Bradford, 1990; Welbaum and Bradford, 1991).

It is generally agreed that seeds must reach a critical water content to trigger cell elongation and to initiate radicle emergence (Bradford, 1995; Obroucheva et al., 1995); however, there is no universal parameter for measuring the threshold of water. Water status in seeds can be measured by water content, water potential (Roberts and Ellis, 1989) and cell turgor pressure (Steudle et al., 1993). Bradford (1986) and Gray

et al. (1990) found that seed water content at the onset of germination was the same among osmotic conditions. Water content was the first parameter used for describing seed water relations during germination. However, variation of seed water content exist among imbibition temperatures in fully imbibed seeds and water content of the embryo is recommended as a better parameter of water status related to germination (Bai et al., 1999). Seed ψ and cell turgor are considered better than water content when determining the water threshold for germination, even though there is a wide variation among species in minimal ψ that permits embryo growth (Obroucheva et al., 1995).

2.5 Ecological significance of seed size

2.5.1 Sources of variation in seed size

Seed size or weight, reflects potential food reserves for seedling growth, and are considered as important traits determining the successful establishment of individual plants (Westoby et al., 1992; Vaughton and Ramsey, 1997; 1998; Zhang, 1998). Variation in seed weight between or within plant species has evolved under different selection pressures, ecologically increasing their potential fitness by producing a large number of seeds or greater allocation of maternal resources to individual seeds (Westoby et al., 1992; Vaughton and Ramsey, 1997; 1998; Zhang, 1998). Although seed weight is commonly considered one of the least variable plant characteristics, it can vary widely among populations of a species (Zhang, 1998) and is often pronounced within individual plants (Vaughton and Ramsey, 1997; 1998). Seed-size variation within an individual plant is attributed to parental environment and fruiting position (Mendez, 1997; Vaughton and Ramsey, 1998; Simons and Johnston, 2000). Vaughton and Ramsey (1998) analyzed sources of variation in seed weight among populations, plants, and years, and within plants and fruiting positions, and concluded that the most pronounced variation is within plants and inflorescence in silver banksia (*Banksia marginata* Cav.).

One explanation of seed weight variation within plants is that resources for seeds are limited and plants are not able to provide resources to all seeds at the

optimum level. Resource constraints for seed mass variation are demonstrated by the trade-off of seed number and seed mass (Wolfe, 1995), seasonal decline in seed mass, and variation with position within fruits and plants (Vaughton and Ramsey, 1997). Other factors inducing changes in seed mass variation include environmental factors during seed development (Vaughton and Ramsey, 1998; Baskin and Baskin, 2001) and pollen availability or origin (Bertness and Shumway, 1992; Vaughton and Ramsey, 1997).

2.5.2 Germination behavior and ecological significance related to seed size

Seed mass represents the amount of maternal investment for individual offspring. Generally, seed weight variation is associated with fitness and population establishment since seed traits are critical elements in the life history of plant. In agronomic species, seed weight is correlated with seed vigor, plant growth, and even yield (Lafond and Baker, 1986; Berdahl and Frank, 1998; Boe, 2003). In wild plants, large seed size is correlated with a higher seedling recruitment (Negri and Facinelli, 1990; Mendez, 1997; Susko and Lovett-Doust, 2000; Dalling and Hubbell, 2002; Debain et al., 2003), bigger seedlings (Hou and Romo, 1998), and greater probability of survival (Simons and Johnston, 2000; Walters and Reich, 2000). Ecologically, seedlings emerging from large seeds often survive longer than that from small seeds under adverse seedbed conditions, such as low light (Simons and Johnston, 2000), low water (Hendrix and Trapp, 1992; Chacon and Bustamante, 2001), nutrient limitations (Vaughton and Ramsey, 1998), and deep burial depth (Yanful and Maun, 1996; Ruiz-de-Clavijo, 2002). Seed mass is also correlated with dispersal (Andersson, 1996). However, correlations between seed weight and emergence may be species- and habitat- specific, and seed weight may not have universal effects on emergence for a species (Hendrix and Trapp, 1992). For example, Paz et al. (1999) compared seven woody species of *Psychotria* in two contrasting habitats, gap and shade, and concluded that seedling emergence was driven more by external ecological factors, such as light condition, than by intrinsic seed weight.

Seed size is one element of a coevolving complex of traits including seed dormancy, seed dispersal, plant mass, longevity, niche specialization, and

competition among species (Leishman et al., 2000; Simons and Johnston, 2000). Because individual seed traits do not evolve independently, fitness consequences of seed-size variance cannot be generalized among species. The correlation between seed size and relative growth rate of seedlings (Westoby et al., 1992; and references therein) is presumably driven by resource allocation to other functions such as stress tolerance (Armstrong and Westoby, 1993). In addition, it has been demonstrated that seed-size variance within species is associated with a variety of fitness-related traits such as the probability and timing of germination (Simons and Johnston, 2000). Positive association between seed size and seedling emergence occurs more in small seeded species than in large seeded species (Paz et al., 1999).

2.6 Seed biology and seedbed ecology of winterfat

2.6.1 Seed botany and characteristics of winterfat

The seed characteristics of the *Chenopodiaceae* form the taxonomic division of the family and can be classified into two groups based on the internal embryo structure. The placement of winterfat embryo gives rise to kidney-shaped or lenticular seeds with a conspicuous hilum notch (Booth, 1988). The embryo is peripheral, encircling a central perisperm, a thin, papery testa (Fig. 1.1), and a utricle enveloped by united bracts. At maturity the bracts have formed fluffy white diaspores (seed-containing dispersal units) that decorate the fruiting spikes and function in seed dispersal, embryo protection, and in promoting establishment and survival of seedlings (Booth, 1988). Bract hairs are 2 to 8 mm long in spreading tufts. Each pair of bracts encloses an indehiscent, pubescent, 1-seeded fruit (utricle) (Booth, 1988). In Saskatchewan, winterfat begins to flower in late-June, with a mean flowering period lasting about 45 days (Budd and Campbell, 1959). Winterfat normally sheds seeds from mid-September through October in the Northern Mixed Prairie (Romo et al., 1995). Winterfat seeds have an after-ripening requirement that can be met by storage at room temperature for 10 to 25 weeks (Springfield, 1972), by cold and moist conditions (Booth, 1992), or by chemical treatments, with ethanol or GA (Hou and Romo, 1998).

2.6.1 Seedbed characteristics and seedling emergence in the field

Winterfat is well adapted to cold seedbed conditions of early spring, and utilizes more available soil water for better seedling survival before experiencing desiccation in late spring (Hou et al., 1999). Seed germination under snow has been observed in natural plant communities (Hilton, 1941; Woodmansee and Potter, 1971). Snow helps push diaspores to the soil surface and seeds usually germinate and emerge in early spring during a relatively short period of abundant soil water (Springfield, 1971; Booth and Schuman, 1983; Romo, 2004). However, freezing temperatures may limit the establishment of winterfat seedlings in the Northern Mixed Prairie (Hou and Romo, 1997). Winterfat seeds usually germinate on the soil surface or at shallow depth in the field (Springfield, 1971; Booth, 1989). The seedbed condition is importance for alleviating mortality of winterfat seedlings, such as litter accumulations, standing dead plant material, and snow cover (Booth, 1987; 1992; Romo, 2004). High seedling mortality has been identified as the major limitation for stand establishment of winterfat from direct seeding (Booth, 1992; Hou and Romo, 1997; Garvin et al., 2004).

2.6.2 Germination requirements and freezing tolerance

Winterfat seeds are capable to germinate over a wide range of temperatures including 0 or near 0^{o}C (Hilton, 1941; Booth, 1987). Seed germination of winterfat is reported to be optimum in alternating temperatures of 0-5^{o}C with 15 to 20^{o}C (Allen et al., 1987). Oxygen has little effect on the utilization of seed reserves during germination, but imbibition temperatures do affect the efficient use of seed reserves and seedling vigor (Booth, 1992). Imbibition temperatures also affect seed water uptake rate, seed water content and cold tolerance (Bai et al., 1999). Thus, cool temperatures (5^{o}C) are recommended for better germination and seedling vigor.

Winterfat is adapted to the climatic conditions of the Canadian Prairie and is extremely tolerant to cold seedbed conditions. Hydrated winterfat diaspores can supercool to –4 to –5^{o}C and fully hydrated winterfat seeds can survive freezing temperatures below –30^{o}C even after the occurrence of low temperature exotherms

(Bai et al., 1998). Although winterfat seeds are cold tolerant, freezing temperatures can reduce germination and seedling vigor. Freezing tolerance of seedlings depends on their age and growth temperature. The younger the seedling and the lower the growth temperature are, the higher the seedling freezing tolerance will be (Hou and Romo, 1997). Considerable genotypic variation in cold tolerance exists among populations, which are correlated to habitat differences (Bai et al., 1998). Metabolic responses to temperature also vary among ecotypes (Thygerson et al., 2002).

2.6.3 Restoration and seeding methods

Interest in the use of winterfat for restoration is increasing. Winterfat can be used for rangeland restoration because it is an important structural component in the Northern Great Plains and is a superior winter forage for livestock and wildlife. Winterfat is extensively utilized by rodents, rabbits, antelope, deer, elk, and big horn sheep. Winterfat can be used for soil erosion control because of its deep taproot and extensive fibrous root system near the soil surface, which help stabilize soil. Because winterfat establishes fairly easily on drastically disturbed soils or poorly developed soils, it can be used for land reclamation. Winterfat germinates readily and growth rapidly under favorable growing conditions. Seeds can be sown in the field in late fall, winter or early spring (Booth, 1987; 1992; Romo, 2004) and seeding in the fall is recommended over spring seeding in Saskatchewan (Romo 2004; Schellenberg, 2004). Broadcasting seeds on snow or on a moist, firm soil surface followed by a packing improves in better stands. Winterfat should be seeded on the soil surface or at shallow soil depths (Springfield, 1971; Booth, 1987). Sowing seeds with a gel carrier onto a wet seedbed can be an alternative sowing method (Booth, 1987). Relatively large seeds are recommended for restoration because of their early emergence and greater seedling establishment than small seeds (Hou and Romo, 1997; Hou et al., 1999).

3. A Thermal Time Model for Seed Germination of Winterfat

Abstract Thermal time has been widely used to quantify and model seed germination. However, several assumptions in the thermal time model remain untested. The purpose of this study was to test variation in parameters of the thermal time model among seed size classes, seed collections, and sub-zero imbibition-temperature treatments in winterfat. Seeds were imbibed at sub-zero temperature for 0, 1, 3 and 5 days and then were germinated at 2, 5, 10, 15, 20, and 25°C. Estimated base temperature (T_b) was lower in the large seed class (-4.5°C) than the small seed class (-3.5°C). Thermal time for 50% germination ($\theta_{T(50)}$) and the standard deviation of thermal times ($\sigma_{\theta T}$) were not significantly different between seed collections and seed size classes. Seeds germinated faster after imbibition at sub-zero temperature, but the enhanced germination rate was mainly attributed to thermal time accumulation during imbibition rather than the often speculated physiological changes. A thermal germination rate index was proposed, which successfully accounted for the thermal time accumulation during imbibition. Sub-zero imbibition-temperature treatments did, however, alter T_b, $\theta_{T(50)}$ and $\sigma_{\theta T}$ for both collections and seed size classes as evidenced by the higher model fit of the modified model than the original model. Base temperature in the thermal time model of winterfat was lower for larger seeds, which enables large seeds to accumulate more thermal time at a given temperature. This is particularly important for species such as winterfat because seeds of this species germinate early in the season.

3.1 Introduction

The timing of germination plays critical roles in seedling establishment in natural ecosystems and cropping systems. The seedling stage usually has the highest mortality rate in the plant life cycle (Fenner, 1987; Booth, 1992) and

mechanisms such as delaying germination and spreading germination in time may result in better seedling establishment (Probert, 1992). Thermal time and hydrothermal time models have considerable potential for characterizing and quantifying the effects of seedbed environments on seed germination and seedling emergence (Forcella et al., 2000; Bradford, 2002). These models have been successfully used to predict field seedling emergence of crops (Finch-Savage and Phelps, 1993), forages (Hardegree and Van Vactor, 1999) and weed species (Roman et al., 2000).

Temperature is the primary environmental factor regulating seed dormancy and germination (Roberts, 1988). Thermal time, the heat unit accumulation above a temperature threshold, is an essential component in germination modeling. One assumption of previous thermal time models is the constancy of T_b within a seed population (Garcia-Huidobro et al., 1982; Gummerson, 1986). Unless dormancy is present (Welbaum and Bradford, 1991), T_b generally is believed to vary little among seed subpopulations (Ellis et al., 1986; Ellis and Barrett, 1994; Dahal et al., 1990; Steinmaus et al., 2000). The base temperature is also assumed to remain constant after seed treatment (Bauer et al., 1998; Meyer et al., 2000). Other reports, however, have shown that T_b changes with water potential (Fyfield and Gregory, 1989; Kebreab and Murdoch, 1999), priming treatment (Hardegree et al., 2002), and germination stage (Fyfield and Gregory, 1989; Lisson et al., 2000). More complex models have been suggested for seed germination behaviour at temperatures near T_b (Kebreab and Murdoch, 1999). The base temperature may be an adaptive characteristic and a species that normally germinates at cool temperatures has a relatively low T_b (Steinmaus et al., 2000). T_b can be below 0^oC in rangeland species (Hardegree and Van Vactor, 1999; Trudgill et al., 2000) and even in crop species (Ellis et al., 1986; Squire et al., 1997). Species with low or subzero Tb that germinate and emerge earlier in the spring may have a competitive advantage for survival and establishment (Hou and Romo, 1998).

Larger or heavier seeds usually germinate faster than smaller or lighter seeds of the same species (Vaughton and Ramsey, 1998; Humara et al., 2002). Because thermal time models are based on the germination rate of subpopulations,

it is reasonable to hypothesize that parameters in the thermal time model vary among different seed portions. Seed treatments such as stratification, priming and after-ripening modify the physiological status of seeds and affect germination percentage, germination rate and/or seedling vigor (Hardegree and Van Vactor, 2000; Shen et al., 2001; Allen and Meyer, 2002). These treatments may also alter the thermal-germination response and thermal time model parameters. The effect of imbibition treatment at temperatures below those ranges (i.e., $<0^{o}C$) on thermal accumulation and model parameters will be particularly important because it may be experienced by seeds in early spring, but has not attracted much attention. Therefore, the testing and validation of assumptions in thermal time model will help improve model accuracy and provide insight to the mechanisms that plants have developed to cope with their environment.

We hypothesized that thermal time requirement for germination varies between seed size classes and imbibition at sub-zero temperature modifies this requirement in winterfat. The objectives of this study were to test the assumptions of the thermal time model using winterfat, specifically: 1) to determine variation in parameters of the thermal time model between seed size classes, and 2) to determine the effect of sub-zero imbibition-temperature on parameters of the thermal time model.

3.2 Materials and methods

3.2.1 Seed sources and characteristics

Two collections of winterfat seeds (diaspores) were purchased from Wind River Seed (Manderson, Wyoming, USA) (Table 3.1). The Cela collection was from a reclamation site in Northern Colorado with seeds originating from Tooele, Utah. The #63 collection was from Delta, Utah. The two sites differ in temperature and precipitation, with the Delta site having lower minimum temperature and less precipitation than the Tooele site (Fig. 3.1). Fruits were stored in a warehouse for approximately 7 months after harvesting in October

2001. They were air-dried at room temperature for at least a week after purchase, and then cleaned by rubbing, fanning and passing over serial sieves and blowers.

Table 3.1. Characteristics of two collections and two seed sizes of winterfat. Values are means ± S.D. Means with the same letter within a parameter and a seed collection are not significantly different at P = 0.05 using FPLSD.

Seed collection	Seed size	Seed weight (mg seed^{-1})	Seed water content (% d.w.)
#63	Large	2.85 ± 0.18 a	5.9 ± 0.1 a
	Small	1.74 ± 0.14 b	6.1 ± 0.4 a
Cela	Large	2.39 ± 0.33 a	5.2 ± 0.1 a
	Small	1.69 ± 0.13 b	5.0 ± 0.2 a

Cleaned seeds were separated into two size classes based on seed mass using a seed blower and seed size is used hereafter instead of seed mass. Seeds that blew to the upper container of the blower were classified as "small" and sediments were "large". Eight random samples from each seed size class were then tested for significant differences in seed weight between the two classes. Seeds were mixed and separated again by adjusting the airflow if the weight of the two classes was not significantly different (P > 0.05). Seed water content was determined after cleaning using gravimetric and oven-drying methods (80^{o}C, 72 h) with four replicates of 10 seeds. Results indicated that seed water content was not significantly different between seed size classes within a seed collection (Table 3.1). Seed weight of all experimental units (50 seeds/unit) was recorded and the difference in seed weight between the large seed size class and the small seed size class was significant (P = 0.05). Cleaned and classified seeds were then sealed in plastic bags and stored at –18^{o}C until use (Booth et al., 1999).

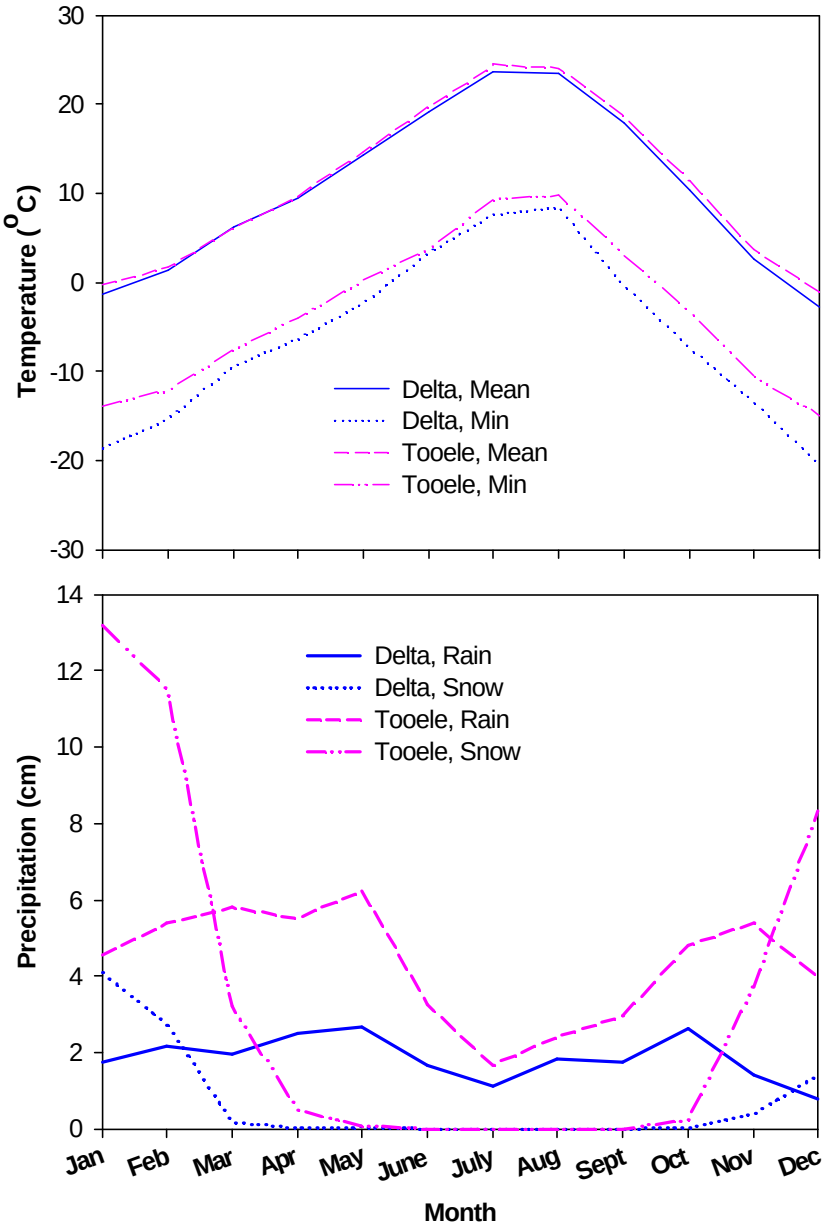


Fig. 3.1. Long-term (1990 -2000) monthly mean and minimum air temperatures and monthly total precipitation of Delta (for collection #63) and Tooele (for collection Cela), Utah, USA, where winterfat seeds originated. Data were from Utah Climate Center, Utah State University, Logan, USA (http://climate.usu.edu/free/, accessed May 16, 2003).

3.2.2 Seed germination test for model construction

Germination tests were conducted in darkness using six incubators (Sanyo Versatile Environmental Chamber MLR-350H, Sanyo Scientific, USA). Temperatures of, 2, 5, 10, 15, 20 and 25^{o}C, were randomly assigned to each incubator. Only the middle three shelves of each incubator, 15 cm apart, were used for the germination test to minimize the temperature difference among shelves within each incubator. Temperatures were measured every 1 min at the center of each shelf, averaged hourly, and recorded by dataloggers (21X, Campbell Scientific Inc., USA). Measured temperature data were used for model development.

A randomized complete block design (RCBD) was used with five replicates at 7-day interval between replicates in each incubator. Seeds, 50/unit, were sprinkled in 9 cm Petri dishes with 5 mL distilled water on top of two layers of filter papers (Whatman No.1), and Petri dishes were randomized within each chamber. Clear plastic bags were used to seal Petri dishes to reduce water evaporation. Two mL of distilled water were added periodically to ensure the saturation of the filter paper throughout the testing period. Seeds were considered germinated when either the emerging radicle or cotyledons was = 2 mm. Germination was recorded at 4, 8, 12, 24 or 48 h intervals, depending on germination rate, for up to 32 days until no germination occurred in 7 consecutive days in all Petri dishes of a replicate. Germinated seeds and rotten seeds were removed after each counting. Seed surfaces were sterilized with 95% ethanol whenever there were signs of microorganism development.

The percentage of viable seeds was estimated at the end of the germination test and adjusted to a scale of 0-100% by dividing a scaling factor (Ellis et al., 1986; Hardegree and Van Vactor, 1999). Scaling factors were based on the maximum mean germination percentage achieved among the six germination temperatures: 96, 83, 88, and 86% for #63 large, #63 small, Cela large, and Cela small, respectively. Application of the scaling factor adjusted the germination

percentage for each seed size class in each collection to a common scale of 0 to 100%.

3.2.3 Sub-zero imbibition-temperature treatments

Seeds, 50/unit, were imbibed in Perti dishes with 6 mL distilled water on two layers of filter paper at -1^{o}C for 0, 24, 72, and 120 h. A RCBD with five replications initiated at 7-day intervals was used. Petri dishes were sealed in clear plastic bags and randomized in a growth chamber (Conviron PGR15, Controlled Environments Limited, Canada). Temperatures inside the plastic bags were recorded with a datalogger (HOBO H8, Onset Computer Corporation, USA). Petri dishes were transferred to incubators for the germination tests after imbibition treatments using the same temperatures and procedures as in the previous experiment. Between 10 and 15% of the seeds germinated at the end of the imbibition treatment and the pre-germinated seeds were included in the total germination count for calculation and the construction of a modified model.

3.2.4 Data analysis and modeling

3.2.4.1 Curve fitting of germination time course

A germination time course curve was constructed for each germination temperature, seed size class, seed collection, and replicate. Cumulative germination percentage was transformed using $\log_{10}$ Probit (Finney, 1971). Probit transformation linearizes the cumulative normal distribution, which facilitates modeling efforts (Bradford, 1995). For modeling purposes, a seed population was considered to be composed of subpopulations based on relative germination rate (Garcia-Huidobro et al., 1982). The PROC PROBIT procedure in SAS (SAS Institute, Inc., 1995) was used to estimate germination time for the 10, 20, 30, 40, 50, 60, 70, 80, and 90% subpopulations. Data for the 90% subpopulation were not used in the subsequent analysis because of the high estimation error and the lack of data at low temperatures. To meet the probit assumptions, cumulative germination percentage of each experimental unit was adjusted using the final germination

percentage of each unit and converted back after the curve fitting. Pearson Chi Square Test was used as the goodness-of-fit test ($P \geq 0.10$). The best-fit equations were obtained by removing germination data over 90% (Kebreab and Murdoch, 1999) and the constant data points at low germination and high germination portions.

3.2.4.2. Estimating thermal time model parameters

According to the thermal time model Eq. 2.2 (Garcia-Huidobro et al., 1982), $GR_{(g)}$, the reciprocal of t_g to a given g, is linearly related to T between T_b and optimal temperature for non-dormant seeds. The linear relationship between $GR_{(g)}$ and T varies among subpopulations in the slope of the linear regression line, which is equal to the reciprocal of $\theta_{T(g)}$.

If the variation of $\theta_{T(g)}$ within a seed population follows a normal distribution (Ellis et al., 1986), then germination time course in terms of thermal time can therefore be described by Eq.2.3. The standard deviation of thermal time ($\sigma_{\theta T}$) is the reciprocal of the linear regression slope of Probit germination for all subpopulations (Bradford, 1995; Kebreab and Murdoch, 1999). Eq. 2.2 and 2.3 assume that all subpopulations have a common T_b and different $\theta_{T(g)}$ for each subpopulation.

Common T_b, $\theta_{T(50)}$ and $\sigma_{\theta T}$ were estimated for each seed size class of each collection for the construction of the thermal time model. In order to estimate a common T_b, regressions of $GR_{(g)}$ on T were derived from Probit analysis of germination time course at each germination temperature. Germination rate (h^{-1}) was calculated by the estimated time to germinate by subpopulations based on Probit analysis and then was regressed on T separately for each replicate and each successive increment of 10% subpopulation. Actual recorded temperatures of incubators were averaged according to the time required for germination of subpopulations and were used for regression analysis. Data used for estimating T_b and $\theta_{T(g)}$ were within the range of suboptimal temperatures based on visual inspection of data. The significance ($P \leq 0.05$) of the linear regression of $GR_{(g)}$ on

T was tested by PROC REG procedure in SAS. The x-intercept of the regression line is defined as T_b (Kebreab and Murdoch, 1999; Steinmaus et al., 2000). The base temperature was computed for each regression line or each subpopulation and their average was used as the common T_b for thermal time modeling of the whole population. Thermal time for a given germination percentile ($\theta_{T(g)}$) was determined from the reciprocal of the regression slope (Kebreab and Murdoch, 1999).

After $\theta_{T(g)}$ had been estimated for each subpopulation, the thermal time course towards germination was established. According to the cumulative normal distribution function, $\sigma_{\theta T}$ is the inverse of the slope of the probit regression line. Therefore, $\sigma_{\theta T}$ was obtained from the regression analysis of thermal times on Probit (g) by PROC REG procedure in SAS.

Thermal time accumulation during the sub-zero temperature imbibition treatment was calculated using the common T_b determined from the non-imbibition treatment for each seed size class and collection. Calculated thermal time was then converted to hours according to each germination temperature and added to the germination time course for further analysis. The parameters of the thermal time model after imbibition treatment (modified model) were estimated as previously described.

3.2.4.3 Testing the variation of parameters of thermal time model

PROC GLM in SAS was used to test differences in T_b between subpopulations. The effects of seed collections, seed size classes and sub-zero temperature imbibition treatment on T_b, $\theta_{T(50)}$, and $\sigma_{\theta T}$ were also tested using PROC GLM. Means were separated with Fisher's Protected Least Significant Difference (FPLSD). Accuracy of thermal time model was measured by coefficient of determination (R^2, R.J. Baker, personal communication). R^2 was calculated as $1-(SS_{residual}/SS_{total})$, while $SS_{residual}$ = (observed value – predicted value) and SS_{total} = (observed value – mean).

Germination rate index (GRI), which is independent on the thermal time model, was calculated as:

$$GRI = (G_{tot} / p)^{*}\Sigma\ (g_i / t_i) \qquad \text{(Eq. 3.1)}$$

Where G_{tot} is the total number of germinated seeds in a dish at the end of the germination test, p is the total number of seeds incubated; g_i is the number of germinated seeds accumulated between time (h) t_{i-1} and t_i (Steinmaus *et al.*, 2000). The germination rate index was modified to incorporate the effect of thermal time (θ_i), resulting a new index, Thermal Germination Rate Index (TGRI):

$$TGRI = (G_{tot} / p)^{*}\Sigma\ (g_i / q_i) \qquad \text{(Eq. 3.2)}$$

TGRI can be calculated if T_b is known. The thermal effect of temperature as well as the effect of T_b on germination rate is accounted for TGRI so the true ability of germination rate of a population can be compared with other populations or with the same population after treatments. The effect of seed size, seed collection and sub-zero imbibition-temperature on GRI and TGRI were also analyzed using GLM.

3.3 Results

3.3.1 Parameter estimation for thermal time model

Germination rates of subpopulations, $GR_{(g)}$, calculated from the predicted germination time course correlated strongly with temperature ($R^2 > 0.90$) at the suboptimal temperature range (Fig. 3.2). Optimum temperatures varied among subpopulations between 20 and 25^{o}C with lower optimum temperature in more slowly germinating subpopulations.

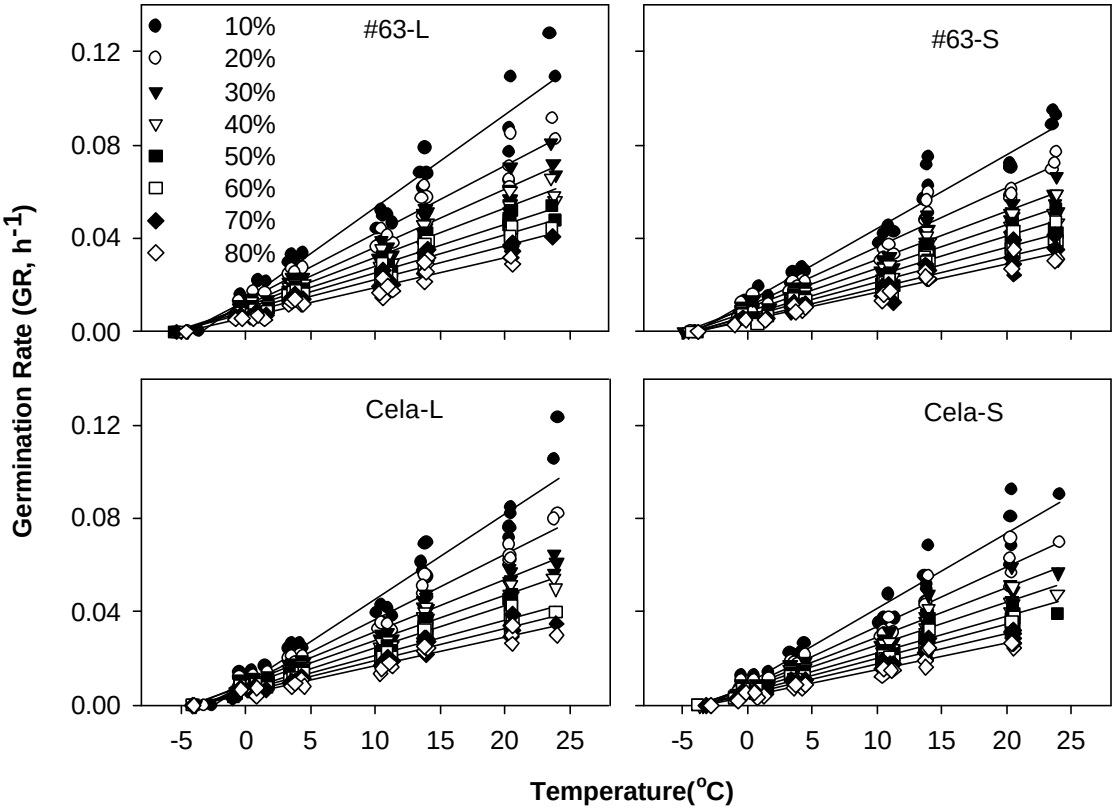


Fig. 3.2. Germination rate (h^{-1}) within the range of suboptimal temperatures as a function of temperature and sub-population in two seed size classes and two seed collections of winterfat. L: large seeds, S: small seeds, $R^2 > 0.90$.

T_b was below 0^oC for both seed collections, and was significantly different between seed size classes and between collections (Fig. 3.2, Table 3.2). Average Tb for collection #63 (-4.5^oC) was about 1^oC lower than that of Cela (-3.5^oC). Large seed class (-4.3^oC) had lower T_b than small seed class (-3.7^oC). The difference between seed size classes was more than 0.5^oC for the two collections and was greater in Collection #63. T_b was significantly different among subpopulations in collection Cela ($P \leq 0.05$), but not in collection #63 (data not shown). There were no significant differences either in $\theta_{T(50)}$ or $\sigma_{\theta T}$ between collections and seed size classes.

Table 3.2. Estimated parameters for the thermal time model in two seed size classes and two collections of winterfat. Means with the same letter within a parameter and a seed collection are not significantly different at P = 0.05 using Fish Protected LSD.

Seed collection	Seed size	T_b (°C)	$\theta_{T(50)}$ (°C h)	$\sigma_{\theta T}$ (°C h)
#63	Large	-4.8 (1.3) a	549.0 (62.1) a	253.8 (32.2) a
	Small	-4.2 (1.3) b	571.1 (105.8) a	247.0 (36.3) a
Cela	Large	-3.8 (1.0) b	567.0 (51.0) a	262.9 (36.2) a
	Small	-3.3 (1.0) c	614.4 (53.7) a	274.3 (25.3) a
LSD		0.45	92.3	44.7

3.3.2 Accuracy of the constructed thermal time model

Thermal time models were constructed separately for each seed size class and collection using parameters listed in Table 3.2. Original germination data at temperatures between 2 and 20°C were used to test the accuracy of thermal time models. R^2 values were generally high (mostly > 0.80) for both collections and seed size classes (Table 3.3). At low temperatures (e.g., 2°C), R^2 was between 0.76 and 0.79 and the departure of the predicted value from the observed one occurred for some subpopulations (e.g., >70% subpopulations) (Fig. 3.3).

Table 3.3. Accuracy of thermal time model at the range of suboptimal temperatures as measured by coefficient of determination (R^2)[1].

Seed collection	Seed size	Temperature (^oC)	R^2
#63	Large	2	0.77
		5	0.89
		10	0.91
		15	0.86
		20	0.96
	Small	2	0.77
		5	0.81
		10	0.83
		15	0.92
		20	0.82
Cela	Large	2	0.79
		5	0.91
		10	0.87
		15	0.91
		20	0.92
	Small	2	0.76
		5	0.83
		10	0.89
		15	0.83
		20	0.89

[1]R^2 was calculated as: 1-($SS_{residual}$ /SS_{total}); $SS_{residual}$ = (observed value – predicted value); and SS_{total} = (observed value – mean).

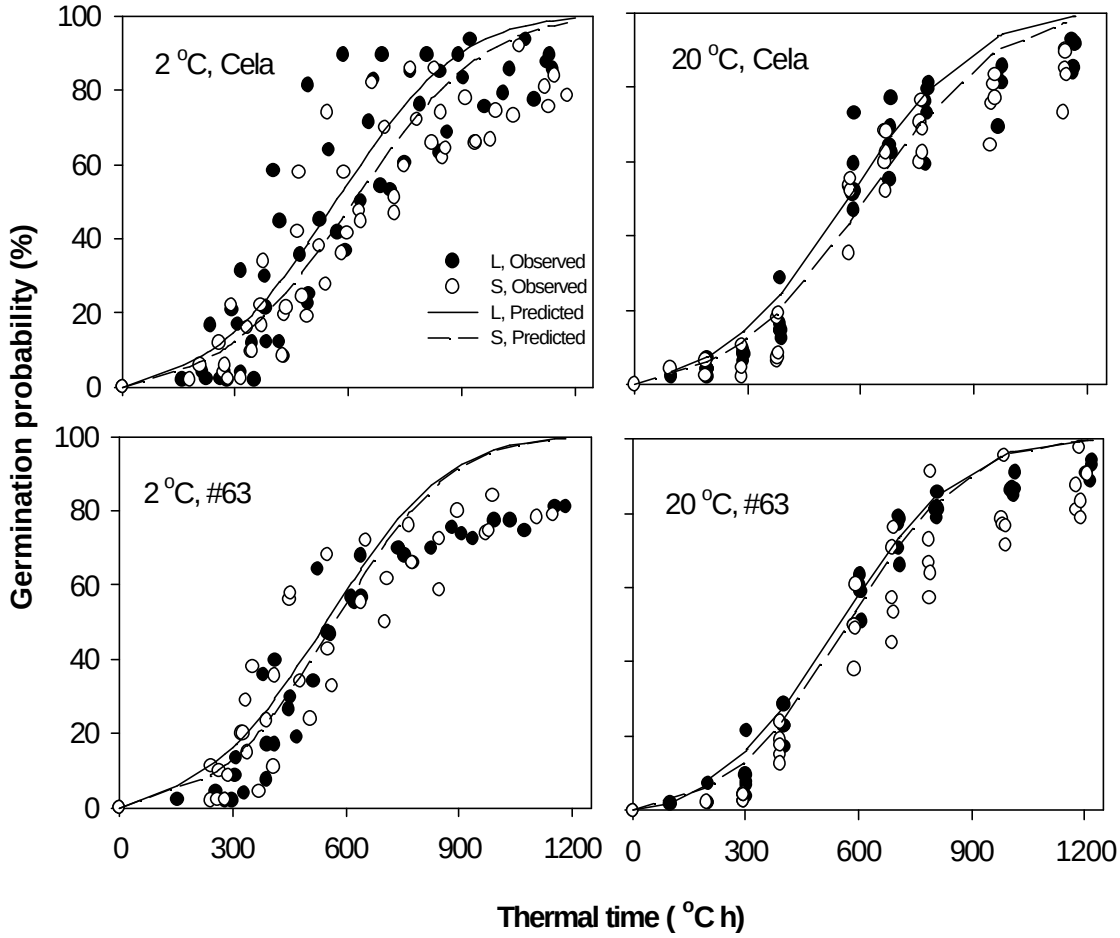


Fig. 3.3. Observed (scattered points) and predicted (regression lines, using Eq. 2.3 and parameters in Table 3.2) thermal time course for two seed size classes and two seed collections of winterfat at 2 and 20°C. L: large seeds, S: small seeds.

3.3.3 Thermal time model as affected by sub-zero temperature imbibition

The average sub-zero imbibition-temperatures were –0.78, -0.78 and –0.91°C for the 24, 72 and 120 h treatments, respectively. Parameters in the thermal time model were altered by sub-zero temperature imbibition treatments. The $\theta_{T(50)}$ decreased slightly after 24 or 72 h imbibition at sub-zero temperatures, but significantly increased after 120 h ($P<0.05$), except for small seeds of Cela (Fig. 3.4).

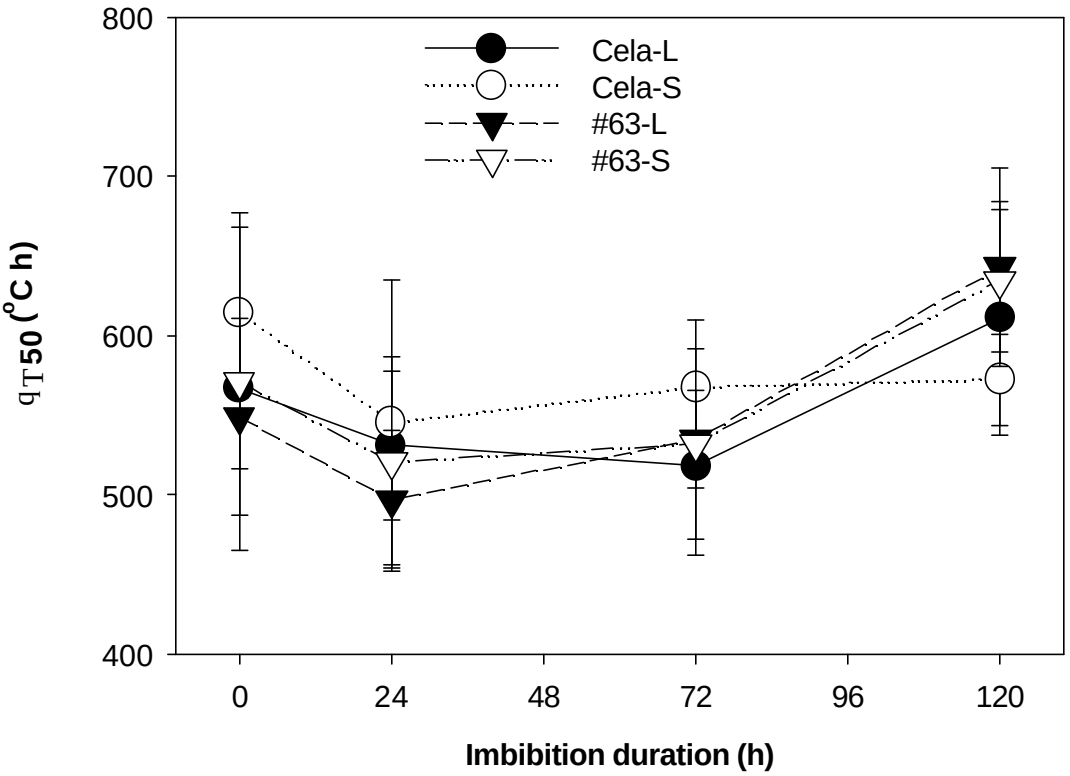


Fig. 3.4. Thermal time of 50% subpopulation ($\theta_{T(50)}$) as affected by sub-zero imbibition-temperature treatment in two seed size classes and two seed collections of winterfat. Values are means ± S.E. L: large seeds, S: small seeds. Average imbibition-temperatures for 24, 72 and 120 h were -0.78, -0.78 and -0.91°C, respectively.

The standard deviation of thermal time ($\sigma_{\theta T}$) was not significantly different among treatments for both seed collections and seed size classes ($P > 0.05$) except for the small seed class of collection Cela, for which $\sigma_{\theta T}$ was higher after 72 h imbibition than the others ($P = 0.08$, Fig. 3.5).

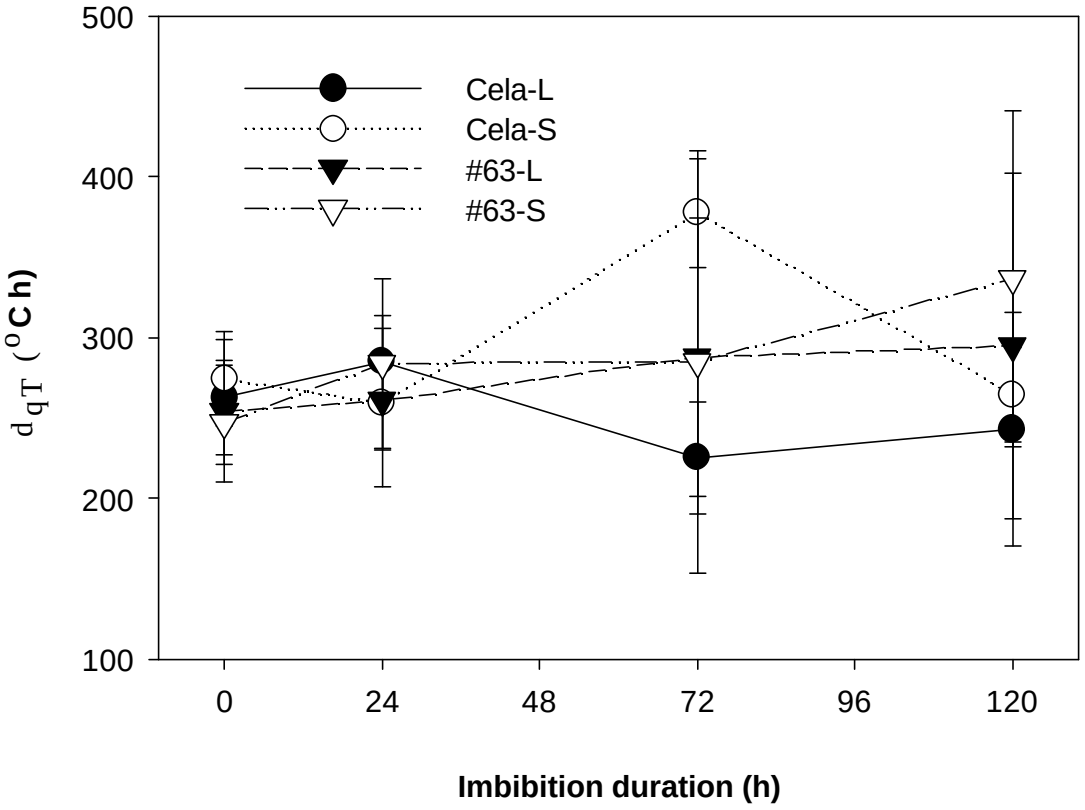


Fig. 3.5. Standard deviation of thermal time ($\sigma_{\theta T}$) as affected by sub-zero imbibition-temperature treatment in two seed size classes and two seed collections of winterfat. Values are means ± S.E. L: large seeds, S: small seeds. Average imbibition-temperatures for 24, 72 and 120 h were -0.78, -0.78 and -0.91°C, respectively.

Among parameters of the thermal time model, T_b was most sensitive to the imbibition treatments (Fig.3.6). T_b increased (less negative) between 1 and 1.5°C with increasing imbibition duration up to 72 h, but decreased to the original level (without imbibition treatment) after 120 h imbibition except for the small seed of Cela in which T_b was not different among imbibition treatments.

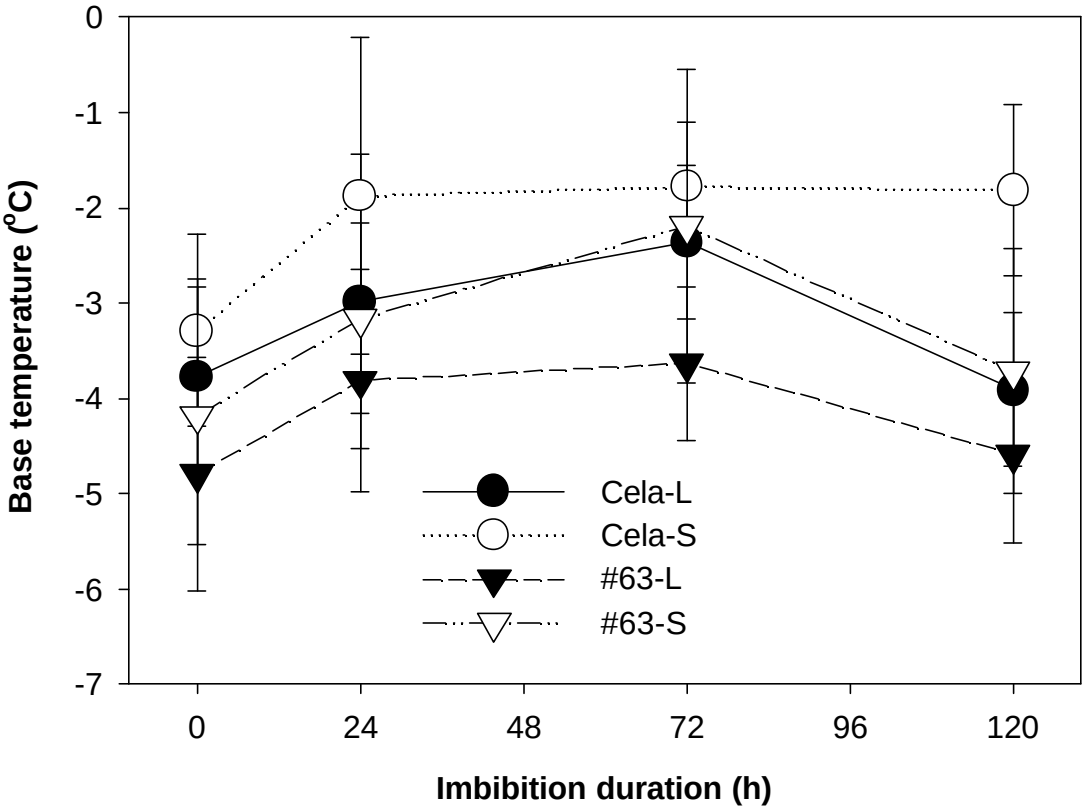


Fig. 3.6. Base temperature (T_b) as affected by sub-zero imbibition-temperature treatment in two seed size classes and two collections of winterfat. Values are means ± S.E. L: large seeds, S: small seeds. Average imbibition-temperatures for 24, 72 and 120 h were -0.78, -0.78 and -0.91°C, respectively.

When using the original model without the including of thermal time accumulation during the imbibition treatment, the model fit decreased with increasing imbibition duration (Fig. 3.7). The model fit was improved greatly with the inclusion of thermal time accumulation, especially for the 72 and 120 h imbibition treatments, indicating that the original model can be used to predict seed germination after the sub-zero temperature imbibition treatment. Only for germination at low temperatures (e.g., 2^{o}C) and usually for the small seed class, model fit was proved by using the modified model, in which all parameters were altered from the original model (data not shown). This indirectly validates the modified model and indicates that the physiological status of seeds may have changed by the sub-zero imbibition-temperature treatment above T_b.

3.3.4 Using thermal germination rate index (TGRI) to link thermal time model to conventional germination rate measures

Seed germination rate as measured by GRI was different between seed size classes and seed collections at various temperatures and GRI was dependent on incubation temperature (Fig. 3.8). There was a 1.5- and 2.1-fold difference between the lowest and highest values within each incubation temperature and a 10-fold difference among temperatures. The large seeds germinated faster than small seeds in both collections ($P < 0.05$) and collection #63 germinated faster than collection Cela ($P < 0.05$) when all data were analyzed together. Large seeds germinated faster than small seeds at all temperatures for collection #63 and at 2 and 25^{o}C for collection Cela ($P = 0.05$).

When time (h) was replaced with thermal time (^{o}C h), the thermal germination rate index was in a similar range for all temperatures (Fig. 3.9). The effect of T_b was accounted for by TGRI and differences in TGRI were explained by thermal time (θ_T). For example, the higher TGRI in large seeds than small seeds indicates a higher percentage of seeds germinated for the same amount of thermal time.

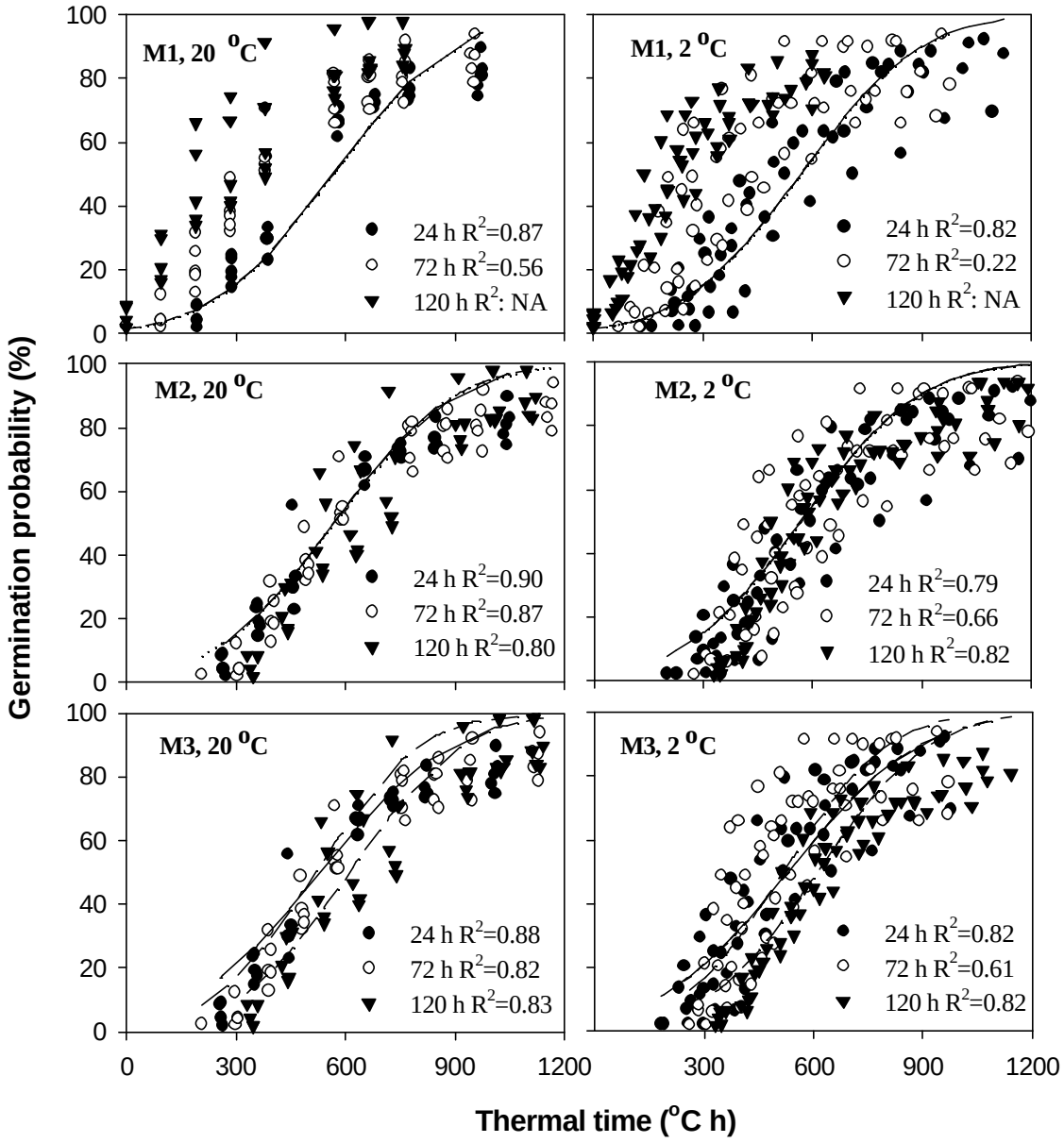


Fig. 3.7. Observed (symbols) and predicted (lines) thermal time course for the large seed size class of collection Cela of winterfat at 2 and 20°C after 24, 72, and 120 h of imbibition at sub-zero temperature. M1: original model without the inclusion of thermal time accumulated during imbibition, M2: original model with the inclusion of thermal time accumulated during imbibition, M3: modified model with altered model parameters.

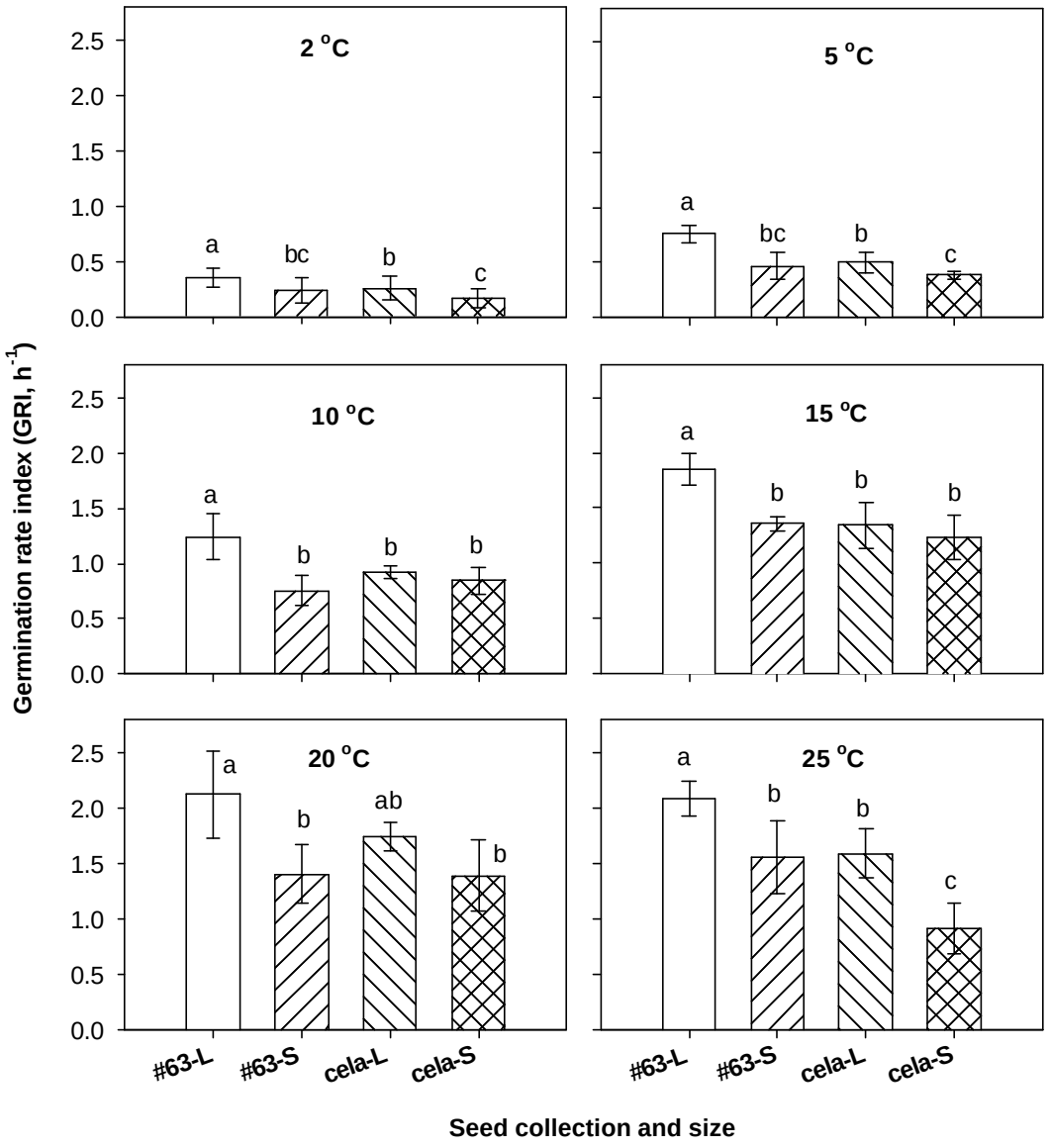


Fig. 3.8. Germination rate index (GRI) of two seed size classes and two seed collections of winterfat incubated at temperatures from 2 to 25°C. Values are means ± S.E. L: Large seeds; S: Small seeds. Means with the same letter are not significantly different at P = 0.05 using FPLSD.

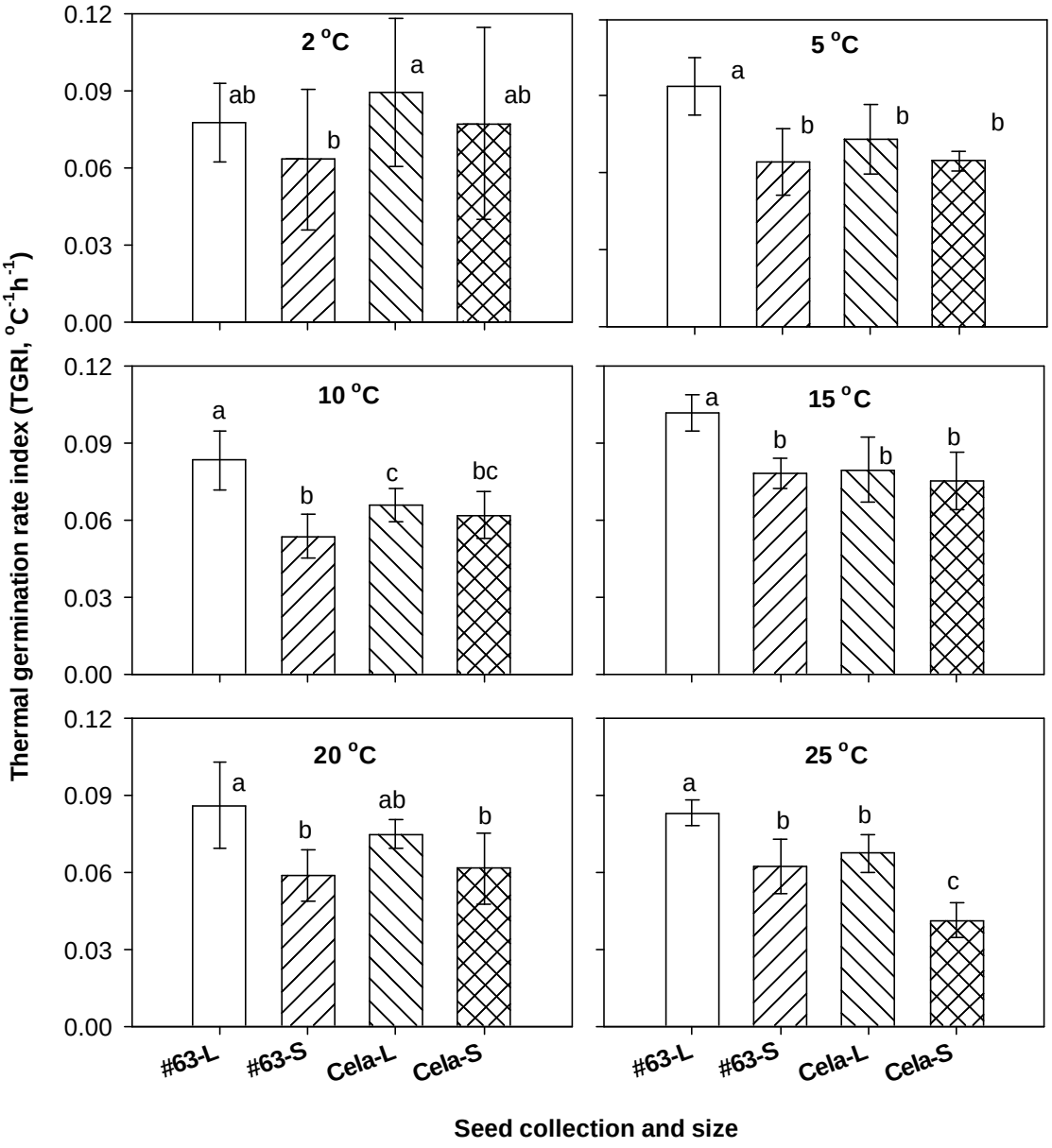


Fig. 3.9. Thermal germination rate index (TGRI) of two seed size classes and two seed collections of winterfat incubated at temperatures from 2 to 25°C. Values are means ± S.E. L: large seeds; S: small seeds. Means with the same letter are not significantly different at P = 0.05 using FPLSD.

Similarly, the values of TGRI were in a similar range among incubation temperatures after the replacement of time with thermal time in GRI in the sub-zero imbibition-temperature treatments (Fig. 3.10). The increased TGRI with increasing imbibition duration indicate seeds germinated faster after imbibition treatment for the same amount of thermal time. After the inclusion of thermal time accumulated during the imbibition treatment to the TGRI calculation, the enhancing effect of imbibition treatment on germination rate became minimal (Fig. 3.11). This implies that most of the variance of the enhanced germination rate by imbibition treatment can be explained by the thermal time accumulated during imbibition at sub-zero temperatures. Even though significant differences existed in TGRI among imbibition durations, no consistent patterns were found.

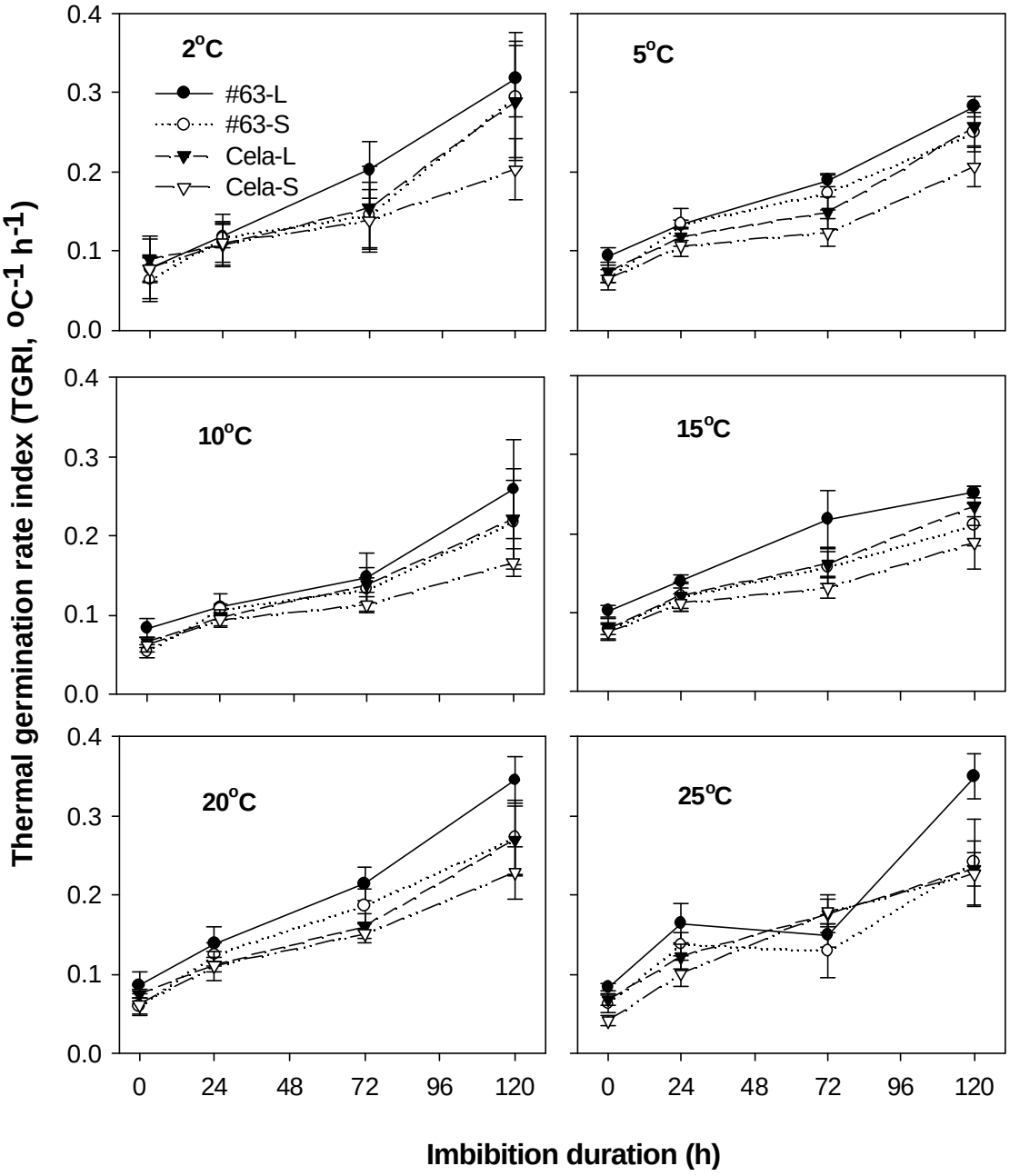


Fig. 3.10. Thermal germination rate index (TGRI) of two seed size classes and two seed collections of winterfat at temperatures from 2 to 25°C as affected by sub-zero imbibition-temperature treatment. Values are means ± S.E. L: large seeds; S: small seeds. Average imbibition-temperatures for 24, 72 and 120 h were -0.78, -0.78 and -0.91°C, respectively.

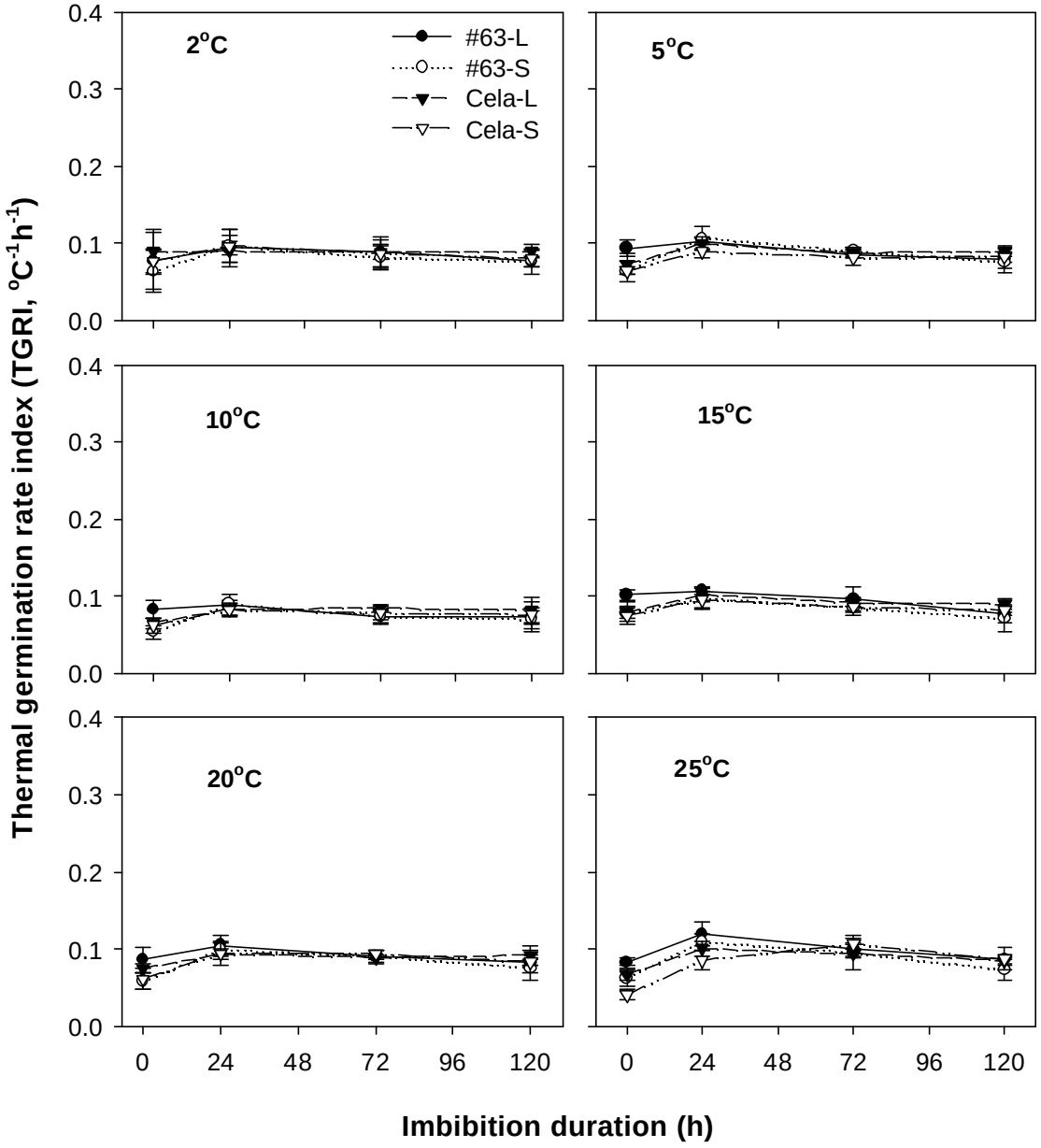


Fig. 3.11. Thermal germination rate index (TGRI) of two seed size classes and two seed collections of winterfat at temperatures from 2 to 25°C as affected by sub-zero imbibition-temperature treatment after the inclusion of thermal time accumulated during imbibition. Values are means ± S.E. L: large seeds; S: small seeds. Average imbibition-temperatures for 24, 72 and 120 h were -0.78, -0.78 and -0.91°C, respectively.

3.4 Discussion

The variation of thermal time among seeds in a population can be either normally (Ellis et al., 1986) or log-normally distributed (Dahal et al., 1990). Our results indicate this is also true for each seed size class within a population. Large seeds germinated faster than small seeds in winterfat possibly due to the ability of large seeds to provide higher energy and nutrients (Vaughton and Ramsey, 1998) for greater germination capacity (Humara et al., 2002). For the two seed collections of winterfat, the thermal time requirement for the 50% subpopulation ($\theta_{T(50)}$) was not significantly different between large and small seed size classes, nor was the standard deviation of thermal time ($\sigma_{\theta T}$). The base temperature, however, was significantly lower in large seeds than that in small seeds, allowing large seeds to accumulate more thermal time units than small seeds under the same temperature and subsequently to germinate faster. Furthermore, T_b was significantly different between seed collections for a given size class. Our results did not agree with previous studies in which T_b was believed to have little variation among individual seeds within a seed lot (Garcia-Huidobro et al., 1982; Dahal et al., 1990), among genotypes (Ellis et al., 1986; Ellis and Barrett, 1994) or seed lots (Cheng and Bradford, 1999) of the same species. The reason that T_b was often considered a constant may be for the ease of modeling (e.g., Meyer et al., 2000). Similar to what we found in winterfat seeds, previous reports indicate that T_b along with other parameters in the thermal time model did not appear stable for non-crop species (Forcella et al., 2000) due to distinct genetic variability within the population (Wang et al., 1995).

Winterfat seedlings from seeds that germinated or emerged at low spring temperature had better survival and establishment (Hou and Romo, 1998). Small variation in spring temperature can lead to significant differences in the rate of seedling emergence of rangeland species (Jordan and Haferkamp, 1989). Therefore, variation in base temperatures between seed size classes within a population of winterfat may favor large seeds especially in early spring when temperatures are low, allowing rapid germination and establishment given suitable

environmental conditions. Collection #63 originated from a colder habitat, (Delta, Utah) had a lower T_b and with annual mean and mean minimal temperature about 10^oC and -6^oC compared to 11^oC and -3^oC for Tooele, Utah. However, conclusive evidence regarding ecotypic variation in base temperature will require the testing of more seed collections.

The base temperatures estimated for both seed size classes and seed collections of winterfat were below 0^oC. Even though negative base temperatures have been reported based on modeling (Ellis et al., 1986; Squire et al., 1997; Hardegree and Van Vactor, 2000), they have never been validated and their biological meaning was seldom discussed possibly because of the lack of direct evidence showing that seeds can actually accumulate heat and germinate under such conditions. Winterfat is among the few species in which seeds can germinate near 0^oC (Booth, 1987). Under naturally occurring cooling rates in the spring, fully hydrated winterfat seeds can supercool to -4 to -5^oC without ice formation (Bai et al., 1998), suggesting that metabolic activities are possible at sub-zero temperatures with the presence of liquid water. Temperatures during the imbibition treatment were close to -1^oC as indicated by the continuous datalogger measurements and 10-15% seed germination was observed after 5 days of imbibition, suggesting that winterfat seeds can accumulate heat and germinate at sub-zero temperatures.

Winterfat seeds germinated faster after imbibition at sub-zero temperatures. By adding the thermal time accumulated during imbibition to GRI, a Thermal Germination Rate Index (TGRI) was proposed. TGRI successfully accounted for the effect of imbibition temperature on germination rate, indicating that thermal time accumulation during imbibition has an overwhelming effect on the rate of germination. Parameters for the thermal time model in winterfat were altered by the imbibition treatment at sub-zero temperatures. The alteration was indirectly verified by the higher model fit than the original model, but the physiological mechanism for the possible changes is unclear. Seed treatments such as priming can modify the base temperature and thermal time, e.g., in two *Elymus* species (Hardegree et al., 2002). The effect of priming on seed is the

physiological advancement for seed germination, such as cell cycle-related events (de Castro et al., 2000), endosperm weakening (Bradford et al., 2000) and mobilization of storage protein and synthesis new protein (Gallardo et al., 2001). Seed treatments, such as stratification, can activate the intracellular repair mechanisms in non-dormant seeds of black spruce (*Picea mariana*) characterized by reserve diminish and organelles development (Wang and Berjak, 2000). The physiological mechanism for the modified parameters in the thermal time model of winterfat after seed treatment at low temperatures and their direct validation warrant further studies.

In summary, we accepted the hypothesis that that thermal time requirement for germination varies between seed size classes and imbibition at sub-zero temperature modifies this requirement in winterfat. Base temperature is the most variable parameter in the thermal time model of winterfat. The large seeds have lower base temperatures and the variation favors large seeds especially in early spring when temperatures are low. Winterfat seeds have base temperatures below 0^{o}C and the thermal time accumulated during imbibition at sub-zero temperatures is mainly responsible for higher germination rate. Parameters related to germination rate, but independent of the thermal time model, such as germination rate index and the proposed thermal germination rate index can be used in conjunction with the thermal time model for the study of seed germination.

4. A Hydrothermal Time Model for Seed Germination of Winterfat at Reduced Water Potentials

Abstract The hydrothermal time model not only quantifies seed germination progress as affected by temperature and water potential, but also has ecological and biological significance. Assumptions of the hydrothermal time model were tested using two non-dormant seed collections of winterfat with two seed size classes. Germination rates of subpopulations were estimated from germination time courses over a water potential range from 0 to –1.33 MPa at 2, 5, 10, 15, 20, and 25°C. Parameters of the hydrothermal time model were estimated from the relationships between GR and temperature at various water potentials and between GR and ψ at various temperatures. Model assumptions were tested using these estimated parameters. The base temperature ($T_{b(50)}$) and base water potential ($\psi_{b(50)}$) of the 50% subpopulation were not independent of temperature and water potential. The $\psi_{b(50)}$ was lowest at temperatures between 10 to 15°C, while T_b decreased linearly with increasing water availability. The estimated shifting rates of $T_{b(50)}$ with ψ were between 2.2 and 3.8°C per MPa for the two collections and large seeds had a greater shifting rate than small seeds. Hydro time (θ_H) was constant among subpopulations only at optimal temperatures. A linear increase of θ_H with subpopulation was found at lower temperatures, especially at 2°C. There were no significant differences in $\psi_{b(50)}$ between large and small seeds, but significant differences were observed in hydrothermal time requirement ($\theta_{HT(50)}$). The predictability of the hydrothermal time model was improved especially at low temperatures when $\theta_{HT(50)}$ was allowed to change with temperature as measured by a modified R^2 value. Changes in other parameters with temperature or water potential did not further improve the predictability of the hydrothermal time model. Therefore, further efforts in improving the hydrothermal time model should focus on variation in θ_{HT}.

4.1 Introduction

Hydrothermal time was first proposed by Gummerson (1986) based on the linear relationship between germination rate and water potential at constant temperature in sugar beet (*Beta vulgaris* L.). The θ_{HT} model and the related assumptions were further developed by Bradford and his coworkers (Bradford, 1990; 1995; Dahal and Bradford, 1990; 1994; Cheng and Bradford, 1999; Alvarado and Bradford, 2002). Hydrothermal time quantifies the combined effects of temperature and water potential on the progress of seed germination, which includes the thermal time accumulation above the thermal threshold (T_b) and the hydro time accumulation above the hydro threshold (ψ_b) towards seed germination. Base temperature varies little among individual seeds or subpopulations and the θ_T is normally distributed within a seed population in the thermal time model (reviewed by Bradford, 1995; 2002). Base water potential in the hydrothermal time model differs among individual seeds and hence must be defined according to a particular germination fraction, while θ_H and θ_{HT} are assumed constant (Bradford, 1990; 1995; Gummerson, 1986). Hydro time is based on the effect of water availability on the germination rate of subpopulations (Hegarty, 1978; Gummerson, 1986). Water has more complicated effects on germination compared to temperature, especially at low water potentials. When ψ is lower than -0.5 MPa, physiological adjustment occurs (Ni and Bradford, 1992), and when ψ is below the threshold of radicle emergence, metabolic advancement or priming effect is observed (e.g., Hardegree et al., 2002; Kaur et al., 2002).

A significant advantage of the hydrothermal time model is that germination progress can be predicted for any combination of temperature and water potential once the model parameters are known. The model can also be applied to entire seed populations. Because of the involvement of water availability and temperature, the two major driving forces in seed germination, the hydrothermal time model has great potential in field application. It has been widely used for predicting seedling emergence in native and introduced species (Christensen et al., 1996; Kebreab and Murdoch, 1999; Grundy, 2000; Roman et al., 1999; 2000; Allen et al., 2000a) and in

crops, such as lettuce (*Lactuca sativa* L.) (Bradford, 1990), tomato (*Lycopercicon esculentum* Mill.) (Dahal and Bradford, 1990; Cheng and Bradford, 1999), and carrot (*Daucus carota* L.) (Finch-Savage et al., 1998; Rowse and Finch-Savage, 2003). Thermal threshold (Steinmaus et al., 2000) and hydro threshold (Allen et al., 2000a) in the hydrothermal time model are ecologically relevant and the hydrothermal time concept is also useful for understanding physiological variation within a seed population and the threshold responses in seed biology (Bradford, 1990).

The accuracy of the hydrothermal time model, however, depends on the water potential to which seeds are exposed. For example, model fit was low when seeds were germinated at low ψ (Bradford, 1990; Ni and Bradford, 1992). Dahal and Bradford (1994) used two sets of parameters specific for lower (< -0.5 MPa) and higher water potentials (> -0.5 MPa), respectively, and obtained a better model fit. The basic assumptions of the hydrothermal time model are the constancy of $\theta_{HT,}$ θ_H, T_b, and $\psi_{b(50),}$ and the normality of ψ_b distribution among subpopulations (Gummerson, 1986; Bradford, 1990; 1995). However, the constancy of θ_H (Dahal and Bradford, 1990), θ_{HT} (Kebreab and Murdoch, 1999), T_b (Fyfield and Gregory, 1989; Kebreab and Murdoch, 1999), and $\psi_{b(50)}$ (Dahal and Bradford, 1994) has been questioned. Kebreab and Murdoch (1999) indicated the two crucial assumptions of the hydrothermal time model: the independence of $\psi_{b(g)}$ and T_b on temperature and water potential, and the constancy of θ_{HT} in a given seed lot, are invalid or speculated in Egyptian broomrape (*Orobanche aegyptiaca* Pers.). The shift of T_b with water potential in mungbean (*Vigna radiata* L.) (Fyfield and Gregory, 1989) and that of $\psi_{b(50)}$ with temperature in tomato (Dahal and Bradford, 1994) and muskmelon (*Cucumis melo* L.) (Welbaum and Bradford, 1991) have also been reported.

Parameters of the hydrothermal time model are generally determined indirectly and the constancy of parameters is usually assumed and rarely tested. Repeated probit analysis for estimating θ_H and θ_{HT} (Bradford, 1990; 1995; Dahal and Bradford, 1990) is a convenient and practical approach, but the procedure ignores the relationship between germination rate and water potential. Gummerson

(1986) demonstrated when germination rate, $GR_{(g)}$, was plotted as a function of water potential, there was a series of parallel regression lines for different subpopulations in sugar beet (*Beta vulgaris* L.). Kebreab and Murdoch (1999) tested the parallelism of $GR_{(g)}$ on water potential in *Orobanche aegyptiaca* seeds and found a good fit when data were analyzed separately for each temperature. However, there was a clear interaction of temperature and water potential on $GR_{(g)}$, resulting in the low predictability of the hydrothermal time model. In addition, $\psi_{b(g)}$ and its standard deviation ($\sigma_{\psi b}$) are usually calculated or estimated using the equivalent relation: $\psi_{b(g)} = \psi - \theta_H / t_g$, in which t_g is the germination time for a germination subpopulation g (i.e., Dahal and Bradford, 1990; 1994; Kebreab and Murdoch, 1999; Alvarado and Bradford, 2002). Thus, θ_H and related assumptions influence the estimation of $\psi_{b(g)}$ and $\sigma_{\psi b,}$ and subsequently the hydrothermal time model.

Since the hydrothermal time model has only been developed recently, more investigations using additional species and seed lots are required to validate the model assumptions (Bradford, 1995). In this study, we used two seed size classes and two collections of winterfat to test assumptions in the hydrothermal time model and the influence of these assumptions on model predictability. We hypothesize the constancy of θ_H and θ_{HT}, and the independency of T_b and ψ_b on germination condition are affected by temperature and water potential. Modified models that can incorporate the inconstancy of these parameters may improve the predictability of the hydrothermal time model. Specific objectives were: 1) to construct the hydrothermal time model for two seed size classes in two collections of winterfat; 2) to test assumptions of the hydrothermal time model, such as the constancy of θ_H and θ_{HT} and the independence of thermal and hydro thresholds on temperature and water potential; 3) to evaluate and compare model predictability based on different assumptions; and 4) to analyze the variation in parameters of the model between seed size classes and seed collections.

4.2 Materials and methods

4.2.1 Germination at various temperature and water potential regimes

Water potential gradients were created using polyethylene glycol (PEG-6000, EM Science, Germany) solutions (Michel, 1983; Hardegree et al., 1990). Designated water potentials of PEG solutions were 0, -0.25, -0.50, -0.75, -1.0, -1.25, and -1.5 MPa. The actual water potentials of PEG solutions were measured using a vapor pressure osmometer (Model 5100C, Wescor Inc., Logan, UT). The measurement was taken 30 min after PEG solutions penetrated into two layers of filter papers (Whatman No. 1) in a Petri dish as suggested by Hardegree et al. (1990) to take the effect of filter paper on water potential into consideration. The value of water potential was adjusted according to each germination temperature for modeling.

Germination tests were conducted in darkness using six incubators (Sanyo Versatile Environmental Chamber MLR-350H, Sanyo Scientific, USA). Designated temperatures, 2, 5, 10, 15, 20 and 25^{o}C, were randomly allocated to each incubator. Temperatures of incubators were monitored every minute and hourly averages were recorded using dataloggers (21X, Campbell Scientific Inc., USA). Recorded temperatures were used in modeling.

A RCBD with five replicates was used and replicates were started at 7-day intervals. Seeds, 50/unit, were sprinkled in 9 cm Petri dishes with distilled water or PEG solutions on top of two layers of filter papers (Whatman No.1), and Petri dishes were randomized within each chamber block (shelf). Five mL of PEG solution or distilled water were added initially to each Petri dish and an extra 2 mL were added after 24 h and periodically as required. Clear plastic bags were used to seal Petri dishes to reduce water evaporation.

Seeds were considered germinated when either the emerging radicle or cotyledon was = 2 mm. Germination was recorded at 8, 12, 24, or 48 h intervals depending on germination rate for up to 50 days until no germination occurred in 7 consecutive days in all Petri dishes of a replicate. Seed surfaces were cleaned using

cotton with 95 % ethanol whenever there were signs of microorganism development. Germinated seeds and rotten seeds were removed after each counting.

The percentage of viable seeds was estimated at the end of the germination test and adjusted to a scale of 0-100% by dividing final germination percentage with a scaling factor (Hardegree and Van Vactor, 1999). Scaling factors were based on the maximum mean germination percentage achieved among the combinations of water potential and temperature. They were 0.94 and 0.87 for the large and small seeds of collection #63 and 0.93 and 0.87 for that of collection Cela, respectively.

4.2.2 Parameter estimation for the hydrothermal time model

To estimate the $GR_{(g)}$, germination time courses of each temperature, water potential, and replicate were fitted separately using probit analysis procedures as described in (Chapter 3). The base temperature for the 50% subpopulation ($T_{b(50)}$) was estimated using the linear function of $GR_{(50)}$ on temperature at the suboptimal temperature range for each water potential (Dahal and Bradford, 1994). Thermal time for subpopulations of 10, 20, 30, 40, 50, 60, and 70% was estimated as the inverse of slopes of linear regression lines.

The linear function of $GR_{(50)}$ on water potential was used to estimate $\psi_{b(50)}$ at each germination temperature, whichis the x-intercept of the linear regression line. Similarly, the linear functions of a specific *g* fraction on ψ for subpopulation 10, 20, 30, 40, 50, 60, and 70% were used for θ_H estimation. The inverse of the each regression slope was θ_H (Bradford, 1995) for each subpopulation at a given temperature. Data were pooled from all replicates and the regression lines were disregarded when they were not significant ($P > 0.05$).

Because $\psi_{b(g)}$ of slow subpopulations at low temperature and water potential was unavailable, $\sigma_{\psi b}$ was estimated by the slope of linear regression of the probit (g) on calculated $\psi_{b(g)}$ as expressed by Eq. 2.5 (Bradford, 1990; 1995) at each temperature, and estimated $\theta_{H(50)}$ was used in the equation.

4.2.3 Comparison of hydrothermal time models based on different assumptions

The basic hydrothermal time model was based on Gummerson (1986) and Dahal and Bradford (1990, 1994) with Eq. 2.6. According to the assumptions of the hydrothermal time model, germination progress can be described by the normal distribution of $\psi_{b(g)}$ within a seed population in Eq. 2.7. (Dahal and Bradford, 1994; Cheng and Bradford, 1999). The hydrothermal time for 50% subpopulation ($\theta_{HT(50)}$) was used in the hydrothermal time model for simplification even though variation existed among subpopulations. The predictability of the hydrothermal time model (Eq. 2.7) based on different assumptions was tested: 1): T_b and θ_{HT} were assumed constant within a seed population (Gummerson, 1986). Base temperature of the 50% subpopulation ($T_{b(50)}$) at 0 MPa was used as the common T_b for the whole seed population. Base water potential of the 50% subpopulation ($\psi_{b(50)}$) was calculated as the average of x-intercepts of the linear relationships of $GR_{(50)}$ on water potential at all germination temperatures. Since germination rates using θ_T instead of real time are proportional to water potential (Gummerson, 1986), the common θ_{HT} was estimated as the inverse slope of the linear regression of $1/\theta_{T(50)}$ on ψ with Eq. 2.8. 2): $\theta_{HT(50)}$ was allowed to change with temperature and estimated using Eq. 2.6. 3): $\psi_{b(50)}$, $\sigma_{\psi b}$, and $\theta_{HT(50)}$ were allowed to change with germination temperature and $T_{b(50)}$ was allowed to change with water potential. The shift rate of $T_{b(50)}$ with water potential was estimated by the slope of linear regression of the $T_{b(50)}$ on water potential.

4.3 Results

4.3.1 Germination percentage as affected by water potential and temperature

Water potential and temperature both affected the final germination percentage of winterfat, especially when $\psi < -0.50$ MPa and $T < 5^oC$ (Fig. 4.1). The final germination percentage was more sensitive to changes in water potential than temperature. Water potential, temperature and their interactions significantly influenced the final germination percentage ($P < 0.001$ to 0.01). Large seeds of both

collections had higher final germination percentage than small seeds at all combinations of T and ψ, and the differences between seed size classes increased with decreasing water potentials and temperatures. About 54% of viable large seeds germinated at –0.89 MPa and 2°C; only 37% of the small seeds germinated under the same condition of collection #63; and 31% and 24 % of the seeds germinated for the large and small seeds of collection Cela, respectively.

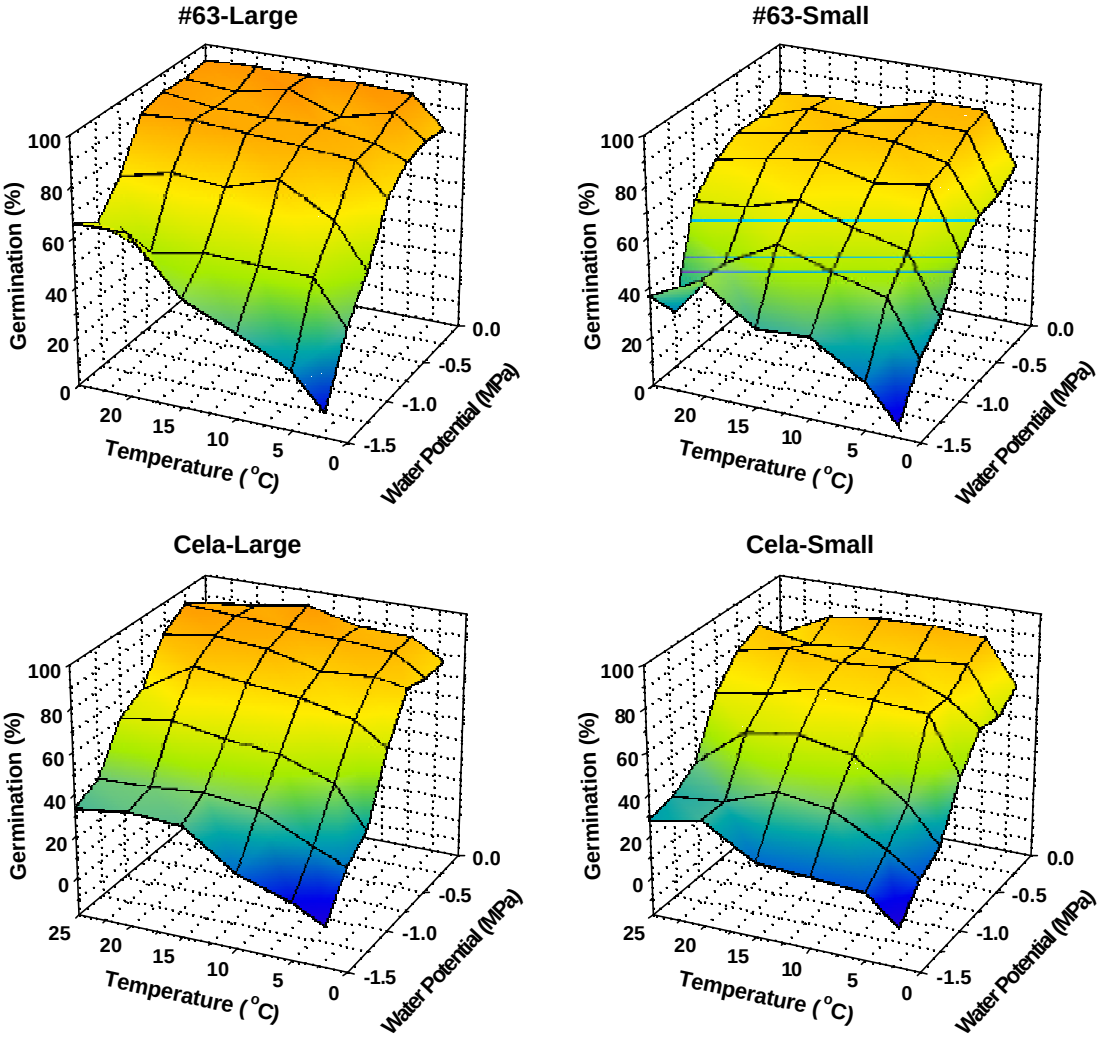


Fig. 4.1. Effects of water potential and temperature on the final germination percentage of two seed size classes and two collections of winterfat.

4.3.2 Effects of water potential and temperature on GR(g), $y_{b(g)}$, and T_b

Germination rates of subpopulations were linearly related to water potential at each temperature ($P < 0.05$, Fig. 4.2). However, these regression lines were not always parallel. Parallelism was found at 20°C, and the regression lines became unparallel or crossed in a common point at the x-intercept at low temperatures (i.e., at 10°C for small seeds of collection #63, Fig. 4.2). Similar patterns were observed in both collections (data not shown for Cela).

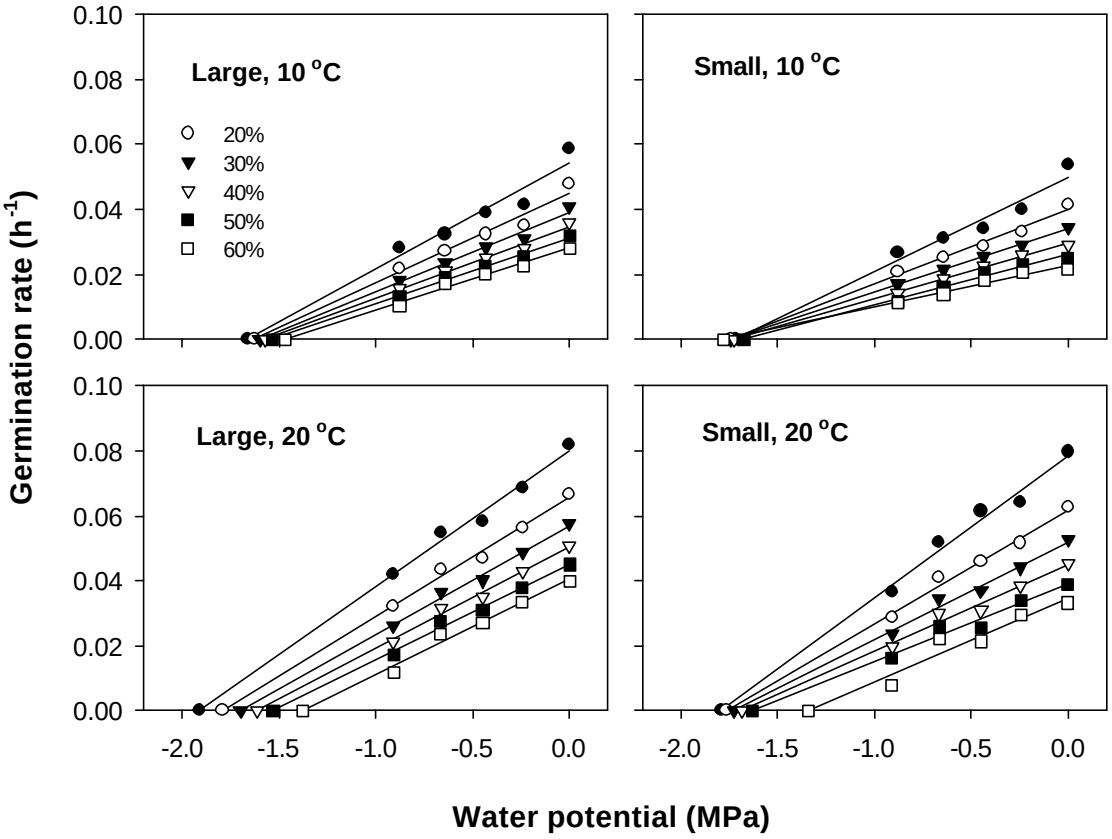


Fig. 4.2. Germination rates (h^{-1}) as a linear function of water potential at various temperatures in two seed size classes with collections #63 of winterfat. Data points at X-interceptions were calculated using linear equations.

Consequently, $\psi_{b(g)}$ estimated from these x-intercepts varied among subpopulations, temperatures, seed size classes, and seed collections (Fig. 4.3). Variation in ψ_b among subpopulations was generally greater at higher temperatures (i.e., 20 or 25°C) than at lower to intermediate temperature (i.e., 2 to 15°C). The variation in $\psi_{b(g)}$ among subpopulations can be as high as 0.98 MPa and the variation among temperatures for a given subpopulation can be as high as 0.37 MPa.

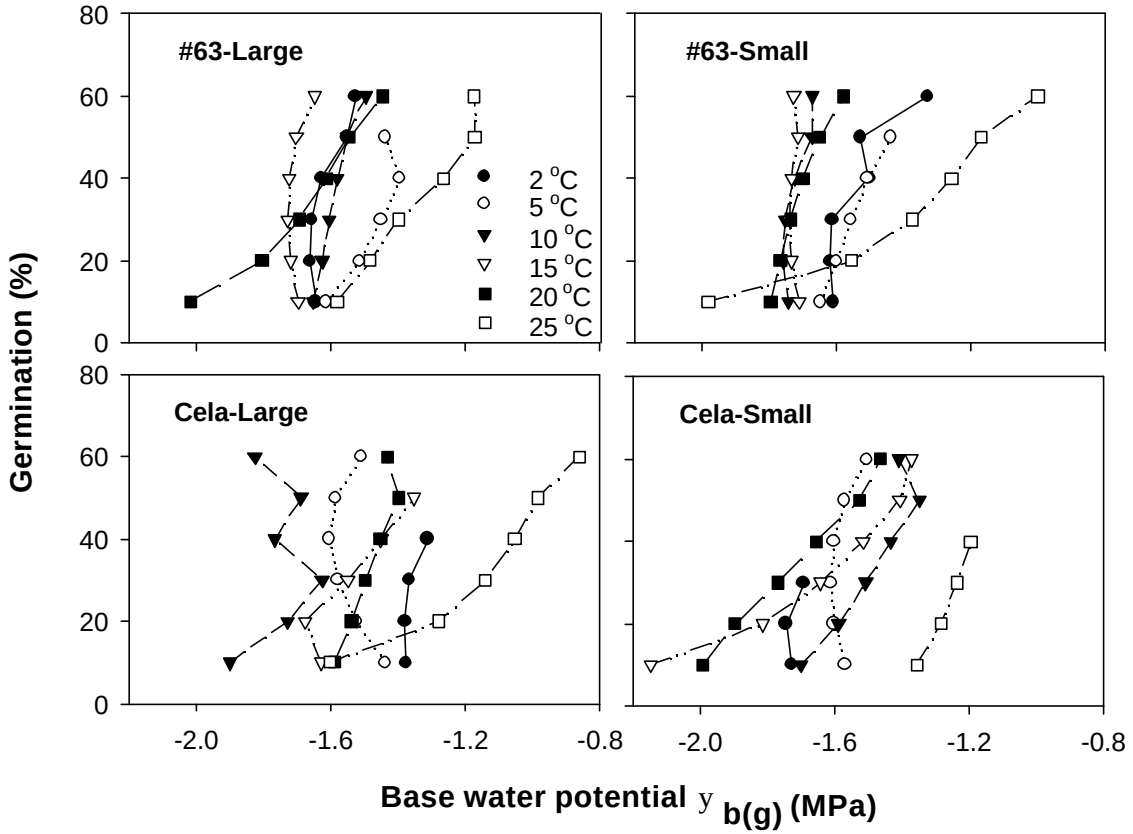


Fig. 4.3. Base water potentials ($\psi_{b(g)}$) among germination subpopulations at temperatures from 2 to 25°C in two seed size classes and two collections of winterfat. The value of $\psi_{b(g)}$ was estimated from pooled data of the X-intercept of the linear relation of $GR_{(g)}$ on water potential.

The estimated $\psi_{b(50)}$ was curvilinearly correlated with temperature in both collections and seed size classes except for small seeds of Cela which exhibited large variability among temperatures (Fig. 4.4). The value of $\psi_{b(50)}$ was lowest at intermediate temperatures (10-15^{o}C) and the difference in $\psi_{b(50)}$ between temperatures was more than 0.5 MPa.

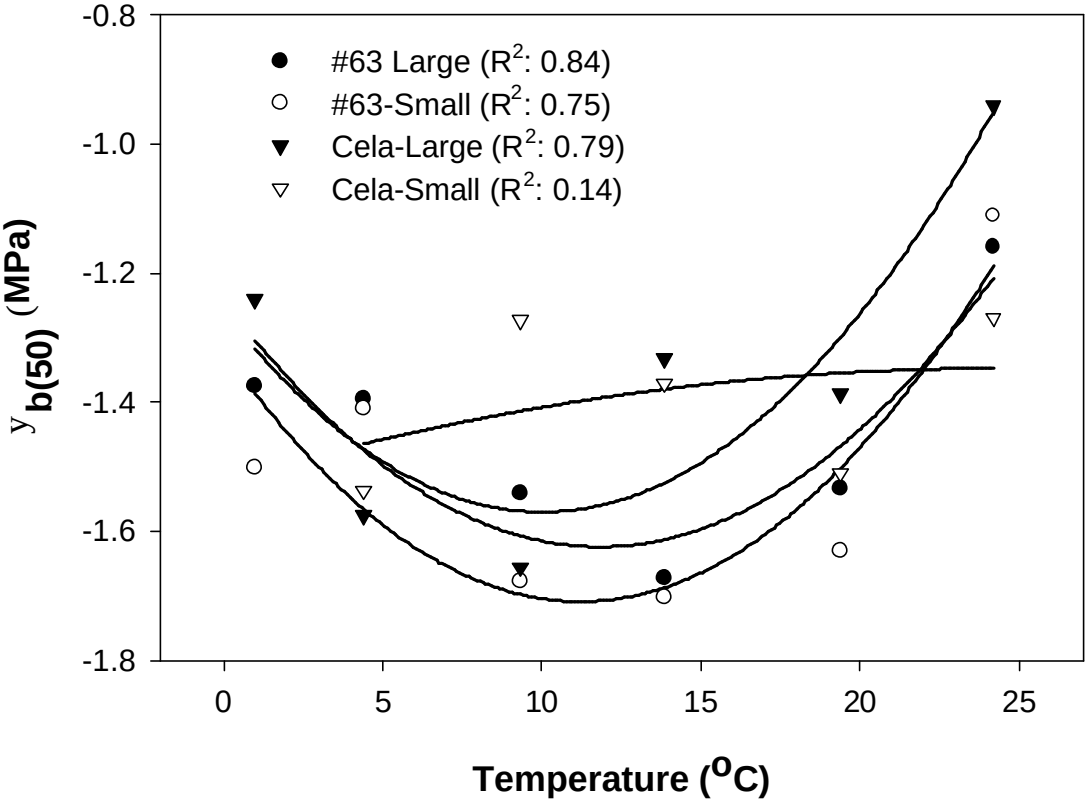


Fig. 4.4. Variation in the base water potential of the 50% subpopulation ($\psi_{b(50)}$) with temperature in two seed size classes and two collections of winterfat. The value of $\psi_{b(50)}$ was estimated by the linear function of $GR_{(50)}$ on water potential at each temperature.

The linear relationship between $GR_{(g)}$ and temperature was altered by water potential, especially for slow germinating subpopulations. The linear temperature range for $GR_{(50)}$ became narrower with decreasing water potential in two seed size classes and collections (Fig. 4.5). The optimum temperature shifted from 20°C at 0 MPa to 10°C at –0.89 MPa. T_b estimation relies on this linear relationship at suboptimal temperatures. The reduced range of suboptimal temperature at lower water potential resulted in fewer data points generating linear regression lines, and subsequently reducing the accuracy of parameter estimation and model predictability.

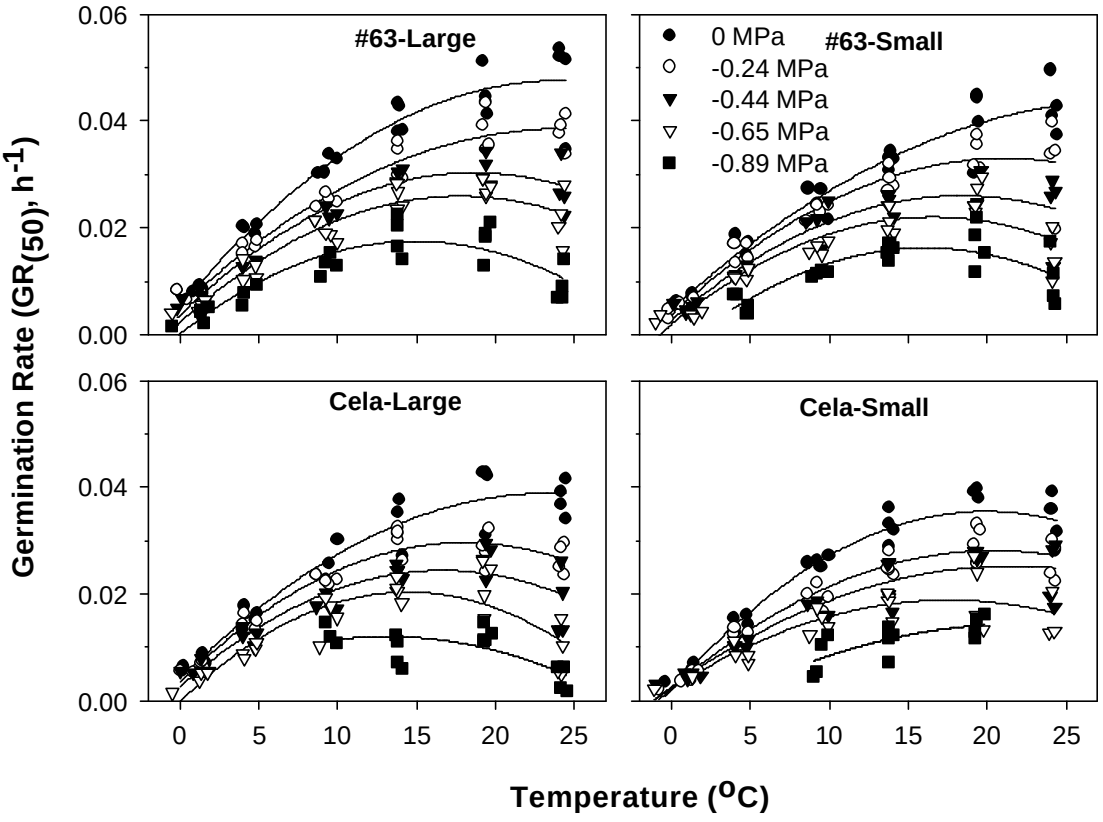


Fig. 4.5. Relationship between $GR_{(50)}$ and temperature as affected by water potential in two seed size classes and two collections of winterfat. Water potentials shown are average values across temperatures, and temperatures were recorded values.

Base temperature was assumed constant among subpopulations for a given water potential. The estimated $T_{b(50)}$ decreased linearly with increasing water potential (Fig. 4.6). This indicates T_b depends on water potential in winterfat and the greater the water availability, the lower the base temperature. The difference in $T_{b(50)}$ within the tested range of water potential can be as high as 3°C. The shifting rate of T_b (mT_b) was estimated from the slope of the linear regression and used to adjust the hydrothermal time model (Table 4.1).

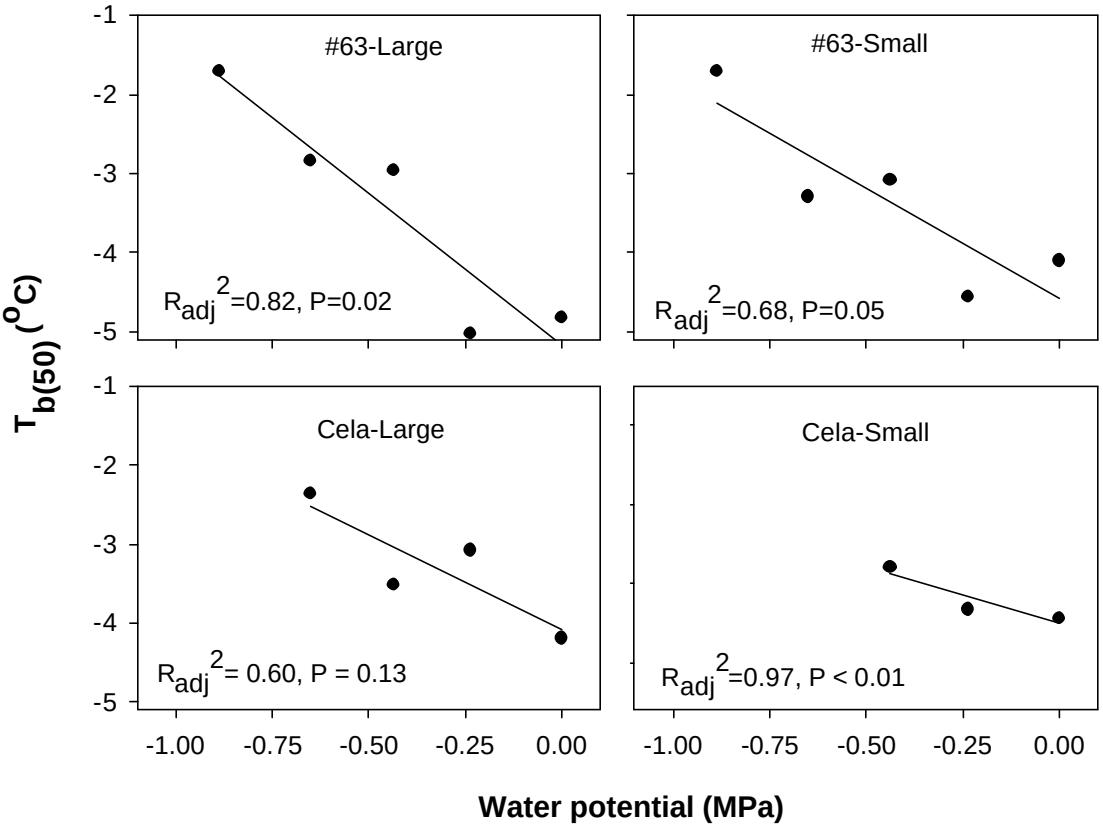


Fig. 4.6. Base temperatures of the 50% subpopulation ($T_{b(50)}$) as affected by water potential in two seed size classes and two collections of winterfat. The value of $T_{b(50)}$ was estimated by the linear function of $GR_{(50)}$ on temperature at each water potential within the suboptimal temperature range.

Table 4.1. Estimated parameters for the construction of the hydrothermal time model in two seed size classes and two collections of winterfat.

Collection	Seed Size	$\theta_{HT(50)}$ (MPa ^{o}C h)[1]	$\psi_{b(50)}$ (MPa)	$\sigma_{\psi b}$ (MPa)	$T_{b(50)}$ (^{o}C)[2]	$mT_{b(50)}$ (°C MPa^{-1})[3]
#63	Large	805	-1.56	0.81	-4.82	3.81
	Small	979	-1.60	1.05	-4.12	2.76
Cela	Large	856	-1.51	0.97	-4.19	2.38
	Small	985	-1.46	0.86	-3.93	2.12

[1] Estimated from the slopes of linear relations of Eq. 2.8 with 50% subpopulation.
[2] Based on germination at 0 MPa.
[3] Estimated using the linear regression of $T_{b(50)}$ on water potential.

4.3.3 Constancy of hydro time (q_H), thermal time (q_T), and hydrothermal time (q_{HT}) at reduced water potential

Hydro time (θ_H), estimated from the inverse of the slope of the linear function of $GR_{(g)}$ on water potential, was not always constant among subpopulations, and the constancy varied with temperature (Fig. 4.7). Hydro time was relatively constant among subpopulations when temperatures were above 10^{o}C, but it increased with increasing germination fractions or subpopulations when temperature was lower than 10^{o}C, especially at 2^{o}C. Generally, θ_H increased with decreasing temperature for a given subpopulation. Hydro time for the 50% subpopulation ($\theta_{H(50)}$) increased with decreasing temperature and the rate of increase was greater at low temperatures (2 to 10^{o}C) than the moderate to high temperatures (Fig. 4.8). Therefore, the assumption of θ_H being constant in the hydrothermal time model was invalid at low temperatures for winterfat seeds. In general, large seeds had less θ_H requirement than that of small seeds and differences in θ_H between seed size classes were greater at low temperatures. Small seeds of collection #63 required about 60 MPa h more hydro time than large seeds at 2^{o}C. Collection #63, originated from a dryer site and required less θ_H than collection Cela, especially at low temperatures.

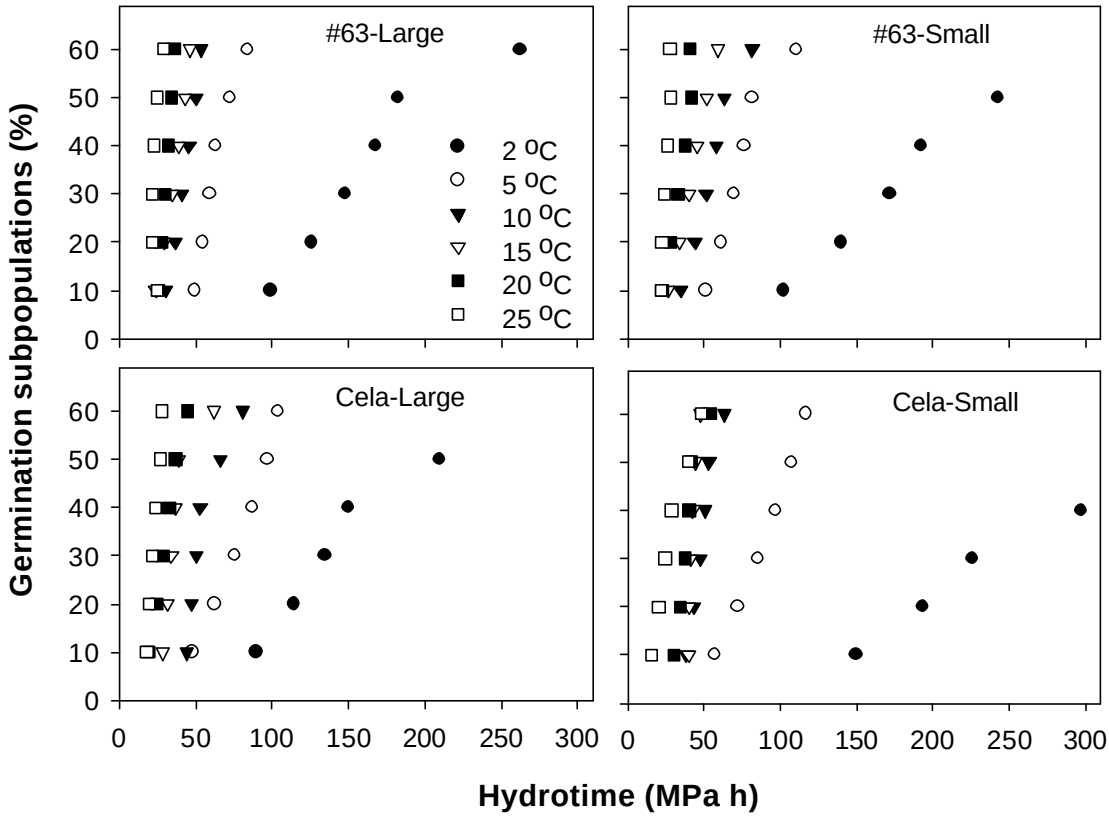


Fig. 4.7. Variation of hydro time (θ_H) among subpopulations at various temperatures in two seed size classes and two collections of winterfat. Hydro time was estimated from the slope (θ_H=1/slope) of the linear function of $GR_{(g)}$ on water potential with pooled data.

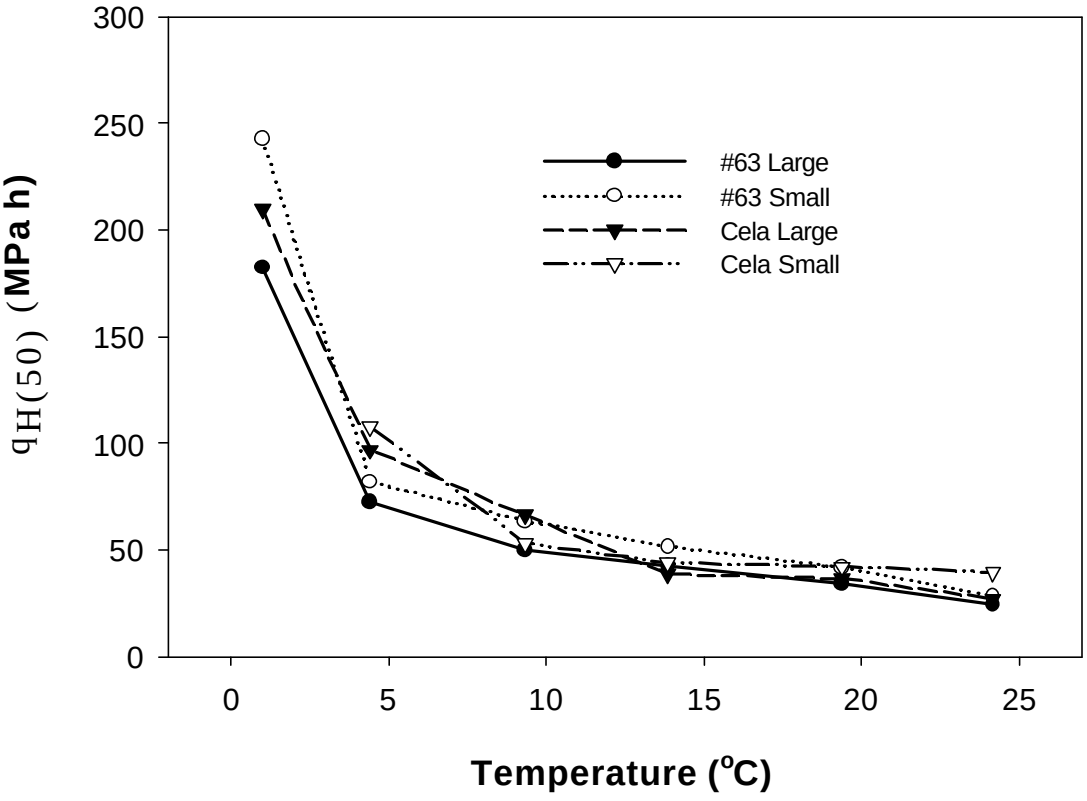


Fig. 4.8. Effect of temperature on hydro time of the 50% subpopulation ($\theta_{H(50)}$) of two seed size classes and two collections of winterfat. The value of $\theta_{H(50)}$ was estimated from the slope (θ_H=1/slope) of linear function of $GR_{(50)}$ on water potential with pooled data.

Thermal time ($\theta_{T(g)}$) increased linearly with decreasing water potential ($P < 0.05$ except for the large seeds of Cela for which $P = 0.09$, data not shown), similar to the relationship between $T_{b(50)}$ and water potential. The rate of change in $\theta_{T(50)}$ with water potential was about 300 to 400°C h per MPa (data not shown). The linear regression lines between $1/\theta_{T(g)}$ and water potential among subpopulations were generally parallel (data not shown). Hydrothermal time (θ_{HT}), which was estimated using the inverse of the slopes of regression lines, was considered constant among subpopulations. Large seeds had smaller $\theta_{HT(50)}$ than the small seeds in both collections (Tables 4.1, 4.2). Similar to the response of $\psi_{b(50)}$ to temperature, $\theta_{HT(50)}$ was lower at intermediate temperatures than either lower or higher temperatures (Table 4.3).

Table 4.2. Variation in $\theta_{HT(50)}$ with temperature in two seed size classes and two collections of winterfat. Values were calculated using Eq. 2.6 using $T_{b(50)}$ and $\psi_{b(50)}$ as listed in Table 4.1.

Temperature	#63		Cela	
(°C)	Large	Small	Large	Small
2	1070 a[1]	1184 a	1039 a	1330 a
5	657 c	792 c	753 c	808 c
10	668 c	756 c	682 c	837 bc
15	664 c	785 c	809 bc	838 bc
20	794 b	900 b	927 ab	936 b
Mean	773 B[2]	871 A	843 B	922 A

[1] Values with the same lower case letters within a column were not significantly different at $P = 0.05$.

[2] Values with the same capital letters within a collection were not significantly different at $P = 0.05$.

Table 4.3. Variation in $\psi_{b(50)}$ (MPa) and $\sigma_{\psi b}$ (MPa) with temperature in two seed size classes and two collections of winterfat.

Temperature	#63				Cela			
(^{o}C)	Large		Small		Large		Small	
	$\psi_{b(50)}$	$\sigma_{\psi b}$	$\psi_{b(50)}$	$\sigma_{\psi b}$	$\psi_{b(50)}$	$\sigma_{\psi b}$	$\psi_{b(50)}$	$\sigma_{\psi b}$
2	-1.55	0.97	-1.53	1.41	-1.55	1.10	--[1]	--
5	-1.44	0.64	-1.44	0.78	-1.59	0.88	-1.57	0.92
10	-1.55	0.76	-1.67	0.96	-1.69	0.89	-1.35	0.71
15	-1.70	0.87	-1.71	1.04	-1.35	0.91	-1.41	0.74
20	-1.55	0.81	-1.65	1.06	-1.40	1.05	-1.53	1.07
Mean	-1.56	0.81	-1.60	1.05	-1.51	0.97	-1.46	0.86

[1] Germination did not reach 50 %.
[2] Values were estimated as X-intercepts of the linear regression of GR_{50} on water potential at each temperature.

4.3.4 Model predictability as affected by parameter assumptions

Hydrothermal time models were constructed using parameters based on different assumptions as listed in Tables 4.1, 4.2, and 4.3 and model predictability was measured by the modified R^2. When all parameters were assumed constant (M 1), model predictability was higher (R^2 >0.70) at moderate water potential range than at higher or lower water potentials at 5 or 20^{o}C (Fig. 4.9). Model predictability was generally reduced when ψ < -0.63 MPa at 5^{o}C and when ψ < -1.20 MPa at 20^{o}C. The predictability was lower at low water potential range and low temperature (i.e., 2^{o}C, data not shown). When θ_{HT} was allowed to vary with temperature (M 2), model predictability was improved at low temperatures and high water potentials, indicating that changes in θ_{HT} with temperature have an important impact on model predictability in winterfat (Fig. 4.10). When changes in T_b with ψ and changes in θ_{HT}, $\psi_{b(50)}$, and $\sigma_{\psi b}$ with temperature were allowed (M 3), model predictability was improved from M 1 but not from M 2 (data not shown). Germination was highly sensitive to the reduced water potential and was highly

correlated with temperature (Fig. 4.10). When ψ decreased by about 0.06 MPa, the germination time course had a minimal difference at 20°C but a great impact at 5°C.

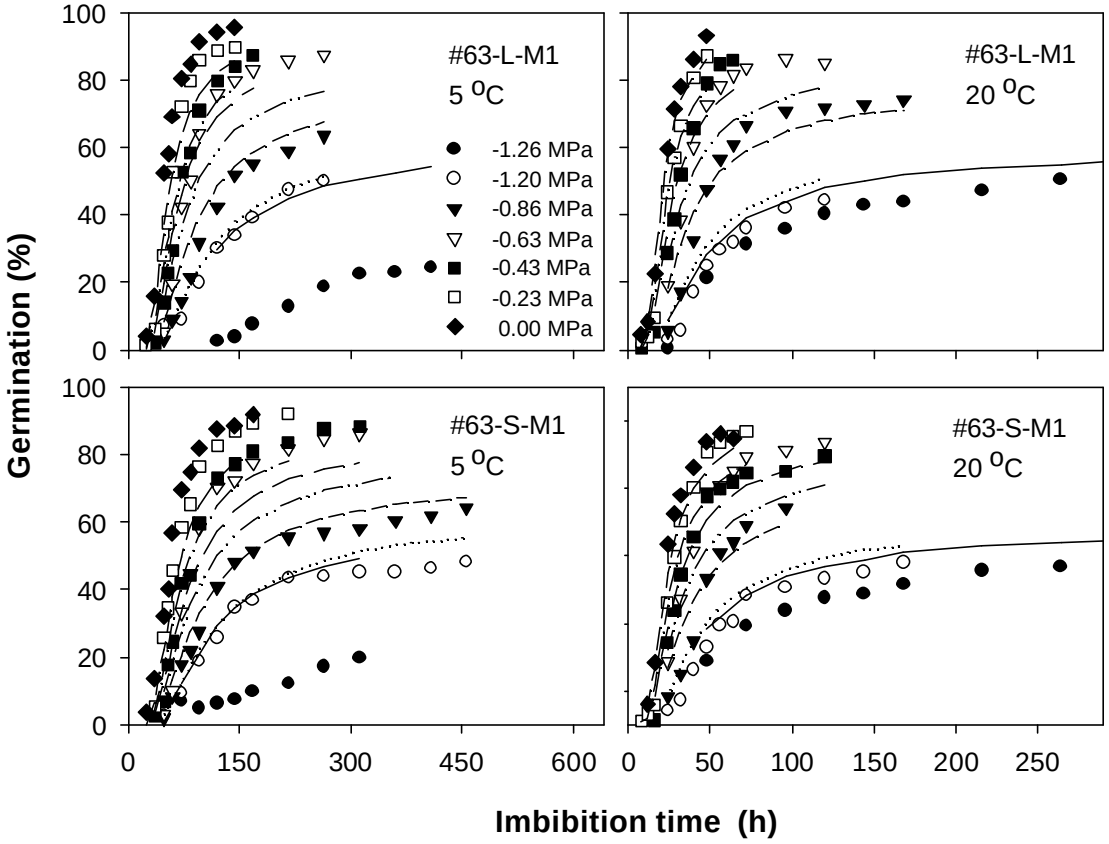


Fig. 4.9. The observed (symbol) and predicted (line) germination time courses of collection #63 of winterfat at various water potentials at 5 and 20°C when all parameters were assumed constant. Water potentials at 20°C were 0.01-0.07 MPa lower than that of the same solutions at 5°C.

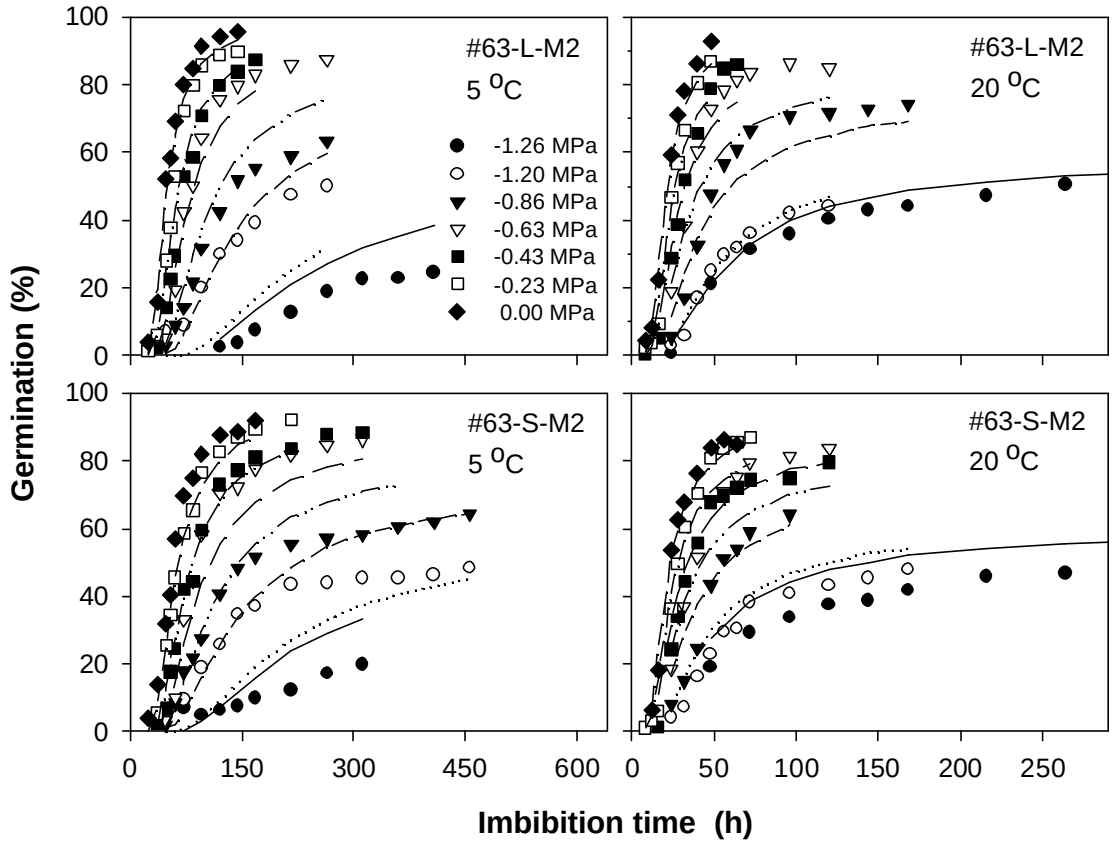


Fig. 4.10. The observed (symbol) and predicted (line) germination time courses of collection #63 of winterfat at various water potentials at 5 and 20°C when $\theta_{HT(50)}$ was allowed to vary with temperature. Water potentials at 20°C were 0.01-0.07 MPa lower than that of the same solutions at 5°C.

4.4 Discussion

Reduced water potential lowered germination rate and final germination percentage in winterfat, similar to other species (Fyfield and Gregory, 1989; Dahal and Bradford, 1990; Shrestha et al., 1999; Grundy et al., 2000). The interactive effect of water potential and temperature on germination rate and percentage of winterfat has also been reported in other species (Ni and Bradford, 1992; Kebreab and Murdoch, 1999; Shrestha et al., 1999). The hydrothermal time model relies on germination rate rather than final germination percentage. However, reduced model fit often occurs at reduced water potentials where germination percentage is low (Dahal and Bradford, 1994). Attempts have been made to include final germination percentage in the hydrothermal time model at reduced water potentials (Grundy et al., 2000), but may be inappropriate (Alvarado and Bradford, 2002; Bradford, 2002). Dahal and Bradford (1990) used final germination percentage to estimate minimal ψ for 50% germination at a constant temperature and obtained a similar result to the $\psi_{b(50)}$ estimated from the x-intercept of GR regression on water potential. However, the observed final germination percentage at a given water potential should be lower than the potential or theoretical final germination since low GR may not be able to complete radicle emergence. As a result, $\psi_{b(g)}$ estimated from the final germination percentage should be higher than the theoretical estimation based on germination rate, which is validated by our results. The differences of minimal ψ estimated from final germination and from germination rate were greater from early to late subpopulations. Thus, final germination and germination rate respond to environmental conditions independently, as reported elsewhere (Bradford, 1995; 2002). The optimum temperatures for final germination percentage were lower than rate optimum in *Orobanche aegyptiaca* (Kebreab and Murdoch, 1999).

The standard deviation of final germination percentage increased with decreasing water potential (data not shown), as well as the sensitivity of germination time course to reduced water potential, especially when water potential was less than –0.50 MPa. The seed-to-seed variation of sensitivity to reduced water potential may lead to the low predictability of the hydrothermal time model. The germination time

courses were more sensitive to reduced water potentials at low temperatures than at high temperatures. The sensitivity to low ψ may be under physiological control (Dahal and Bradford, 1990; Welbaum and Bradford, 1991) or due to the physiological adjustment of seeds to conditions near thermal and/or water potential threshold. Embryo cell turgor and cell wall expansion are required to complete radicle emergence or embryo expansion in seed germination. Since the embryo of winterfat is not enclosed by the endosperm (Booth, 1988), there is no barrier from surrounding tissues during germination. By contrast, endosperm weakening in tomato is the primary determinant at reduced ψ during germination (Foolad and Jones, 1991). However, cell wall modification during germination may be the common process in both species because hormones, such as GA (Chen et al., 2002), are involved in the endosperm cup weakening through regulating cell wall expansin (Chen and Bradford, 2000) and cell wall hydrolases (Chen et al., 2002; Mo and Bewley, 2003). If seeds have adjusted or modified their physiological processes according to germination conditions, variation of hydrothermal model parameters may occur since this model has a strong physiological relevance (Meyer et al., 2000; Alvarado and Bradford, 2002).

The hydrothermal time model assumes a fixed set of model parameters under any germination condition in a seed population (Gummerson, 1986). This approach simplifies the modeling process with relative constant germination responses under varied environmental conditions. However, these parameters were variable with germination conditions (e.g., Kebreab and Murdoch, 1999; Alvarado and Bradford, 2002). Bradford (2002) illustrated the parameter adjustment for the hydrothermal time model according to seed physiological status, priming effects and germination initiators. The present study demonstrated parameters of the hydrothermal time model were highly variable with germination conditions and with seed sizes within a seed collection in winterfat. Estimated thermal and hydro thresholds for seed germination of winterfat both shifted with water potential and temperature, an interactive effect of temperature and water potential also influenced the variation of these parameters. Therefore the basic assumption of the hydrothermal time model, constant thresholds, is invalid in winterfat. Since T_b linearly shifted to higher values

with reducing water potential, it can be adjusted by incorporating water potential effect ($T_{b(\psi)} = T_{b(0)} + mT_b\psi$). The $\psi_{b(50)}$ of winterfat varied with temperature, but the magnitude of change was not as high as in other species (Bradford, 1995; Kebreab and Murdoch, 1999). The quantification of $\psi_{b(g)}$ shifting with temperature was developed at supraoptimal temperature range in potato (*Solanum tuberosum* L.) seeds (Alvarado and Bradford, 2002), carrot and onion (Rowse and Finch-Savage, 2003).

The θ_H was not constant among subpopulations at low temperatures in winterfat, indicating the alteration of water relations at low temperatures. Hydro time determines germination rate at a given temperature, which is a temperature specific parameter (Kebreab and Murdoch, 1999). Large increases in θ_H with decreasing temperature have been reported in many other species (Dahal and Bradford, 1994; Kebreab and Murdoch, 1999; Alvarado and Bradford, 2002). However, the variation of θ_H was not only associated with decreasing temperature, but also with subpopulations in winterfat. The θ_H for late subpopulations was generally high at low temperatures, which may limit germination at low water potentials and low temperatures. The inconsistency of θ_H indicates the estimation of $\sigma_{\psi b}$ is problematic because the $\sigma_{\psi b}$ is usually estimated with calculated $\psi_{b(g)}$ values from Eq. 2.5. Large seeds generally had a lower θ_H requirement than that of small seeds and the differences were greater at low temperatures in both collections. The θ_H can be an indicator of seed physiological quality or vigor in a seed lot and genetic differences among genotypes where $\psi_{b(g)}$ is not changing markedly (Dahal and Bradford, 1990). Physiological advancement of the priming treatment was due primarily to a smaller θ_H requirement, and aged tomato seeds have a higher θ_H value than freshly harvested seeds (Dahal and Bradford, 1990). Low θ_H requirement, especially at low temperature in large seeds of winterfat, shows advantages that large seeds have for germinating under lower temperatures.

Gummerson (1986) tested the linear relationships of $1/\theta_{T(g)}$ on ψ for subpopulations from 5% to 60% in sugar beet (*Beta vulgaris* L.) and found a common slope, thus a constant θ_{HT} was assumed. A similar assumption was adopted

in θ_H with a common slope for linear regression of $GR_{(g)}$ on ψ among subpopulations (Dahal and Bradford, 1990). In the present study, greater parallelism of these regression lines was found in θ_{HT} than in $\theta_{H,}$ but variation existed in both. Hydrothermal time varied among germination subpopulations when single ψ_b (i.e., $\psi_{b(50)}$) was assumed for different subpopulations (Shrestha et al., 1999; Grundy et al., 2000), indicating this may be an inappropriate approach as explained by Bradford (2002). Mathematically from Eq. 2.8, the $1/\theta_{T(g)}$ at reduced ψ is influenced by the variation of θ_{HT} and $\psi_{b(g)}$. The θ_{HT} also varied according to temperature in winterfat. The θ_{HT} in winterfat was estimated from either the slope of linear relations of $1/\theta_{T(g)}$ on ψ, or Eq. 2.6. The θ_{HT} was significantly different among temperatures and seed size classes in both collections from the estimation of Eq. 2.6. The θ_{HT} for large seeds was lower than small seeds using both estimation methods. Since large and small seeds have a similar θ_T requirement at 0 MPa (Chapter 3), the difference in θ_{HT} likely resulted from the differences in θ_H. In addition, the linear relation between GR and temperature was also changed by the reduced ψ, especially for late subpopulations, affecting the accurate estimation of T_b and θ_T in the hydrothermal time model. The altered linear relationship of $GR_{(50)}$ on temperature at reduced water potentials in winterfat is similar to that in carrot and onion (Rowse and Finch-Savage, 2003). The temperature range for linearity was reduced at low water potentials, so was the optimal germination temperature, indicating that winterfat has great plasticity over different environmental conditions at the germination stage. The great plasticity in native plants over a wide range of environments shows adaptive responses to their respective habitats (Bradshaw, 1965; Allen and Meyer, 2002). Germination phenology of *Bromus tectorum* L. populations from less predictable environments (e.g., cold desert) showed greater variation than that from more predictable, extreme environments (Allen and Meyer, 2002).

The general model (M 1) with a set of fixed parameters had the lowest predictability among three models in most temperature and water potential combinations. The predictability of general model (M 1) was high only in a

narrower water potential and temperature range in winterfat. In two tomato genotypes, similar models accounted for 75% of the variation in germination time (Dahal and Bradford, 1994). Species adapted to natural and unpredictable conditions are more likely able to adjust germination response to their environment than crop species that are grown under optimal cultivation conditions. Adjustment and modification of model parameters according to germination conditions improved the predictability of the hydrothermal time model at reduced water potential. The adjustment of θ_{HT} to temperature had a large impact and improved the model predictability. Dahal and Bradford (1994) used two sets of parameters for higher or lower water potentials, respectively and obtained a better model fit, which showed that θ_{HT} was not constant for any combination of temperature and water potential. The model with more parameter adjustments (M 3) did not further improve the fit under most conditions. The inconsistency of θ_H at low temperatures may cause the incorrect estimation of $\sigma_{\psi b}$. The increment of θ_H among subpopulations at low temperatures gives an over estimates of the $\sigma_{\psi b}$ from Eq. 2.7. It was demonstrated that the variation of $\psi_{b(g)}$ in low temperatures was smaller than higher temperatures from the $\psi_{b(g)}$ estimation of x intercepts (Fig. 4.3), but not in the estimation from Eq. 2.7 (Table 4.3). The parameter adjustment with subpopulations should further improve the model predictability, but over parameterization may lose the advantage of the simplification of hydrothermal time model.

In summary, water potential influenced germination rate and final germination percentage in winterfat. The hydrothermal time model detected the physiological adjustment during seed germination under reduced water potentials and variable temperatures. Basic assumptions of hydrothermal time, such as the constancy of model parameters, are invalid for seed germination of winterfat. Model parameters varied with water potentials, temperatures and the seed sizes within a seed collection. Thermal and hydro threshold both varied with germination conditions, and θ_{HT} was altered by low temperature. Hydro time was not just a temperature-specific parameter because variation among subpopulations at low temperature was present. The altered θ_H may lead to incorrect estimations of $\psi_{b(g)}$ and $\sigma_{\psi b}$ at low temperatures if calculated using a constant θ_H. Large seeds had

lower requirements of θ_H and θ_{HT} than small seeds, which may result in faster germination of large seeds under low temperature and low water potentials in winterfat. The general model (M 1) had the poorest model fit among tested models due to the assumption of constant parameters. The adjustment of θ_{HT} according to temperature improved the predictability of the hydrothermal time model, while the inclusion of T_b variation with water potential improved predictability at low water potentials. The non-linear relations of $GR_{(g)}$ with temperature at reduced water potentials may also account for reduced model fit at reduced water potentials.

5. Predicting Seedling Emergence in the Field Using the Constructed Hydrothermal Time Models

Abstract Thirteen seedbed treatments were created with 2 to 3 sowing dates and irrigation levels in the field over two years to quantify the effect of soil temperature and soil water potential on seedling emergence. Seedling emergence ranged from 3% to 69% of viable seeds in these field conditions. Watmer seedbed temperatures associated with late sowing dates greatly reduced seedling emergence. An exponential relation of thermal time requirements between seed germination and seedling emergence was established. The constructed hydrothermal time model had variable accuracy in predicting field emergence, depending on soil temperature and soil water potential. The correlation between predicted and observed emergence can be as high as $R^2 = 0.79$. Generally, the hydrothermal time model fits well for emergence when soil temperatures are 10 to 15^{o}C and soil water is > -0.5 MPa. These seedbed conditions occur in the spring when snow melts, favouring seedling emergence of winterfat. Results from this study provide practical information for seeding dates and seedbed conditions in restoration using winterfat.

5.1 Introduction

Seedling emergence is controlled by species-specific requirements and the availability of favorable seedbed conditions (Harper, 1977). Seedbed conditions including soil temperature, soil water potential, soil air quality and soil compaction affect seed germination, seedling elongation, seedling emergence, and seedling mortality (Benech-Arnold et al., 1990; Finch-Savage et al., 1998; Whalley et al., 1999; Roman et al., 1999; Shrestha et al., 1999; Forcella et al., 2000). These factors can be modulated by litter residues on the soil surface and management practices (Vleeshouwers and Kropff, 2000; Romo, 2004). In addition, interactions among climatic conditions, soil, seeds and seedling characteristics on seedling emergence

are complex under field conditions (Whalley et al., 1999; Vleeshouwers and Kropff, 2000).

Environmental conditions directly surrounding a seed determine germination success and subsequent seedling emergence and establishment (Harper, 1977). Soil temperature and soil water potential are major driving factors for seed germination and seedling emergence (Gummerson, 1986; Finch-Savage et al., 1993; Dahal and Bradford, 1994). The hydrothermal time model is based on temperature and water potential and provides a theoretical framework for quantifying their effects on seed germination and seedling emergence under controlled and natural environments (Allen et al., 2000; Bradford, 2002; Allen, 2003; Hardegree et al., 2003). Although there are some successes in predicting seedling emergence using the hydrothermal time model under variable field conditions (Finch-Savage et al., 1993; 1998; Grundy et al., 2000; Roman et al., 1999; 2000), direct application for field conditions is still limited. Extreme seedbed conditions, created by soil mechanical impedance and reduced oxygen supply, can have overriding effects on seedling emergence (Whalley et al., 1999; Kolb et al., 2002). Disparities between seed germination and field emergence are common even under similar temperature regimes (Romo and Young, 2002).

Pre-germination and post-germination events both affect seedling emergence processes such as radicle protrusion, seedling elongation, and seed and seedling mortality. At the post-germination stage, the physical environment of the soil (Roman et al., 1999; Shrestha et al., 1999; Whalley et al., 1999), seed reserves (Finch-Savage et al., 2001) and the depth of seed burial (Prostko, et al., 1998; Boyd and Acker, 2003) influence seedling emergence percentage, emergence rate and emergence time. Establishing solid, functional relationships between germination rate and environmental factors should be fulfilled when attempting to predict seedling emergence using models (Benech-Arnold, 1990), and environmental factors must be studied in detail.

Seedbeds in the Northern Mixed Prairie are characterized by high fluctuations in temperature and soil water potential, especially at the soil surface during the spring. High seedling mortality is identified as a major limitation for establishing

winterfat from direct seeding (Booth, 1992; Hou and Romo, 1997; Garvin et al., 2004). Objectives of this study were to: 1) determine the effect of soil temperature and soil water potential on seedling emergence of winterfat under various seedbed conditions in the field created by years, sowing dates and irrigation treatments, and 2) to test the predictability of a laboratory-developed hydrothermal time model on seedling emergence in the field. We hypothesized that soil temperature and soil water are the primary factors controlling the emergence of winterfat seedlings in the field.

5.2 Materials and methods

5.2.1 Site description

Field emergence studies were conducted at the research farm of Agriculture and Agri-Food Canada, Saskatoon Research Centre. The soil texture is clay and the contents of sand, clay and silt in the soil were 23%, 45% and 33% respectively. The field capacity was 66%, and the soil bulk density was 1.14 g/cm^3. Field plots were prepared into similar soil conditions and the site was free of tested species before sowing.

5.2.2 Seedling emergence under field conditions

Seeds were sown on May 2 and May 29, 2003, and on May 8, May 20 and June 3, 2004. Two irrigation treatments, natural precipitation and daily irrigation, were applied in 2003. Three irrigation treatments, including natural precipitation, irrigation once daily and irrigation twice daily were applied in 2004. Irrigation plots were watered once or twice daily with a maximum of 10 mm each time unless there was a significant precipitation event (>2 mm) in the previous 24 hours. A RCBD with 5 replicates (blocks) was used and the plot size was 1.0×1.0 m. There was a 1-meter spacing between plots. Two hundred seeds in 2003 and 100 seeds in 2004 seeds were hand-planted at a 1 cm depth in 4 rows within each plot.

Soil temperatures at the sowing depth (1 cm) were measured every minute using dataloggers (21X, Campbell Scientific Inc., USA). The average hourly soil

temperature was used for thermal time calculation. Air temperatures and rainfall were also recorded at the Saskatoon Research Center weather station at the research farm. Soil samples in the top 2-cm were collected on alternate days unless it was raining. Soil water potential of five soil samples was measured using a water potential meter (WP4 Dewpoint PotentiaMeter, Decagon Devices, Inc. Pullman, WA, USA) in 2003. Soil water content was determined gravimetrically after oven-drying at 100°C for 48 h in 2003 and 2004. Soil water potential in 2004 was derived from a nonlinear Hyperbola function using soil water content:

$$Y = a-b / (1+c*X)^{1/d} \qquad \text{(Eq. 5.1)}$$

where a, b, c, and d are constants, X is percent soil water content, and Y is soil water potential (MPa). Eq. 5.1 was developed using soil water content and water potential data for 2003 (Fig. 5.1).

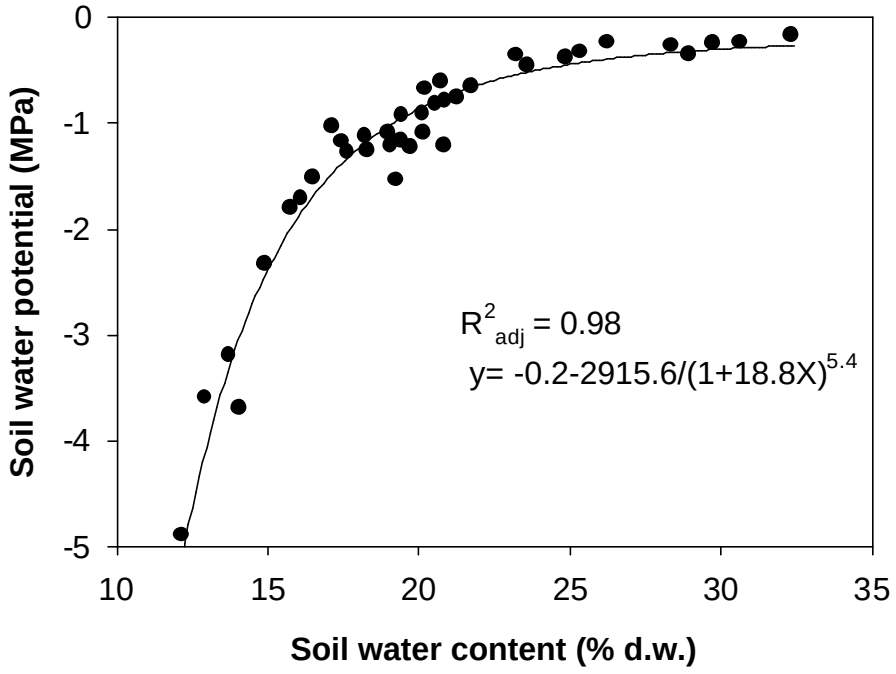


Fig. 5.1. Correlation of soil water content and soil water potential for soil from field experiment plots. Soil water content was measured gravimetrically (100°C, 48 h) and water potential was measured using a water potential meter using the same soil sample.

Seedling emergence was monitored daily during peak periods and at 2-day intervals during non-peak periods. Seedlings that emerged from the soil were counted and removed after each counting.

5.2.3 Comparison between seed germination and seedling emergence

A growth chamber experiment was designed to establish the relationship between seed germination from Petri dishes and seedling emergence from soil. Seed germination from Petri dishes and seedling emergence from pots filled with soil collected from field plots were compared in growth chambers (Conviron PGR15, Controlled Environments Limited, Canada) at constant temperatures of 10 and 20^{o}C in dark. Soil was passed through a 3.35 mm (No. 6) sieve to remove litter and large particles before placing the soil in 10 x 10 x 8 cm pots. Water was soaked into pots from a tray, covered with the tray cover, and put in growth chambers for 2 days for temperature equilibrium before sowing. Five replications of 50 seeds per unit were used with the two seed collections and the two seed size classes. Seeds were imbibed in Petri dishes on top of filter paper (Whatman No. 1) or at 1 cm depth of soil in pots. Water was periodically added to the trays and Petri dishes as required. Germination and emergence were checked daily for 30 days.

5.2.4 Data analyses

The relationship between seed germination from Petri dishes and seedling emergence from soil in the growth chamber was established based on differential thermal time requirements to achieve the same percentage. This relationship was best described by an exponential equation:

$$Y = a*e^{bX} \qquad \text{(Eq. 5.2)}$$

where Y is the thermal time for seedling emergence from soil ($\theta_{T(E)}$), X is the thermal time for seed germination from Petri dishes ($\theta_{T(G)}$), and a and b are constants.

Since soil water potential in irrigated and natural precipitation plots was below 0 MPa, a hydrothermal time model was used to predict seedling emergence under field conditions. Thermal time or hydrotime accumulation was assumed to be 0 when soil water potential or soil temperature was below their germination

thresholds (Finch-Savage and Phelps, 1993; 1998) or above the optimum temperature. The average soil water potential during seedling emergence was used and Eq. 5.2 was incorporated into the following hydrothermal model:

$$\text{Probit (g)} = [\psi-(\theta_{HT(50)}/\theta_{T(G)})- \psi_{b(50)}]/ \sigma_{\psi b} \qquad \text{(Eq. 5.3)}$$

where θ_{HT50} is the thermal time requirement for achieving 50 % germination, $\theta_{T(G)}$ is the thermal time requirement for germination, $\psi_{b(50)}$ is the base water potential for 50 % germination, $\sigma_{\psi b}$ is the standard deviation of base water potential for all germination subpopulations, and Probit (g) is germination percentage in probit scale (Dahal and Bradford, 1994; Finch-Savage et al., 1998). These parameters were previously estimated and varied between seed collections and between seed size classes (Chapter 4). The model predictability was evaluated by the R^2 that was calculated as 1-($SS_{residual}$ /SS_{total}), while $SS_{residual}$ = (observed value – predicted value) and SS_{total} = (observed value – observed mean) (Chapter 3).

Mean maximum seedling emergence in the field was scaled by the mean maximum germination percentage in Petri dishes for each seed collection and each seed size class, which ranged from 78 to 88 %. Percentage data were arcsine transformed. GLM procedure in SAS® Windows version 8.2 was used to analyze treatment differences at a significance level of 0.05. Software SigmaPlot® was used for linear and non-linear regression analyses.

5.3 Results

5.3.1 Comparison of seedling emergence from soil and seed germination in Petri dishes

Seedling emergence of winterfat from the 1 cm depth in soil in the growth chamber required more thermal time accumulation than seed germination in Petri dishes (Fig. 5.2). Most seeds (> 80%) germinated in Petri dishes at 50 degree days at that time seedling emergence from soil had just begun. The final percentage of seedling emergence was 40 to 64% lower compared to the final germination percentage at the same thermal time.

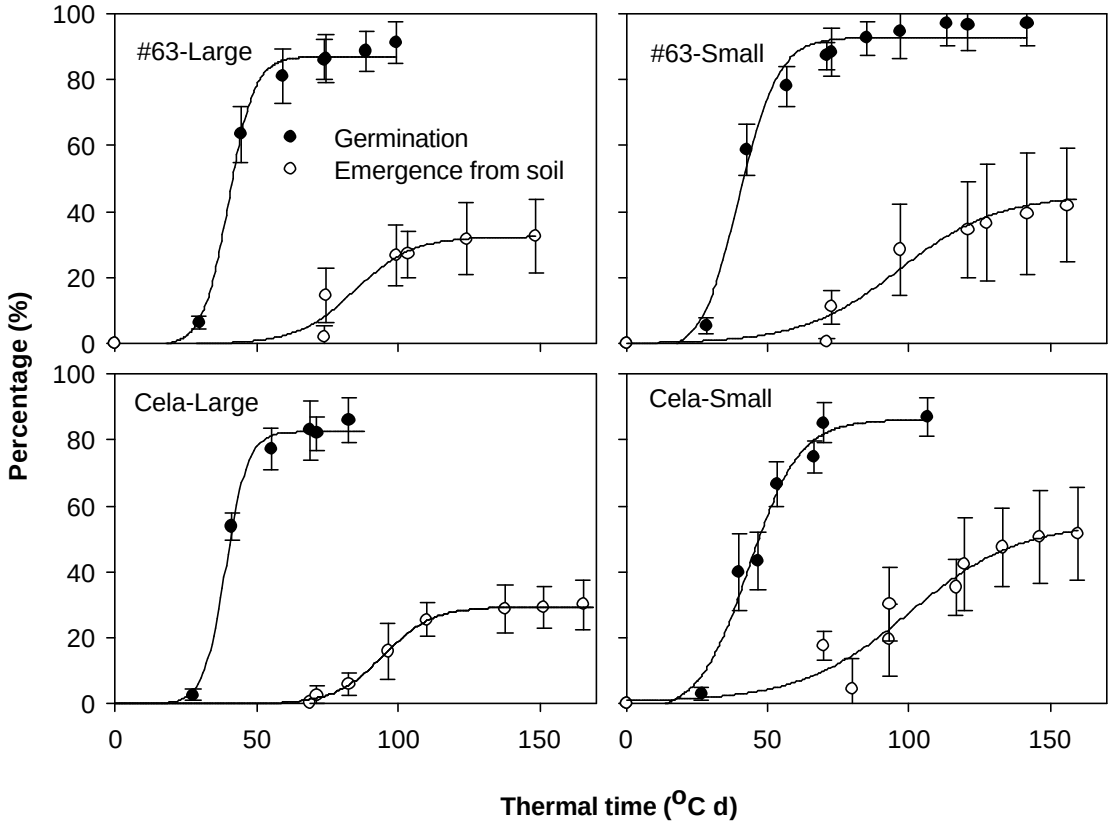


Fig. 5.2. Thermal time courses of seed germination in Petri dishes and seedling emergence from the 1 cm depth in soil at 10 and 20°C for two collections and two seed size classes of winterfat in growth chambers.

Seedlings required 2 to 5 times more thermal time accumulation to achieve the same emergence percentage as seed germination (Fig. 5.3). Thermal time requirements for 10% up to 50% seedling emergence and seed germination were well correlated (R^2>0.98) by an exponential relationship for the two collections and seed size classes.

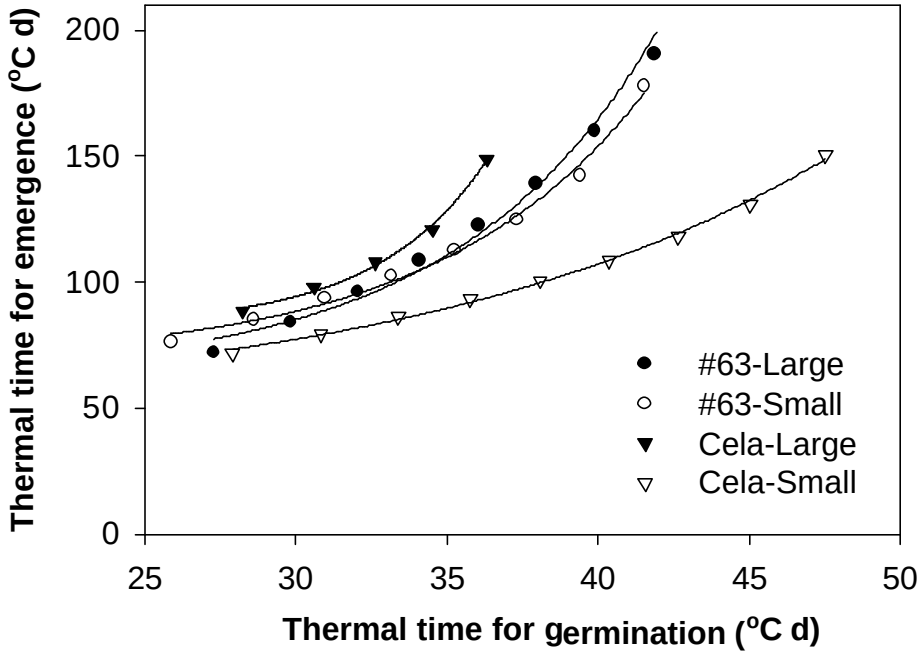


Fig. 5.3. Correlation of thermal time requirements of winterfat between seed germination in Petri dishes and seedling emergence from the 1 cm depth in soil to achieve the same percentage from 10% to 50% for two seed collections and two seed size classes.

5.3.2 Dynamics of soil temperature and soil water in the field

Soil temperature at the 1-cm depth in the field was influenced by soil water because the irrigated treatments generally had lower soil temperatures than the plots with natural precipitation especially when precipitation was low (Figs. 5.4 and 5.5). The diurnal temperature fluctuation at the 1-cm depth was about 10^{o}C. Only after a significant rain event (>5mm), was soil water in the natural precipitation plots above the germination threshold (≈-1.5 MPa, Chapter 4) and seedling emergence of winterfat was possible. A precipitation event of 18 mm in 24 h could only maintain the topsoil (1 cm) above –1.5 MPa for 1-2 days (Fig. 5.4). Fluctuation in soil water was also high in irrigated plots when mean daily temperatures were greater than 15^{o}C. The soil water potential at 1 cm dropped about -1.5 MPa when mean daily air temperatures were close to 25^{o}C in irrigated treatments. The 2004 growing season was characterized by lower temperatures and higher precipitation compared to 2003. Soil water fluctuated less in the plots with irrigation than with natural precipitation under the cooler temperature regimes in 2004.

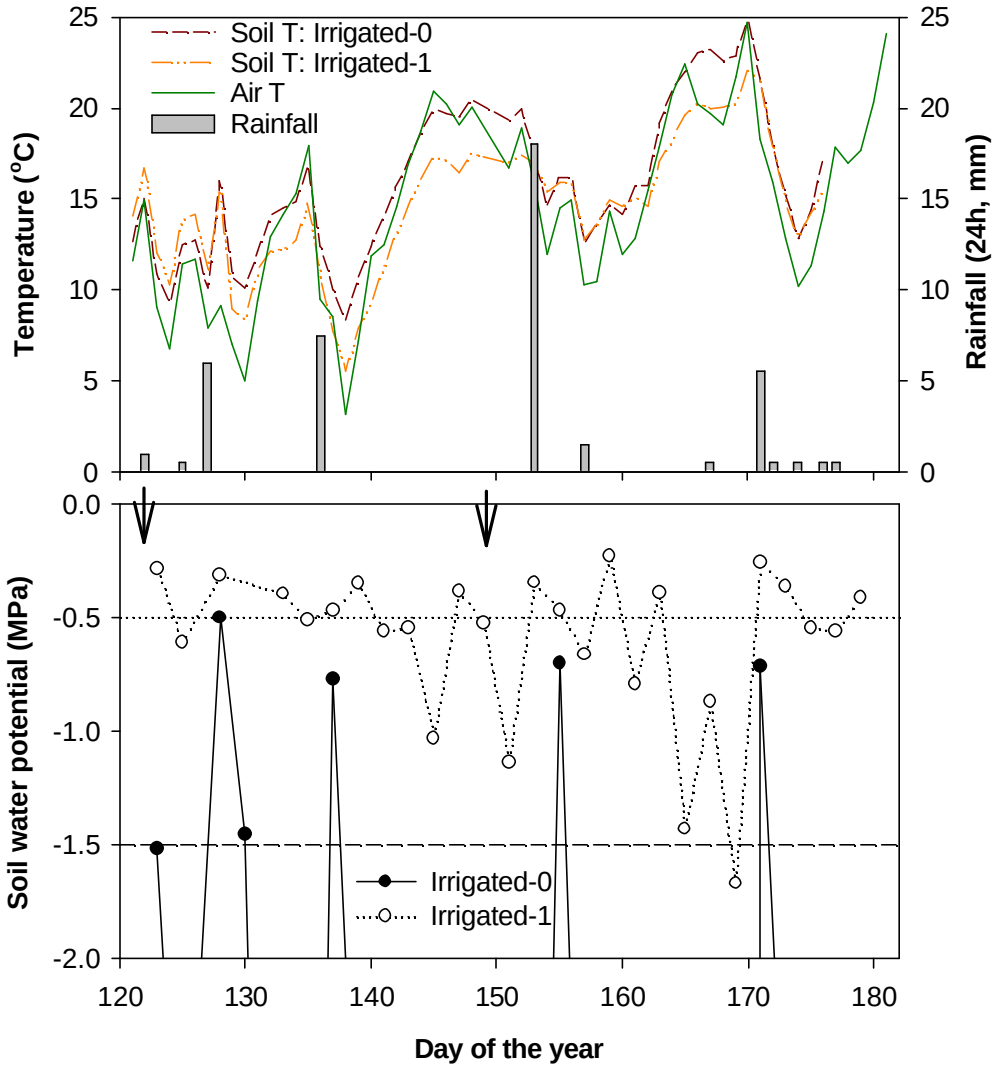


Fig. 5.4. Daily mean air temperature (T) and soil temperature, soil water and daily precipitation during the field experimental periods of 2003. Arrows indicate the two sowing dates (May 2 and May 29). Irrigation treatments are natural precipitation (0) and irrigated once daily (1).

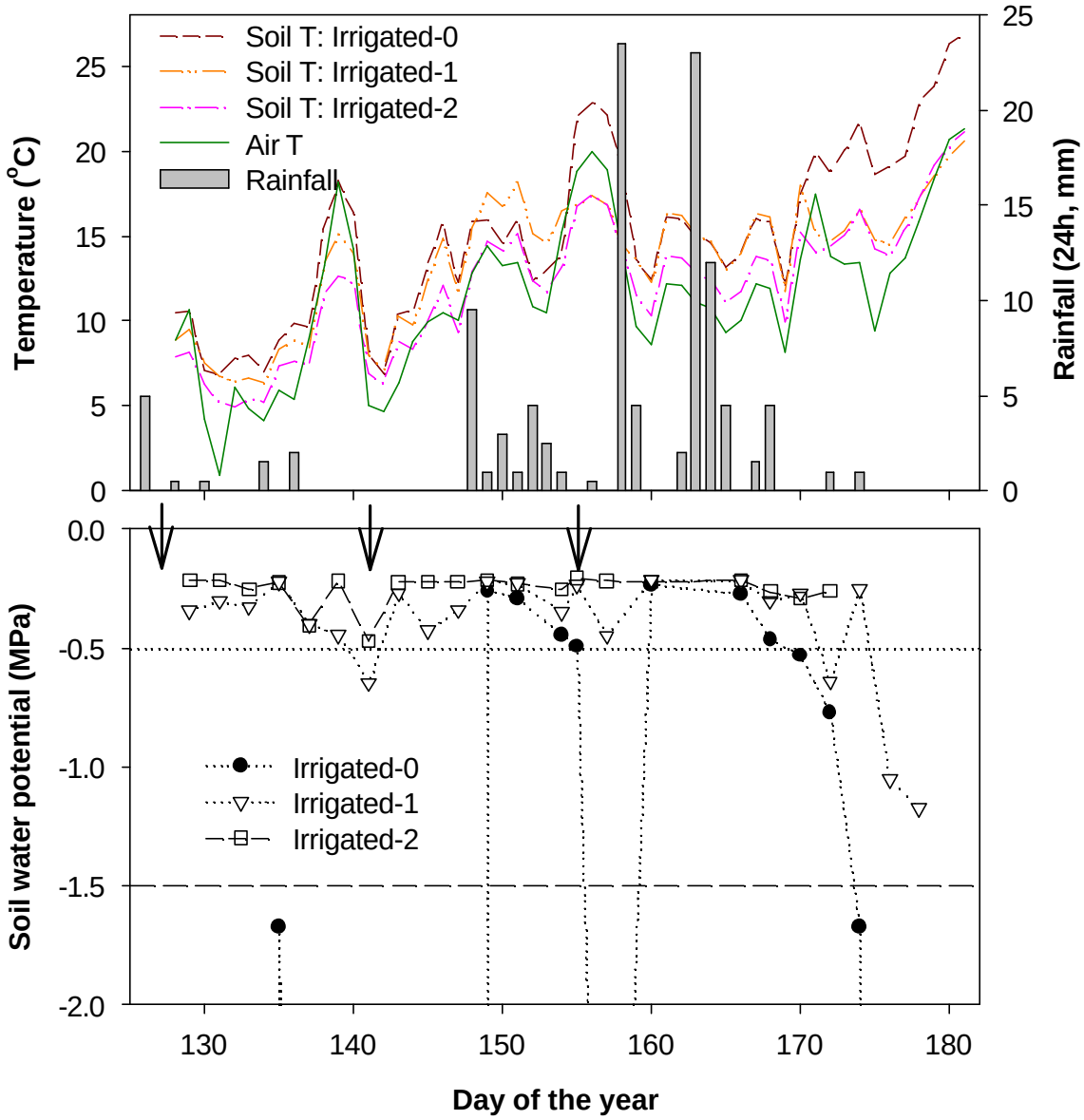


Fig. 5.5. Daily mean air temperature (T) and soil temperature, soil water and daily precipitation during the field experimental periods of 2003. Arrows indicate the three sowing dates (May 6, May 20, and June 3). Irrigation treatments are natural precipitation (0), irrigated once daily (1), and irrigated twice daily (2)

5.3.3 Seedling emergence as affected by soil temperature and soil water in the field

The final percentage of seedling emergence ranged from 3 to 69 % in the field depending on year, sowing date, irrigation treatment, collection and seed size (Table 5.1). Early sowing when soil water potential was high, a result of either high precipitation (sowing dates: May 6 and 20, 2004) or irrigation (May 2, 2003), had greater final emergence (>50%) than others. Seedling emergence was less for late sowing, for example, at the end of May or the beginning of June, generally less than 30% even with irrigation. Seedling emergence from large seeds was greater than seedling emergence from small seeds, especially in collection #63 in 2003. Collection #63 also had greater seedling emergence than collection Cela on the early sowing date (May 6) in 2004.

Table 5.1. Effects of seedbed conditions on final seedling emergence (%) of two collections and two seed size classes of winterfat in 2003 and 2004. Means (S.E.) followed by the same letter within a column are not significantly different at P = 0.05 using FPLSD.

Seedbed condition			Seed collection and size class			
Year	Sowing date	Irrigation	#63-Large	#63-Small	Cela-Large	Cela-Small
2003	May-02	0	21.2 (8.2) dfe	22.4 (8.2) b	16.3 (6.5) cefd	13.4 (6.8) b
		1	68.8 (10.5) a	59.0 (5.2) a	60.0 (10.8) a	48.4 (5.3) a
	May-29	0	15.9 (5.3) dfe	11.1 (3.8) c	9.9 (2.9) ef	10.8 (3.8) b
		1	27.9 (5.3) dc	12.9 (8.5) cb	23.3 (16.0) cebd	18.8 (7.0) b
2004	May-06	0	57.4 (21.2) ab	-[1]	53.3 (3.7) a	-
		1	41.2 (18.0) bc	-	27.7 (21.7) cbd	-
		2	57.4 (9.8) a	-	36.2 (21.5) b	-
	May-20	0	61.7 (7.4) a	-	62.1 (4.2) a	-
		1	23.0 (13.2) de	-	23.1 (7.7) cebd	-
		2	29.5 (12.9) dc	-	31.8 (3.4) cb	-
	Jun-03	0	26.1 (5.2) dce	-	14.4 (7.6) efd	-
		1	3.8 (5.0) f	-	5.4 (5.7) f	-
		2	8.1 (15.7) fe	-	3.3 (3.2) f	-

[1] Data not available.
[2] Irrigation treatments are natural precipitation (0), irrigated once daily (1), and irrigated twice daily (2).

Table 5.2. Days to 10% seedling emergence under field seedbed conditions of two collections and two seed size classes of winterfat in 2003 and 2004.

Seedbed conditions			Seed collection and size class			
Year	Sowing date	Irrigation	#63-Large	#63-Small	Cela-Large	Cela-Small
2003	May-02	0	9.8	11.1	12.0	12.5
		1	4.6	5.0	5.1	5.6
	May-29	0	9.0	10.5	11.8	10.4
		1	3.4	6.5	4.8	4.5
2004	May-06	0	21.9	-	22.2	-
		1	8.3	-	8.9	-
		2	7.4	-	8.3	-
	May-20	0	7.5	-	8.3	-
		1	5.2	-	5.2	-
		2	3.5	-	3.0	-
	Jun-03	0	6.4	-	10.4	-
		1	-[1]	-	-	-
		2	-	-	-	-

[1] Data not available.

[2] Irrigation treatments are natural precipitation (0), irrigated once daily (1), and irrigated twice daily (2).

[3] Data were derived from germination time courses using fitted polynomial functions.

The time required for 10% seedling emergence was generally less with irrigation than with natural precipitation across years and sowing dates (Table 5.2). With irrigation, 10% emergence was achieved within 10 days after sowing. The more the available soil water, the faster the emergence in the two irrigation levels of 2004, except for the third sowing date (June 3). The time required for 10% seedling emergence under natural precipitation varied between 6 and 22 days depending on soil water content.

5.3.4 Using the hydrothermal time model to predict seedling emergence

The previously constructed and modified hydrothermal time model (Eq. 5.3) had different success in predicting seedling emergence of winterfat under field conditions (Figs. 5.6 and 5.7). The model predictability was higher for collection #63 than for collection Cela for the first sowing date in 2003 (Fig. 5.6). The R^2 values for large and small seeds under irrigation were 0.66 and 0.43, respectively, for collection #63 (Fig. 5.6). The model overestimated emergence from the second sowing date in 2003 with irrigation. In 2004, the model predictability was highest in natural precipitation for the first two sowing dates (Fig. 5.7), and R^2 ranged from 0.61-0.79 for the two collections. The model overestimated emergence with irrigation for the first and the second sowing dates and in all treatments for the third sowing date. Seedling emergence was delayed approximately 3-5 days compared to that predicted for irrigation. Model-predicted emergence and actual emergence diverged largely for the third sowing date. The actual seedling emergence was less than 20% and the predicted seedling emergence was more than 60% on the third sowing date.

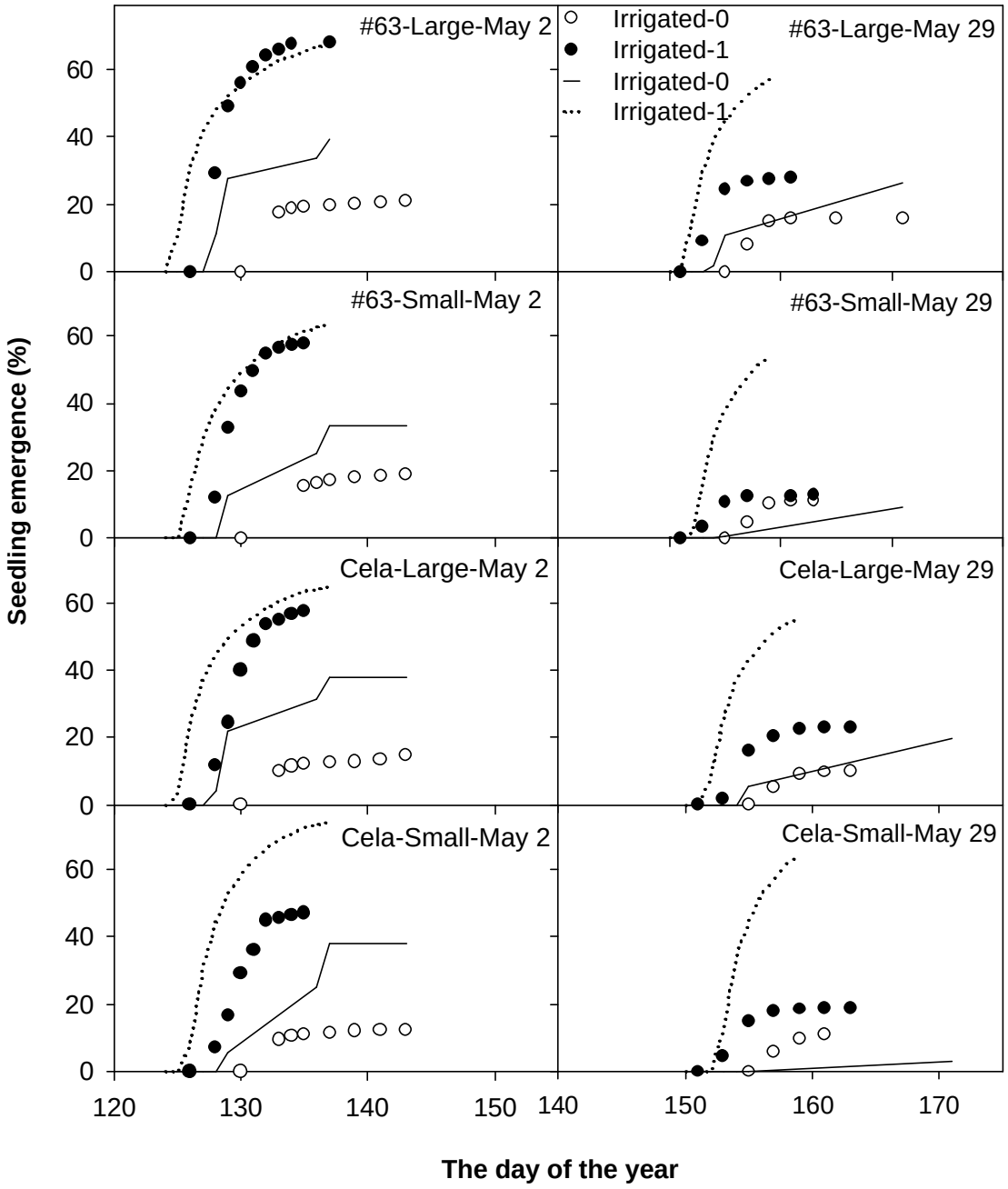


Fig. 5.6. Time courses of seedling emergence of winterfat in 2003 as affected by irrigation treatments, sowing dates, seed collections, and seed size classes. Irrigation treatments are natural precipitation (0) and irrigated once daily (1). Symbols are observed values and lines are predicted emergence using the hydrothermal time model.

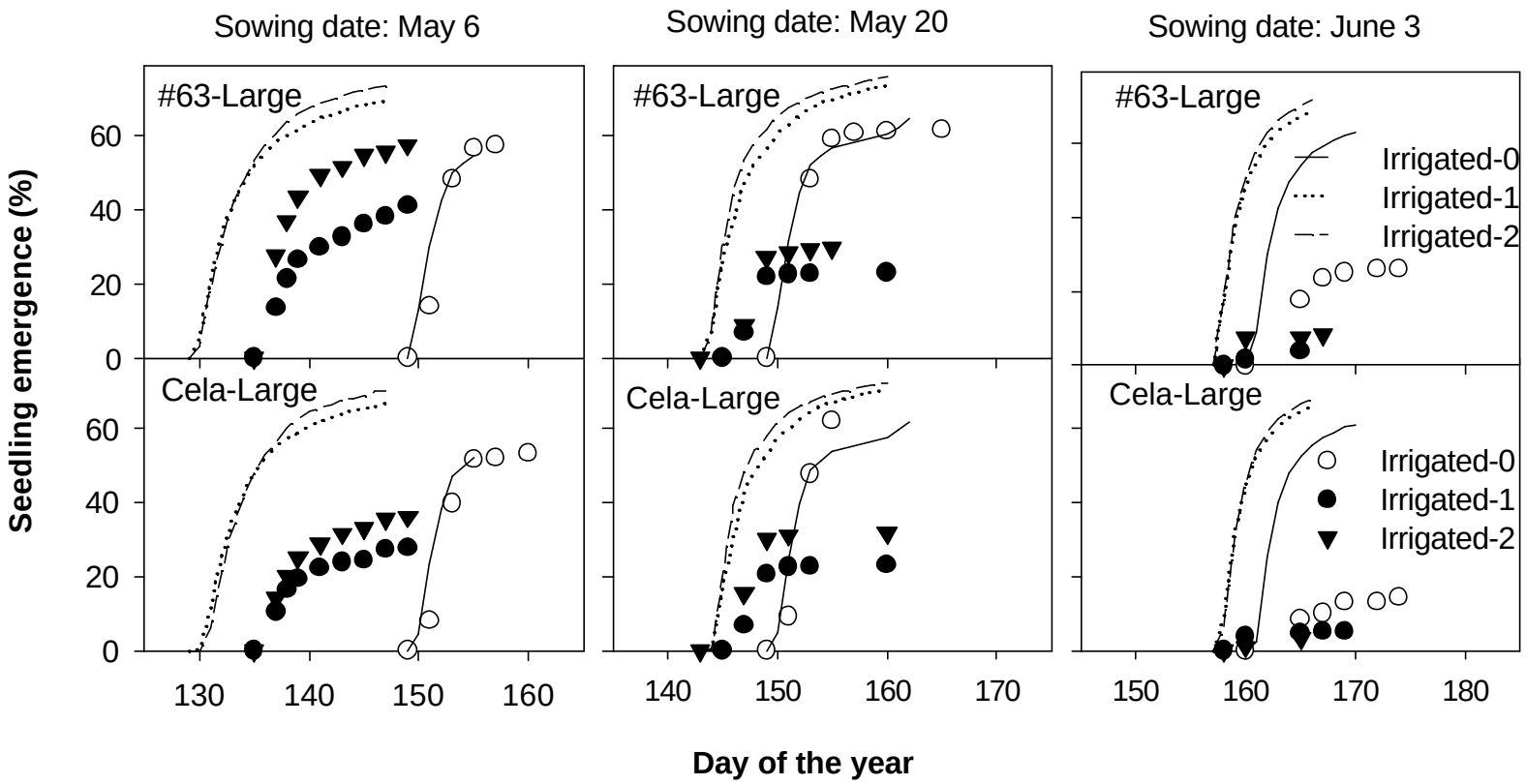


Fig. 5.7. Time courses of seedling emergence of winterfat in 2004 as affected by irrigation levels, sowing dates, and seed collections. Irrigation treatments are natural precipitation (0), irrigated once daily (1), and irrigated twice daily (2). Large: large seed size class. Symbols are observed values and lines are predicted emergence using the hydrothermal time model.

5.4 Discussion

Final percentage of seedling emergence from the soil was greatly reduced compared to germination in Petri dishes under the same moisture and thermal conditions. Winterfat seeds usually germinate on the soil surface or at shallow depths in the field (Springfield, 1971; Booth and Schuman, 1983) with preference for little to moderate amounts of litter (Romo, 2004). Winterfat seedlings may have limited ability to emerge through soil. Furthermore, not all seedlings could emerge from the soil due to post germination events such as viability loss, which in turn affects the number of emerging seedlings (Finch-Savage et al., 1998). The large differences of germination and emergence tested here may indicate that seed deteriorated and/or seedlings died in winterfat. The clay soil texture may partially be responsible for the high mortality since mechanical impedance reduces elongation of germinated seeds (Whalley et al., 1999). However, the seed structure and characteristics of winterfat may largely determine the sensitivity to post germination events. Winterfat seed is relatively small and the perisperm is less than 25% of the total seed weight (Wang et al., Chapter 6). Seed reserves in the perisperm or the endosperm contribute mainly to radicle elongation (Bewley, 1997). Obviously, the limited perisperm reserve in winterfat seeds will be associated with high seedling mortality in a prolonged seedling elongating process caused by adverse seedbed conditions. Booth and Schuman (1983) concluded that the radicle growth of winterfat intact fruits was almost twice longer than that of seeds. Thus, the use of seeds instead of diaspores in our study may be another reason for the low seedling emergence in the field. Using the unprocessed diaspores, rather than naked seeds for winterfat restoration should be considered. In addition, biotic factors in the soil, such as pathogenic fungi can significantly reduce seedling survival of winterfat (Garvin et al., 2004).

Seedling emergence of winterfat from early sowing dates was about 60% under favourable conditions, however, emergence was greatly reduced to less than 20% from late sowing dates even when soil water conditions were favourable. Therefore, winterfat seedling emergence in the field was mainly affected by seedbed conditions. Winterfat normally sheds seeds from mid-September through October in

the Northern Mixed Prairie (Romo et al., 1995). Snow helps push diaspores to the soil surface and seeds usually germinate and emerge in early spring during a relatively short period of high soil water (Springfield, 1971; Booth and Schuman, 1983; Romo, 2004). Winterfat is well adapted to the cold seedbed conditions (Chapter 3) of early spring, and can utilize available soil water for better seedling survival before experiencing desiccation in late spring (Hou et al., 1999). Conversely, higher temperature may adversely affect seedling growth at late sowing dates. Imbibition of winterfat seeds at cold temperatures (0-5^{o}C) reduces dry weight loss of seeds and has longer seedling axis (Booth, 1992). Therefore, the probability is higher for successful seedling establishment and survival when winterfat seeds are imbibed at low temperatures. Large seeds of winterfat had greater seedling emergence at high temperatures (for example, late sowing dates of 2003) than small seeds, perhaps because of higher seed reserve in large seeds. Large seeds also germinate faster than small seeds (Chapter 3). A positive correlation between seed size and seedling vigor has been found in many species including winterfat (Zhang, 1996; Hou and Romo , 1998; Smart and Moser, 1999). Although higher temperature can also induce secondary dormancy in many spring-emerging species, such as in summer annuals (Honek et al., 1999; Baskin et al., 1995) and oil seed rape (*Brassica napus* L.) (Momoh et al., 2002), no secondary dormancy has been reported in winterfat.

The predictability of seedling emergence using the hydrothermal time model varied among seedbed conditions. The model fit best when the mean soil temperatures were between 10 to 15^{o}C, but the model commonly overestimated emergence when temperatures were higher or lower. The optimal temperature for germination was between 20 and 25^{o}C with high water potentials for the two collections of winterfat (Chapter 3 and 4). Because of the diurnal temperature fluctuation at the sowing depth, a mean daily temperature of 20 oC would very likely have a daily maximum temperature >25^{o}C, and surface temperatures of seedbeds in this environment can reach 50^{o}C (Romo and Young, 2002). Temperatures higher than the optimum for seed germination reduce germination rates and seedling elongation rates, changing model parameters (Rowse and Finch-Savage, 2003).

Therefore, additional variables may have been introduced which in turn reduced the predictability of hydrothermal time model. Temperature can affect the percentage and the rate of germination through its effects on seed deterioration and the germination process itself (Roberts, 1988). Seedling emergence involves several biological processes, including seed germination and radicle and shoot elongation. Each of these processes responds differently to environmental conditions (Fyfield and Gregory, 1989). For example, the estimated base temperature was higher for shoot elongation than for germination in lambsquarters (*Chenopodium album* L) (Roman et al., 2000). The timing of germination can account for much of the variation in the timing of seedling emergence in onion (*Allium cepa* L) (Finch-Savage and Phelps, 1993), while the post germination phase accounts for most pre-emergence seedling losses and the spread of seedling emergence time (Finch-Savage et al., 1998). The linear relation between developmental rate and temperature or water potential may also be altered during the post germination (Finch-Savage et al., 2001). Thus, to predict seedling emergence, additional models or sub-models besides germination may be necessary, including the effects of seedbed conditions on seedling elongation, seed deterioration and seedling mortality. Biotic factors and the interactions with environment should also be included because pathogenic fungi can reduce the survival of winterfat seedlings (Garvin et al., 2004).

The use of average daily soil temperatures in the hydrothermal time model of field seedling emergence is common (Hardegree, 1999; Forcella et al., 2000). However, the high magnitude of temperature fluctuation is harmful for germination (Garcia-Huidobro et al., 1982) and may reduce seedling emergence. Thermal time accumulation is assumed as zero when water potential is below the base water potential (Finch-Savage and Phelps, 1993), but water dry-wet cycles and water stress (from < -1.5 to -50 MPa) may affect physiological processes of germination and post germination. This may also account for some of the over- or under-estimation of seedling emergence using the hydrothermal time model in this study.

In summary, the percentage and timing of seedling emergence in winterfat were greatly variable according to seedbed conditions, particularly soil temperature and soil water potential. Therefore, seeding date is critical in restoration using

winterfat. Recommended seeding date for winterfat would be from late fall to early spring when soil temperatures are lower than 15^{o}C and soil water potential is high (> -0.5 MPa). Generally, the hydrothermal time model fits well for emergence when temperatures are low and soil water is available, resulting in an optimal seedbed condition for the natural regeneration of winterfat in the spring after snow melt. Adverse seedbed conditions such as high soil temperature and limited soil water reduced predictability of the hydrothermal time model in seedling emergence. Pre- and post-germination events that affect seed deterioration, seedling mortality and seedling elongation may override the effects of temperature and soil water potential on the predictability of the hydrothermal time model for winterfat.

6. Variation in Low Temperature Germination between Two Seed Sizes and its Physiological Mechanisms

Abstract The effect of seed size on germination at low and subzero temperatures and the associated physiological mechanisms were investigated. Winterfat seeds achieved 43 to 67% germination at – 3°C, a temperature slightly above the base temperatures estimated using thermal time models. While models have inferred the potential of seed germination at subzero temperatures in native seeds, this is one of the first direct evidences of this phenomenon. Small seeds required approximately twice as long to reach 50% germination at subzero temperatures as large seeds. Large seeds maintained stable water uptake rate for the seed and the embryo when temperatures decreased from 5 to –1°C. In contrast, faster water uptake and greater K^+ leakage in small seeds indicated possible damage to membrane integrity at subzero temperatures. Metabolic heat production rate (Rq) was greater in small seeds than in large seeds at high (20°C) and low temperatures (10°C), but the respirational CO_2 production rate (R_{CO2}) was similar between large and small seeds at the low temperature. Higher carbohydrate conversion efficiency (Rq/R_{CO2}) of large seeds was only apparent at the lower temperature. Higher cold tolerance in large seeds was possibly correlated with higher contents of glucose, raffinose and sucrose during germination. Greater germination by large seeds compared to small seeds at low temperatures may be due to maintenance of membrane integrity, more soluble cryoprotective sugars and greater carbohydrate conversion efficiency by large seeds under freezing temperatures.

6.1 Introduction

Seed mass can vary widely within a population of a species (Zhang and Maun, 1990; Zhang, 1998) depending on biological and environmental constraints (Vaughton and Ramsey, 1997; 1998). Populations with different mean seed weights are thought to have evolved under different selection pressures, increasing their potential fitness

(Westoby et al., 1992). Variance in seed-size also is pronounced within an individual plant and is attributed to parental identity and fruiting positions (Mendez, 1997; Vaughton and Ramsey, 1998; Simons and Johnston, 2000). Seed weight is correlated with seed vigor (Lafond and Baker, 1986; Berdahl and Frank, 1998), seedling recruitment (Negri and Facinelli, 1990; Mendez, 1997; Susko and Lovett-Doust, 2000; Dalling and Hubbell, 2002; Debain et al., 2003), plant size and the probability of survival (Hou and Romo, 1998; Simons and Johnston, 2000; Walters and Reich, 2000).

Early-emerging winterfat seedlings grow faster and survive better than late-emerging plants (Hou and Romo, 1998). Thus, relatively heavier seeds are desirable for restoration. The selection for increasing seed size was positively associated with faster germination (Charlton, 1989) and greater seedling vigor at low temperatures (Kelman and Forrester, 1999). However, the physiological mechanisms for the different performance among seed sizes, especially under stress conditions such as low temperatures, are rarely studied in spite of its importance for understanding the adaptation of plants to local environments.

Physiological mechanisms related to plant cold tolerance include accumulation of compatible solutes including sugars and proline (Hoekstra et al., 2001), dysfunction or the damage of cell membranes and changes in membrane fluidity and integrity (Leborgne et al., 1992; Sharom et al., 1994; Saltveit, 2002; Wisniewski et al., 2003), and higher metabolic rates under stress temperatures (Massardo et al., 2000). Dramatic changes in physiological and biochemical activities take place during seed germination and can be correlated with phases in water uptake (Bewley and Black, 1994; Bewley, 1997). Generally leakage peaks at the initial imbibition stage during cell membrane repair (Bewley and Black, 1994). Ionic leakage may be a more sensitive measurement of membrane integrity than bulk conductivity (Ouyang et al., 2002), and potassium is a better indicator of cell membrane integrity (Dias et al., 1996; and references therein). Therefore, the ionic leakage of seeds during imbibition affects not only membrane repair and functional recovery, but also freezing tolerance. Besides cellular function recovery, respiration is the first detectable metabolic activity providing energy for germination (Bewley and Black, 1994). Plant metabolic activities can be studied using calorimetry, which measures the metabolic heat production rate (R_q) from catabolism and anabolism

(Thygerson et al., 2002). Plant growth rates are correlated with heat production rates and respiration rates, and the latter can be measured by CO_2 production rate (Rco_2) from respiration (Criddle et al., 1991). Metabolic heat and CO_2 production rates have been used to measure metabolic responses to environmental stress (Hemming et al., 1999; Smith et al., 2000; Thygerson et al., 2002), respiratory substrates (Criddle et al., 1997; Edelstein et al., 2001) and growth rates (Hansen et al., 1994). The ratio of Rq/Rco_2 provides a reference point of respiratory substrates utilized by the tissue (Hansen et al., 1997). Seed reserves and its composition affect respiration, metabolism and stress tolerance. Soluble sugars, such as sucrose and the raffinose family, are compatible solutes for maintaining enzyme structure and function, and membrane integrity under stress (Bailly et al., 2001; Hoekstra et al., 2001; Uemura and Steponkus, 2003). The possible physiological mechanisms to cold stress between seed sizes, therefore, were investigated in water uptake, membrane leakage, metabolic rates and soluble sugar contents.

The objectives of the study were to determine physiological differences between large and small seeds of winterfat at low temperatures and to study the mechanism associated with these differences, specifically: 1) To determine seed germination at subzero temperatures and correlate germination with seed size and the thermal time model; 2) To examine membrane integrity, soluble sugars and water uptake characteristics at low and subzero temperatures as affected by seed size; and 3) To determine metabolic activities at low temperatures as affected by seed size. We hypothesize that large seeds of winterfat are more resistant to low temperature stress than small seeds and the different germination characteristics are the result of different physiological mechanisms.

6.2 Materials and methods

6.2.1 Seed size and embryo ratio

Cleaned seeds from two seed collections were separated into large and small using a seed blower based on seed mass and were then sealed in plastic bags and stored at $-18^{o}C$ until use (Chapter 3). The ratio of embryo to seed for the two seed size classes

was calculated gravimetrically. Ten seeds of each seed size class and each collection were hydrated for 30 min on top of two filter papers in Petri dishes at room temperature (22.5 ± 1°C). The hydrated seeds were excised under a microscope into embryo and perisperm with the seed coat. Seeds oven-dried at 80°C for 48 h, and a microbalance (± 1 μg) was used for determining the seed dry weights.

6.2.2 Germination tests at subzero temperatures

Large and small seeds of both collections, Cela and #63, were germinated at -1 or -3°C, respectively, using a circulating liquid bath (ethylene glycol, temperature stability ± 0.01°C, Cole Parmer, Vernon Hills, IL, USA). Three replicates of 50 seeds each were used. Seeds were placed in 5 cm Petri dishes on top of two layers of Whatman No. 1 filter paper. Polyethylene glycol (PEG) solution at -0.20 MPa was added to Petri dishes to prevent ice formation at subzero temperatures. Preliminary tests indicated that ice did not form in this solution and seed germination rate and percentage at -0.20 MPa were similar to that at 0 MPa. Germination was monitored at 7-d intervals for –1°C and 15-d intervals for –3°C up to 30 days. Germination was checked on an ice bed.

6.2.3 Seed water uptake at low temperatures as affected by seed size

Twenty seeds of five replicates from each seed size class and collection were imbibed in distilled water on top of the saturated filter paper at 5 and -1°C, respectively. The weight of individual seeds from each replicate was repeatedly weighed using a microbalance (± 1 μg) at 2, 4, 12, 24, and 48 h intervals during imbibition. Embryos were excised and weighed from different seeds of each replicate at each time interval. Seeds or embryos were blotted gently with tissue paper to remove surface water before weighing until germinated. Dry weights of seeds and embryos were determined gravimetrically after oven drying at 80°C for 48 h; and changes in water content were calculated on a dry weight basis.

6.2.4 Cell membrane integrity at low temperatures as measured by K^+ leakage

Ten seeds free of morphological damage were selected for each seed size class and collection and weighed individually to determine the air-dry-weight of seeds. Each seed was imbibed with 1 mL deionized water in 1.5 mL micro-centrifuge tubes at 30°C for 1.5, 3.5, 5.0, and 6.5 h, at 10°C for 4, 8, 12, and 16 h, and at -1°C for 24, 48, 72, and 96 h, respectively. Imbibition intervals for different temperatures were determined based on time to reach similar thermal time accumulation (Chapter 3) and comparisons of different temperatures were expressed on a thermal time course. Seeds were removed and the leaching solutions were kept at 5°C until use at the imbibition time points. To prevent ice formation at subzero temperatures, two drops of PEG solution of -0.20 MPa was added to deionized water. An atomic absorption spectrometer (Spectr AA-220, Varian Scientific Instrument, Palo Alto, USA) was used to measure K^+ concentration in each solution. Deionized water and the deionized water with the PEG solution were used as the control for temperatures above and below 0°C, respectively. Relative K^+ leakage was calculated based on maximum leakage, which was the K^+ concentration after boiling seeds for 30 min. Air-dry-weight of seeds was used to calculate relative K^+ leakage as recommended by the Seed Vigor Test Committee of the Association of Official Seed Analysts (1983).

6.2.5 Heat production rate and respirationary CO_2 production rate

Microcalorimetry (Multi-cell Differential Scanning Calorimeter, MDSC, 4100DSC, Calorimetry Science Corporation, American Fork, Utah, USA) was used to study the heat production rate from seeds. Five large seeds and eight small seeds based on similar weights from collection #63, were weighed and placed in plastic cassettes and soaked in 1% sodium hypochlorite for 10 min for surface sterilization. Rinsed seeds were placed in a 5°C incubator for 16 and 24 h, respectively. Metabolic heat production rates, Rq, were measured up to 3000 sec at 10°C and 2000 sec at 20°C after 10-20 min equilibrium, respectively (Smith et al., 2000). The Rq was measured before and after measuring the respirational CO_2 production rate and the average value was used. Respirational CO_2 production rate, Rco_2, was measured by the rate of heat production

from respired CO_2 forming carbonate in a NaOH trap. The Rco_2 was thus calculated from the differences of the heat production rates with and without the carbonation. The reaction of CO_2 with NaOH solution to form carbonates produces –108.5 kJ mol^{-1} and there was 25 µL of 0.4 M NaOH placed in the trap (Criddle et al., 1997). The Rq and the R_{CO2} were converted to µW per mg air-dry-weight and the ratio of Rq/R_{CO2} was multiplied by 108.5 KJ mol^{-1} (Criddle et al., 1997).

6.2.6 Changes in soluble sugar content during imbibition at low temperatures

Four replicates of 10 seeds from large and small seed size classes of collection #63 were imbibed in distilled water for 0, 12, 24, 48, and 72 hours at 5°C. Soluble sugars were extracted from these seeds with seed air-dry weight of 15 to 30 mg. Two mL distilled deionized water (dd H_2O, Millipore, Milli Q^{TM} water system, Milford, MA, USA) at 98 ± 2°C were added to seed samples and the samples were grounded to fine mixture using a pestle and mortar. The mixture was incubated at 90°C for 10 min to inactive enzymes in a microtube incubator (incuBlock, Denville Scientific Inc. Metuchen, NJ, USA) and then centrifuged at 13,000 rpm for 10 min. The supernatants were diluted in an appropriate concentration and passed through a 13 mm diameter nylon syringe filter (0.2 µm pore size, Chromatographic Specialties Inc., Brockville, ON, Canada) before analysis.

Fructose, glucose, raffinose and sucrose concentrations were determined with a Dionex Bio LC 4000 gradient liquid chromatography unit (Dionex; Sunnyvale, CA) equipped with a pulsed amperometric detector (PAD; Dionex). The working gold electrode, during operation, was maintained at the following potentials and durations: E_1=0.05 V (t_1=0.299); E_2=0.60 V (t_2=0.299); and E_3=-0.80 V (t_3=0.499) at a sensitivity of10K nA. Isocratic (80 mM NaOH) separation was achieved using a Dionex CarboPac PA1 anion exchange column (250 x 4 mm) equipped with a Dionex CarboPac PA1 Guard column (50 x 4 mm). The mobile phase flow rate was 1 mL/min and the sample volume was 50 mL. Standard curves for glucose, fructose, raffinose and sucrose were constructed at concentrations ranging from 10 to 188 ppm. The standard curves had correlation coefficients of 0.99 or better. Approximate retention times for fructose, glucose, sucrose and raffinose were 5.5, 6.3, 9.8, 25.8 minutes, respectively. The

presence of raffinose in seeds was confirmed by the analysis of two samples with raffinose standard (100 ppm) and spiking experiments. Lyophilized samples were derivatized by adding 0.5 mL of Tri-Sil Z (Chromatographic Specialties Inc., Brockville, ON). The vials were capped, and the solutions heated at 80°C for 1h. The trimethylsilylated carbohydrates were analyzed with a gas chromatography (HP6890; Hewlett-Packard, Mississauga, ON) equipped with an auto sampler.

6.2.7 Data analysis

Thermal time, biological time rather than actual time, was used in germination time courses and other associated time expressions to make germination or physiological responses at different temperatures under the same time scale. The θ_T was calculated according Eq. 2.1. The T_bs were from –4.8 to –3.3°C for large and small seeds of the two collections of winterfat (Chapter 3).

Germination percentage was scaled by the mean maximum germination percentage which ranged from 83 to 95% for each seed collection and each seed size class. Percentage data were arcsine transformed for mean separation. General Linear Model (GLM) procedure, ANOVA and FPLSD at significant level of p= 0.05 were used for mean separation in SAS® Windows version 8.2. SigmaPlot® was used for curve fitting and estimation of days to 50% germination (D_{50}).

6.3 Results

6.3.1 Seed mass and relative embryo mass allocation in large and small seeds

Seed mass was significantly different between the two seed size classes in both collections ($P < 0.0001$). However, the ratios of the embryo to seed were not significantly different ($P = 0.119$) between large and small seeds for the two collections indicating that the embryo to seed ratio is a conservative trait in winterfat. More than 70% of the seed mass was allocated to the embryo (Fig. 6.1).

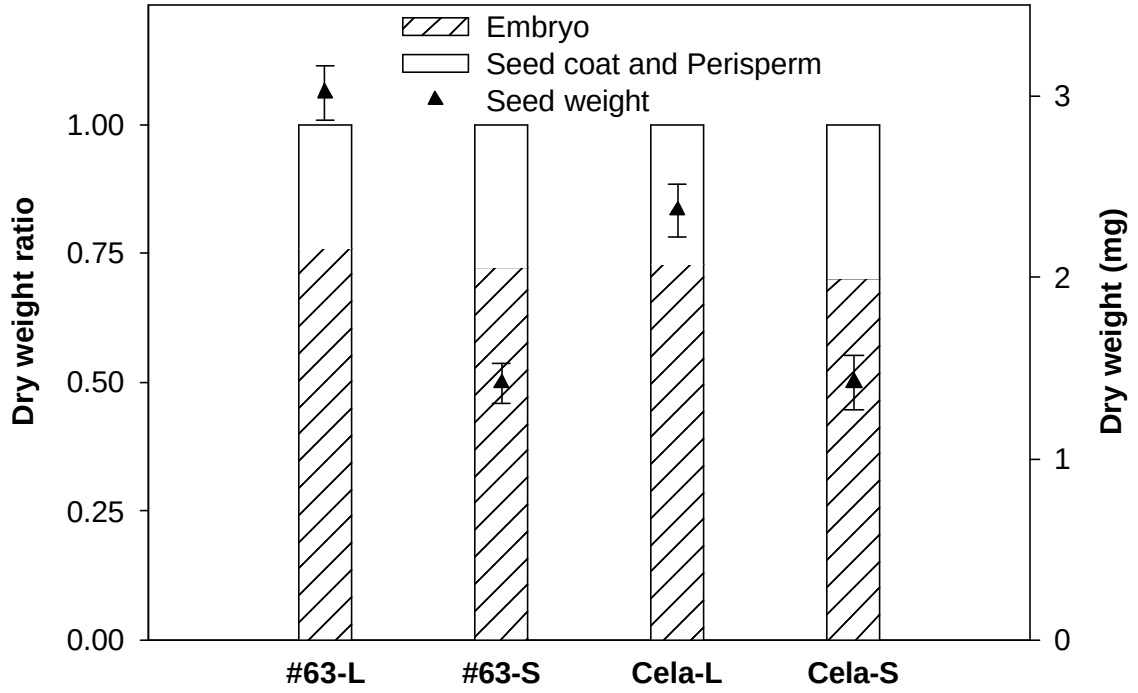


Fig. 6.1. Seed mass and its allocation to the embryo in large (L) and small (S) seeds of collections, Cela and #63, in winterfat. Means with the same letters were not significantly different within a collection at P = 0.05.

6.3.2 Germination at subzero temperatures

Winterfat seeds germinated to a high percentage at subzero temperatures (-1 and -3°C) after 30 days (Fig. 6.2). The germination percentage was 20 to 40% lower than that predicted by the thermal time model (Chapter 3). Parameters of the thermal time model were estimated using germination data at > 0°C temperatures. The observed germination time courses diverged from the predicted using thermal time model. The thermal time model did not estimate germination accurately at –1 and –3°C, respectively.

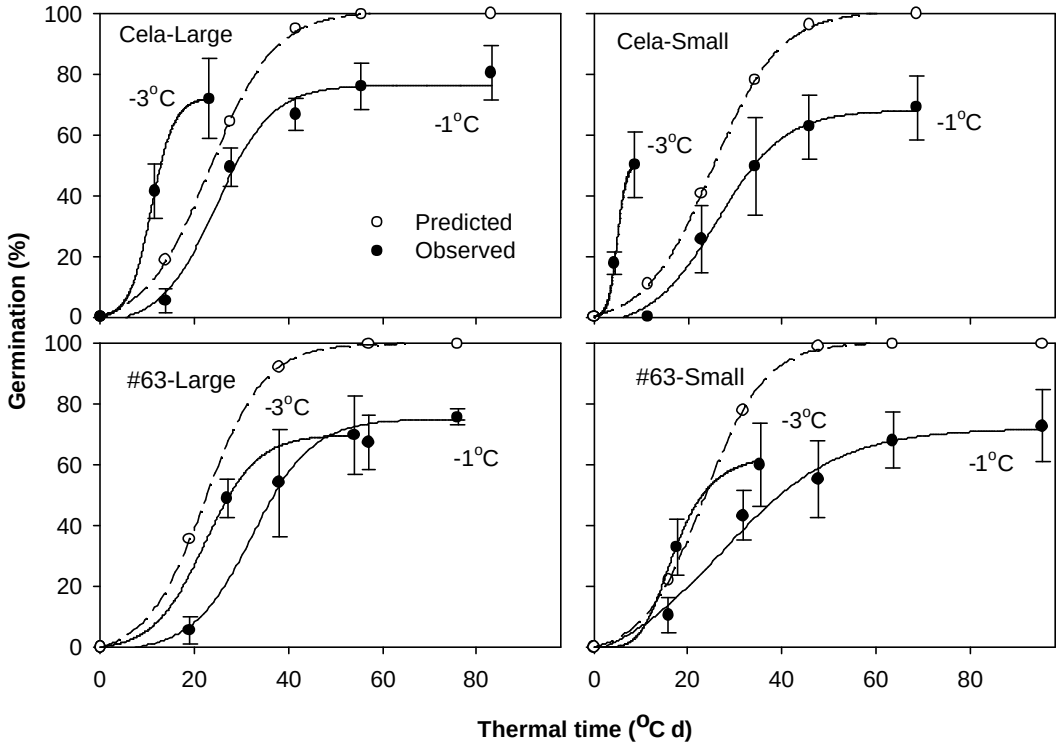


Fig. 6.2. Predicted and observed thermal time courses of germination at -3 and -1°C for large and small seeds of collections, Cela and #63, in winterfat. Predicted values were calculated from thermal time models with estimated parameters in chapter 3. Fitted lines were based on the least squares fit sigmoid function.

Large seeds had a significantly higher germination percentage than small seeds at –1 and – 3°C in collection Cela, but not in collection #63 in spite of the 7 to 10 % higher germination in large seeds (Fig. 6.3). However, the average D_{50} were much longer in small seeds than in large seeds. The D_{50} were 12 and 6 days, and 16 and 9 days for small and large seeds, respectively, in collection #63 and Cela at –1°C. The D_{50} increased at –3°C to 24 and 16 days, and 30 and 19 days in small and large seeds, respectively, in collection #63 and Cela.

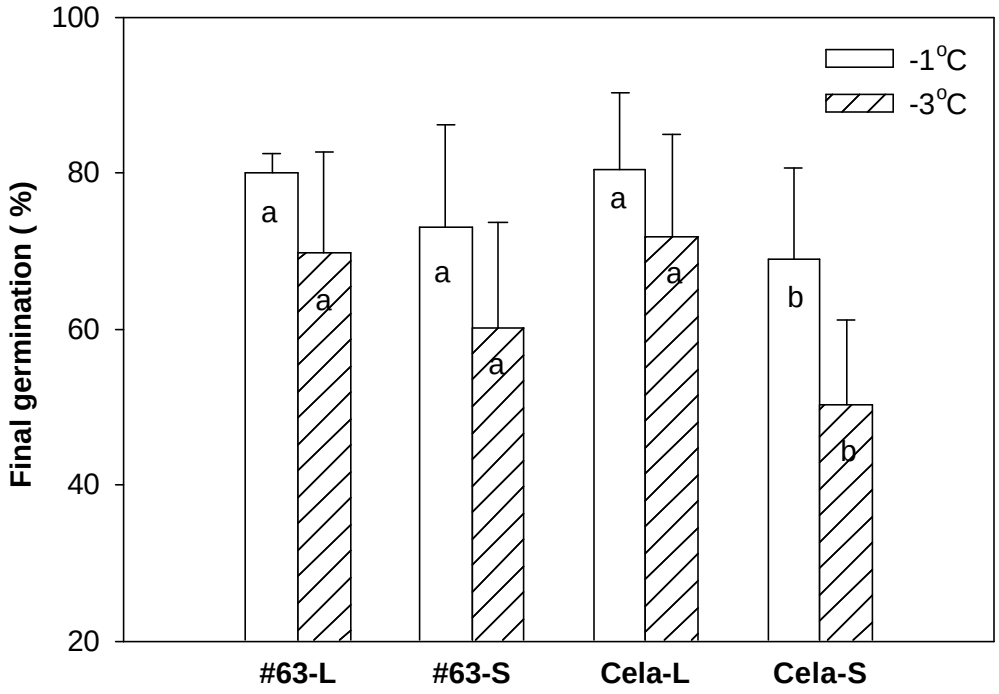


Fig. 6.3. The total germination percentage of viable seeds (maximum germination at optimum temperature) at –1 and -3°C of large (L) and small (S) seeds of collections, Cela and #63, in winterfat. Means with the same letters were not significantly different within a collection at P = 0.05.

6.3.3 Seed water uptake as affected by seed size

The thermal time course of water uptake was similar in large and small seeds and between whole seeds and embryos at 5°C (Fig. 6.4). Small seeds generally had faster water uptake at subzero temperature (-1°C) when expressed in thermal time, especially for embryos. Slopes of the thermal time course curves at the early stage of water uptake were much steeper for small seeds than for large seeds in both collections at -1°C. Water content in small seeds and their embryos increased to approximately 50% at 400 degree-hours in collection Cela and 40% in the embryos of small seed in collection #63 at -1°C. Conversely, the water content of large seeds increased little at –1°C compared to 5°C in both collections.

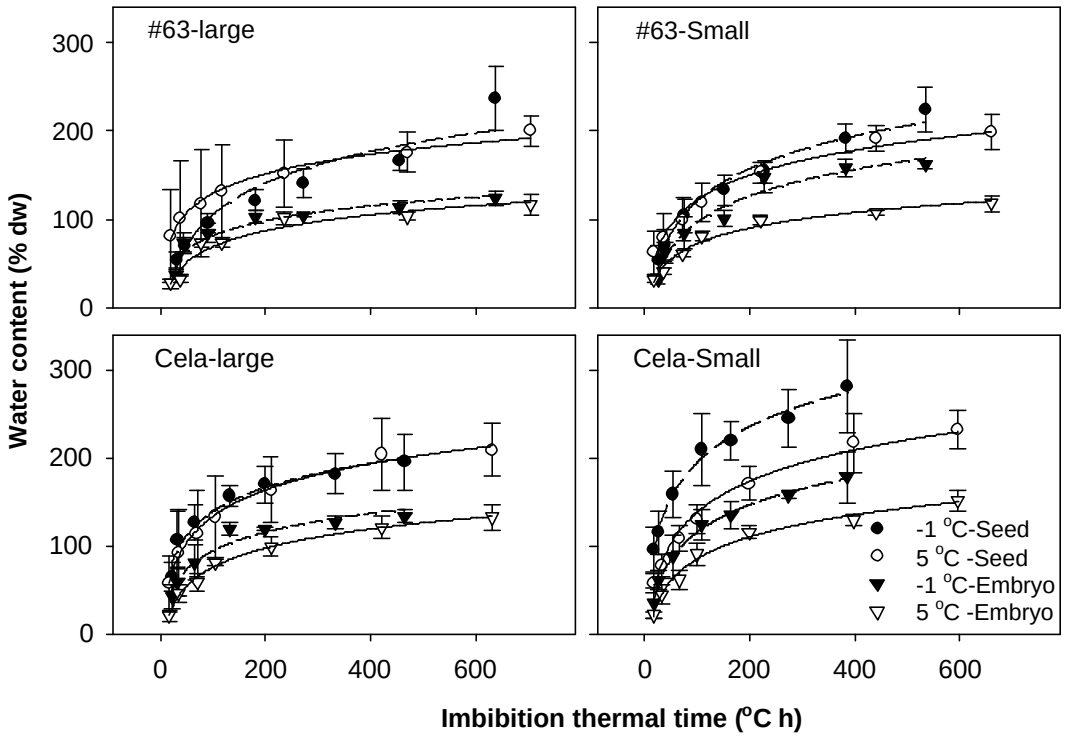


Fig. 6.4. Water uptake with increment of imbibition thermal time at 5°C and –1°C for large and small seeds of collections, Cela and #63, in winterfat. Thermal time was based on estimated base temperatures (T_b) in Chapter 3. The estimated T_b values for large and small seeds were -4.8 and -4.2°C for collection #63 and -3.8 and –3.3°C for collection Cela. Fitted lines were based on the least squares fit sigmoid function.

6.3.4 The leakage of K^+ as affected by seed size

The K^+ leakage of large and small seeds was similar when imbibed at 10 and 30°C, but was different at -1°C for both collections (P = 0.02 and 0.03, respectively, for #63 and Cela) (Fig. 6.5). Large seeds had lower K^+ leakage than small seeds at the early stage of imbibition, suggesting differential membrane integrity between large and small seeds at subzero temperatures.

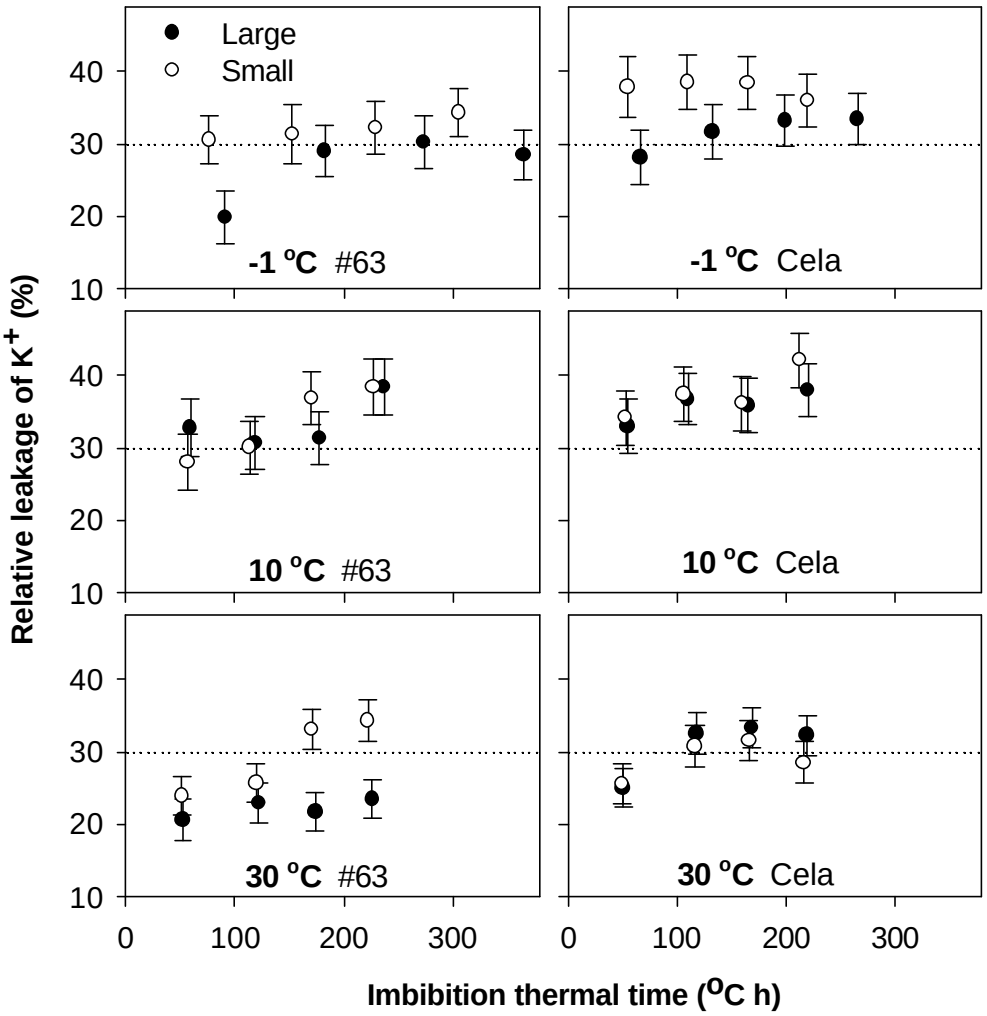


Fig. 6.5. The relative K^+ leakage at different imbibition time intervals at 30, 10 and –1°C for large and small seeds of collections, Cela and #63, in winterfat. The relative leakage was calculated as the percentage of maximum leakage, which was the leakage when seeds were boiled for 30 min.

6.3.5 Metabolic rates of large and small seeds during imbibition

Heat production rates were significantly correlated with seed mass at 10 and 20°C following 16 and 24 h imbibition at 5°C (Fig. 6.6). Heat production rates per mg air-dry weight was significantly lower in large seeds than in small seeds, especially at 20°C.

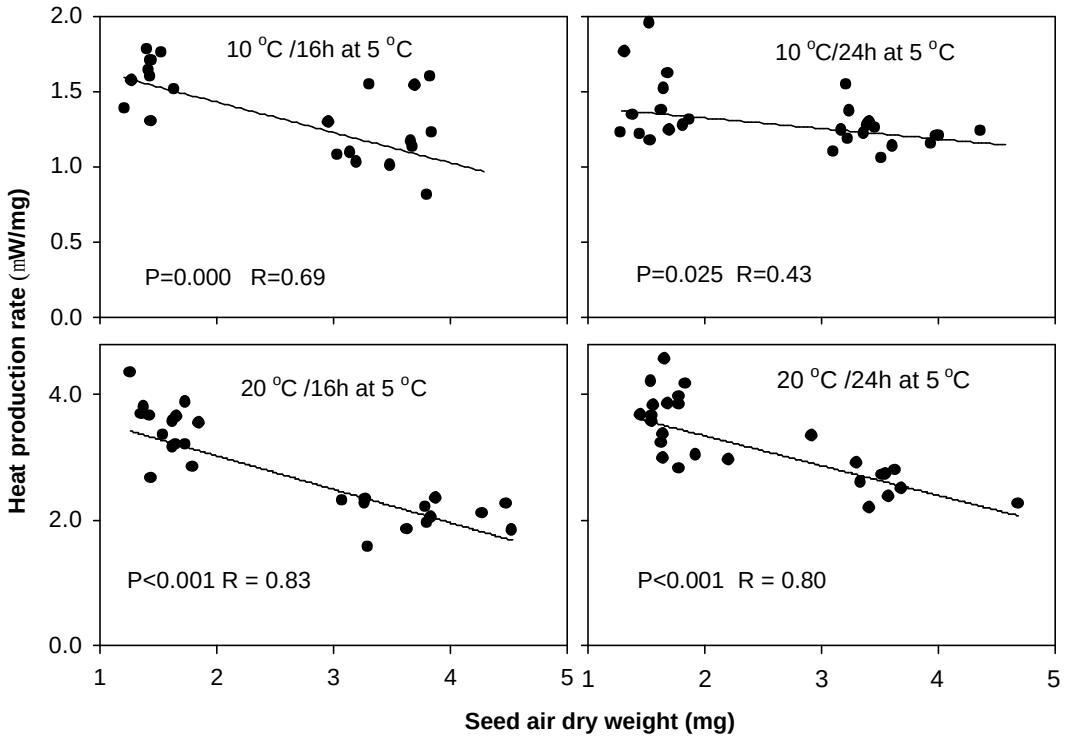


Fig. 6.6. Relationships between heat production rates (Rq) and seed mass at 10 and 20°C following 16 and 24 h imbibition at 5°C in winterfat (collection #63).

Respirational CO_2 production rates were significantly correlated with seed mass, and large seeds had lower respiration rates at 20°C after 16 and 24 h imbibition at 5°C (Fig. 6.7). Small seeds had a significantly higher Rq, but not R_{CO2}, at 10°C after 16 h imbibition at 5°C (Figs. 6.6 and 6.7). Rq and R_{CO2} were temperature dependent, increasing with temperature. The lower the value of Rq/R_{CO2}, the higher the efficiency. The ratios of Rq/R_{CO2} greatly varied among seeds with no significant differences between large and small seeds, except at 10 °C after 24 h imbibition at 5°C (P = 0.01) (Table 6.1). The ratio of Rq/R_{CO2} ranged from 359 to 458 KJ mol^{-1} for large and small seeds and close to the value of carbohydrate oxidation (Hansen et al., 1997).

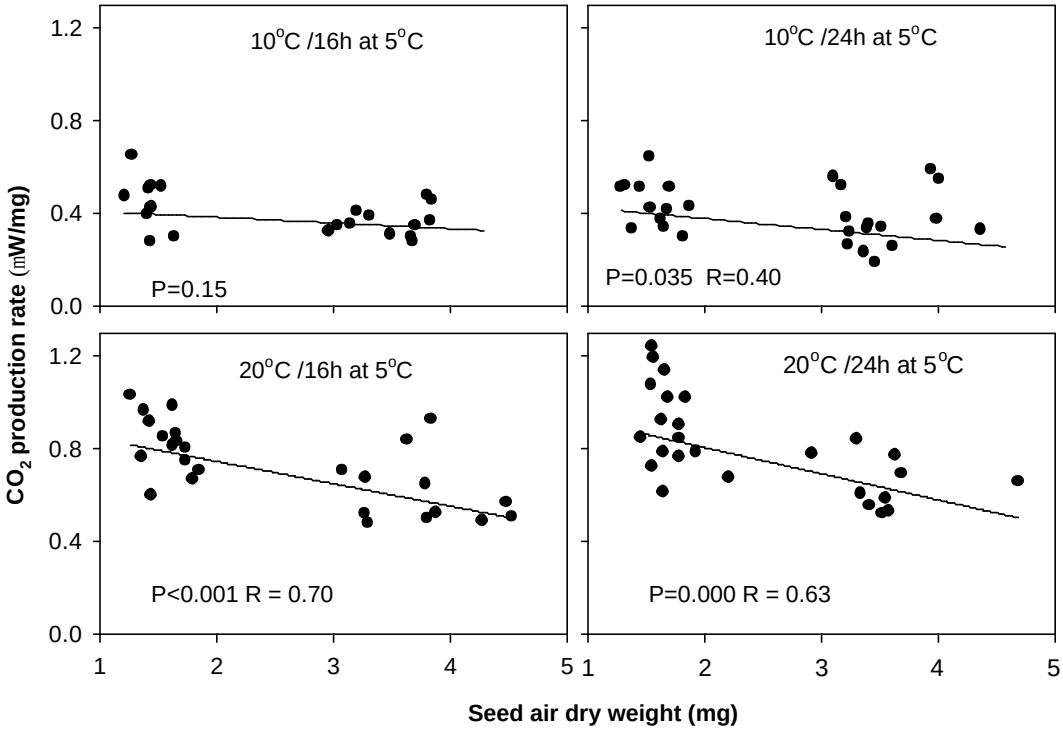


Fig. 6.7. Relationships between respiration CO_2 production rates (R_{CO2}) and seed mass at 10 and 20°C after 16 and 24 h imbibition at 5°C in winterfat (collection #63).

Table 6.1. The metabolic efficiency measured by the ratio of metabolic heat production rate, Rq, and respirational CO_2 production rate R_{CO2} at different temperature and imbibition time in collection #63 with large and small seeds.

Seed Size	Temperature (^oC)	Imbibition Time (h)	Rq/R_{CO2} (KJ mol^{-1})	S.E.	P< 0.05
Large	10	16	369.3[1]	28.9	a
Small	10	16	389.4	33.7	a
Large	10	24	382.2	18.6	a
Small	10	24	458.1	17.2	b
Large	20	16	399.0	30.7	a
Small	20	16	359.4	34.4	a
Large	20	24	443.4	21.0	a
Small	20	24	438.2	16.6	a

[1]: Value are least square mean (lsmean), mean separated by p-value from GLM of SAS.

6.3.6 Dynamics in soluble sugar content during imbibition as affected by seed size

Initial contents of glucose and raffinose per unit seed mass were less in small seeds than in large seeds, while the content of sucrose was similar in the two seed size classes (Fig. 6.8). Glucose and raffinose contents remained low in small seeds during imbibition up to 72 h. The sucrose content decreased from 24 h and a dramatic drop occurred at 72 h in small seeds. The decrease in raffinose content was greater in small seeds than in large seeds. The fructose content was not significantly different between large and small seeds, with highly variable concentration peaks between 24 and 48 h imbibition at 5°C.

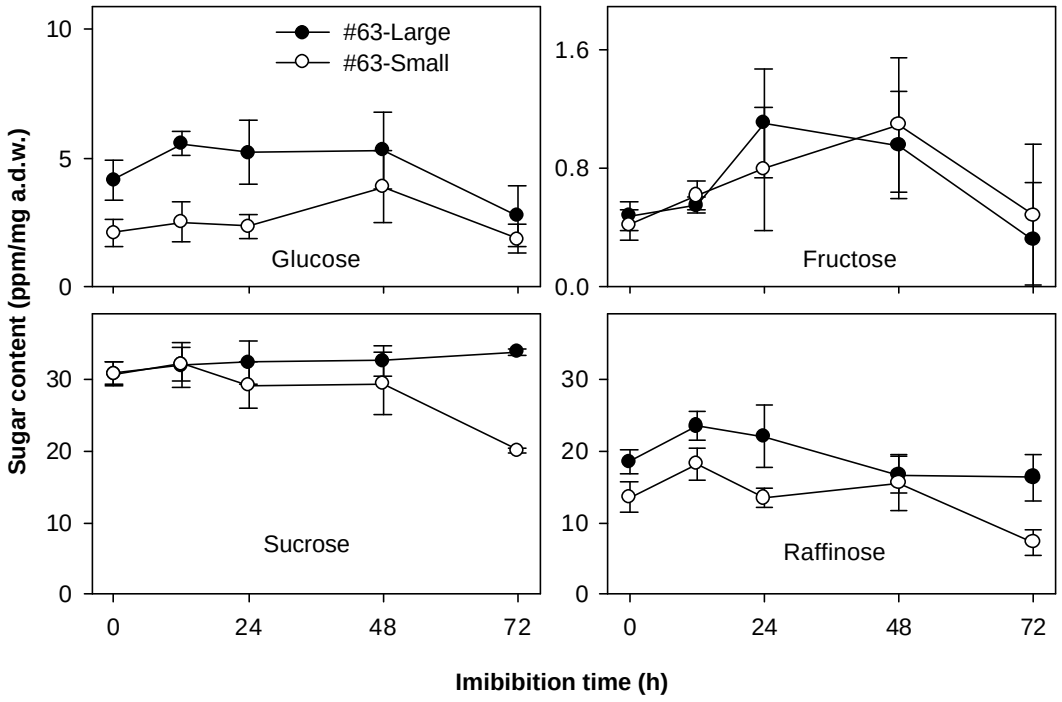


Fig. 6.8. Changes in soluble sugar contents during imbibition at 5°C for large and small seeds of winterfat (collection #63).

6.4 Discussion

Large seeds of winterfat germinated faster and had higher germination percentage at low temperatures than small seeds. This is in agreement with previous reports that germination percentage increased with increasing seed mass (Hou and Romo, 1998) and large seeds had a faster germination rate in winterfat (Springfield, 1973; Chapter 3). This study demonstrates that a high germination percentage of winterfat seeds can be achieved at subzero temperatures, as long as the temperature is above the base temperature that was estimated using the thermal time model. Winterfat is thought to germinate at near 0^oC temperatures (Booth, 1987). Under naturally occurring cooling rates in the spring, fully hydrated winterfat seeds can supercool to -4 to -5^oC without ice formation (Bai et al., 1998).

The small but significant difference in base temperatures ($< 1^oC$) for large and small seeds (Chapter 3) caused as high as a 2-fold difference in the time to achieve the same percentage of germination at subzero temperatures. The lower the temperature, the greater the differences in germination time between seed size classes at sub zero temperatures. Because germination time (D_{50}) and final germination percentage are more reliable characteristics for cold tolerance or sensitivity at the early development stage (germination) than LT_{50} (Massardo et al., 2000). Greater germination of large seeds in winterfat at low and freezing temperature showed higher resistance to low temperature than small seeds.

Germination of winterfat at subzero temperatures provides direct validation of the thermal time models, especially the different parameters for large and small seeds. However, the divergent estimation of the thermal time course indicates physiological changes may have occurred when imbibition temperatures approached base temperatures and the modification of model parameters may be necessary to reflect these changes. The linear relationship between germination rate and temperature can be altered near the base temperature (Marshall and Squire, 1996; Kebreab and Murdoch, 2000). Seeds may adjust physiological responses and processes if they stay in a long period of adverse germination condition such as water stress (Dahal and Bradford, 1990; Welbaum et al., 1998). One of the significant aspects of threshold

thermal and hydrothermal time models is that the models closely correlate with the physiological status of seeds than the empirically models (Meyer et al., 2000; Bradford, 2002).

Little difference in water uptake was observed between 5 and -1^{o}C imbibition temperatures for seeds and embryos of large seeds, but water uptake was much higher at -1^{o}C than at 5^{o}C for small seeds, especially in the embryo. Slower water uptake in large seeds at the subzero temperature may be due to less damage caused by this temperature. This, in turn, may enable larger seeds to better resist low temperatures, because fast water uptake at low temperatures can cause imbibition injury for seeds (Bedi and Basra, 1993; Bewley and Black, 1994). Cold stress can cause membrane lipid phase separation and phase transition (Sharom et al., 1994) and change membrane fluidity associated with cold signal transduction (Orvar et al., 2000), altering membrane permeability (Wanner et al., 1999; Saltveit, 2002). Conditions encouraging a low rate of water uptake also improved the rate and uniformity of seedling emergence in cauliflower (*Brassica oleracea* L.) (McCormac and Keefe, 1990).

The negative correlation between electrolyte leakage per unit seed mass and seed mass was observed in cowpea (*Vigna unguiculata* L.) due to the large surface to volume ratio of small seeds (Ismail and Hall, 2002). The higher surface/volume ratio of small seeds may cause faster water uptake. But this effect should be consistent across temperatures, which is not supported by our results. Therefore, the major influence of seed size on winterfat seed germination at low temperatures is related to the different degrees of damage on membrane function by subzero temperatures. The significantly high K^{+} leakage at initial imbibition stages in small seeds at subzero temperature indicates membrane damage. Increased membrane permeability allows less controlled water flux, or faster seed water uptake, which results in more membrane damage by the physical force. Ion leakage has also been used to measure membrane damage and repair in response to seed aging and osmotic priming in maize (*Zea mays* L.) (Ouyang et al., 2002), and chilling tolerance in cowpea (*Vigna unguiculata* L. Walp.) (Ismail et al., 1997; Ismail and Hall, 2002).

Negative correlations between seed weight and metabolic heat production rate (Rq) or respiration CO_2 production rates (R_{CO2}) per unit seed weight were observed at different temperatures and imbibition stages. The values of Rq and R_{CO2} correlate to metabolic activities and the ratio of Rq and R_{CO2} reflects substrate carbon conversion efficiency (Criddle et al., 1997). The ratio of Rq/R_{CO2} was not significantly different between large and small seeds in higher temperature (20^oC) in winterfat, possible due to the large variation among seeds. Generally, small seeds had higher metabolic activities but similar carbon conversion efficiency per unit seed mass than large seeds, especially at the lower temperature (10^oC). Carbohydrate oxidation results in the Rq/R_{CO2} values of 455 KJ mol^{-1}, while the oxidation of lipids releases 700-800 KJ mol^{-1} depending on their degree of instauration (Edelstein et al., 2001). The respirationary substrate for winterfat seeds was in the range of carbohydrates. Rq and Rco_2 are temperature-dependent (Hansen et al., 1994) and can be used to determine optimum and stress temperatures, metabolic rates and metabolic efficiency (Criddle et al., 1997; Taylor et al., 1998; Hemming et al., 1999; Thygerson et al., 2002). Cold tolerance mechanisms among different genotypes include maintaining high metabolic rates at stressed temperature (Edelstein et al., 2001). However, the higher Rq and R_{CO2} in small seeds than large seeds of winterfat do not agree with the greater cold tolerance in large seeds. The mechanisms in cold tolerance may vary among stages in the life history of plant. Diverse adaptation to stress exists among species (Rathinasabapathi, 2000).

The decrease in sucrose and raffinose contents of small seeds of winterfat at low temperatures may be related to their high metabolic rates compared to large seeds at the late germination phase (at 72 h). Low energy reserves for seedling growth after germination may cause low seedling vigor in small seeds, reducing the rate and percentage of seedling emergence in the field (Chapter 5). Even though the relative amount of perisperm reserve was similar between large and small seeds in winterfat as indicated by the similar embryo to seed ratio, the amount of glucose and raffinose per unit mass was significantly higher in large seeds than in small seeds. In addition, accumulation of compatible solutes, such as sugars and proline, has been proposed to protect macromolecules, thus, cellular structure and function under stress both at the

early radicle stage (Massardo et al., 2000) and the plant development stage (Wanner et al., 1999; Hoekstra et al., 2001; reference within). Germination is an anabolic and metabolically demanding stage and the higher reserves of compatible solutes may be particularly critical. Acquisition of desiccation tolerance in orthodox seeds is associated with the accumulation of raffinose and oligosaccharide reserve deposition during seed maturation (reviewed in Peterbauer and Richter, 2001), such as in seeds of pea (*Pisum sativum* L.) (Corbineau et al., 2000), maize (*Zea mays* L.) (Brenac et al., 1997), and wheat (*Triticum aestivum* L.) (Black et al., 1999). Sucrose, in particular, has been suggested to be important in preventing liquid crystalline to gel phase transition in the membrane lipid bilayer (Hoekstra et al., 2001). The tolerance mechanisms for abiotic stresses are interconnected (e.g., Wang et al., 2003), and the high content of sucrose, glucose and raffinose in winterfat seeds, especially in large seeds should contribute to its cold tolerance and germination at low temperatures.

In summary, large seeds of winterfat germinated faster and in higher percentage than small seeds at subzero temperatures. The germination of winterfat seeds at subzero temperatures validates estimated subzero T_b using thermal time model. The hypothesis that the variation in germinability between seed size classes at low temperatures is associated with physiological mechanisms is accepted. Carbohydrate conversion efficiency is higher in large seeds at low temperatures. Higher cold tolerance in large seeds was correlated with greater membrane integrity, less imbibition damage, higher contents of soluble cryoprotective sugars, glucose, raffinose and sucrose during germination at low temperatures.

7. General Discussion and Conclusions

7.1 Modeling seed germination and seedling emergence

7.1.1 Environmental factors on modeling germination

The results presented in this thesis show that θ_T and θ_{HT} models explain the most variance in the germination rate of subpopulations of winterfat under controlled environments as previously reported for other species (Garcia-Huidobro et al., 1982; Gummerson, 1986; Forcella, 1993; Benech-Arnold and Sánchez, 1995; Dahal and Bradford, 1994; Grundy, 1999; Kebreab and Murdoch, 1999; Roman et al., 1999; 2000; Allen et al., 2000a; Meyer et al., 2000; Rowse and Finch-Savage, 2003). Winterfat seed germination can be well quantified ($R^2 > 0.70$) by these models at temperatures between 2 and 20°C and ψ between -0.6 and -1.2 MPa. The constructed θ_T and θ_{HT} models are reliable and quantitative tools for describing winterfat seed germination time and germination fractions in general. This leads to the acceptance of the hypothesis that θ_T and θ_{HT} can be used to predict seed germination time of winterfat. However, the model assumptions are not always valid in winterfat and the major modification is the parameter consistency across environmental conditions, seed sizes, and collections. The parameter of T_b varied with seed size, seed collection (Chapter 3), seed imbibition temperature, and water availability (Chapter 4). Although the difference in T_b between seed sizes is only about 1°C, it causes faster germination in large seeds especially at low temperatures (Chapter 6).

Fundamental to the success of threshold models is the reliable, quantitative relationships of GR with T and/or ψ, because the estimation of model parameters is based on these relationships. As a result, model parameters have their physiological determinants of germination response to T and ψ (Bradford, 1995; 2002; Meyer et al., 2000). The linear relationship between GR and T in winterfat changes when T is near

T_b, such as at subzero temperatures (Chapter 6) and when ψ is reduced from 0 MPa (Chapter 4). The alteration of GR response to T or ψ often occurs when conditions approach germination thresholds. Decreasing T at suboptimal temperatures increased θ_H in winterfat seed germination (Chapter 4). The non-linear relationship as T approaches T_b has been reported in other species (e.g., Squire et al., 1997; Trudgill et al., 2000). The $\psi_{b(g)}$ distribution shifted to more positive values with increasing T when T was higher than T_o, instead of being constant at sub-optimal temperatures. The shifting explained the reduction of GR and germination percentage (Alvarado and Bradford, 2002; Rowse and Finch-Savage, 2003). Therefore, the physiological and biochemical responses to increasing T can be detected indirectly by the changing in model parameters. The shift of $\psi_{b(g)}$ of a seed population is related to physiological responses that are regulated by environmental factors, hormonal conditions (Ni and Bradford, 1992; Dahal and Bradford, 1994), and dormancy release (Meyer et al., 2000). It is not surprising that germination behavior changes at T over T_o and near T_b because multiple physiological responses including stress tolerance to these marginal temperatures may be involved other than merely rate response.

This study also provides evidences of the interactive effects of ψ and T on: 1) the shift of model parameters including T_b, $\psi_{b(50)}$, θ_T and θ_{HT}; 2) the modification of the linear relations of GR and T; and 3) the sub-optimal T range (Chapter 4). The relationship between GR and ψ can be linear (Gummerson, 1986; Dahal and Bradford, 1994) or non-linear (Squire et al., 1997; Rowse et al., 1999), depending on the temperature range, especially near T_o (Rowse and Finch-Savage, 2003). There is substantial evidence of interactions between ψ and T on germination (Cheng and Bradford, 1999; Kebreab and Murdoch, 1999; Roman et al., 1999; 2000; Larsen et al., 2004). Therefore, θ_{HT} model cannot be fulfilled in those cases when non-linear relations between GR and T or ψ and interactive effects exist. New model assumptions are necessary for those species when the interactive effects cannot be ignored (Kebreab and Murdoch, 1999; Bradford, 2002). The modification of model assumptions and the use of varied model parameters indeed increased the model predictability in this study (Chapter 4), accepted our hypothesis. This also indicates

that wild plant species with broad genetic background and natural adaptation history have more complex responses to temperature and water potential during germination than crops.

7.1.2 Modeling seedling emergence in the field

The θ_{HT} model can be used to accurately predict seedling emergence under some T and ψ conditions in the field using a simple correlation of θ_T between germination and emergence for winterfat (Chapter 5). Therefore, the hypothesis that θ_T and θ_{HT} are the major contributors to seedling emergence under field conditions is accepted for optimal conditions or with rapid germination events. To date, common approaches to predict seedling emergence are still imprecise (Forcella et al., 2000). From a practical point of view, models for predicting seedling emergence in the field should be balanced between simplicity and accuracy even though they often conflict. Furthermore, the physiological relevance of model parameters must be addressed so the most promising models can be developed for seedling emergence in the field. A generalized model across species and habitats or sites could be a challenge because of variation of species-specific mechanisms of adaptation and different dominant factors in environment at a particular habitat or site. However, threshold models provide a basic framework for modeling seedling emergence in the field (Forcella et al., 2000; Vleeshouwers and Kropff, 2000; Bradford, 2002; Rowse and Finch-Savage, 2003). Threshold models have been recognized as important approaches to predict seedling emergence in integrated weed management and crop production under variable field conditions (e.g., Forcella et al., 2000; Vleeshouwers and Kropff, 2000; Bradford, 2002; Rowse and Finch-Savage, 2003). When processes other than rate response also drive seedling emergence, the lack of model fit occurs. With different factors to be addressed or focused upon, models that quantify dominant environmental factors for specific applications could give a satisfactory prediction in seedling emergence. Sub-models for various dominant environmental factors have been developed to accommodate mechanical impedance of the soil (Whalley et al., 1999), soil burial depth (Prostko et al., 1998; Kolb et al., 2002), dormancy (Meyer et al., 2000), and priming effects (Finch-Savage et al., 2000; Hardegree and Van Vactor, 2000).

7.2 Physiological and ecological significance of threshold models

7.2.1 Physiological significance of threshold models

Differences in T_b between seed sizes of winterfat (Chapter 3) were related to the tolerance to low temperatures (Chapter 6). Large seeds had a lower T_b than small seeds because large seeds are more cold tolerant. The greater cold tolerance was accounted by less membrane damage, higher content of compatible sugars, and higher metabolic efficiency at low or subzero temperatures. In addition, at subzero temperatures, large seeds have a stable rate of water uptake while the rate of increase water content was greater in small seeds (Chapter 6). Therefore, the model parameters have physiological foundations. Because of the physiological relevance, the parameters of θ_{HT} model have been used as indices for comparing seed lots (Covell et al., 1986; Benech-Arnold et al., 1990), species with different adaptations or evolutionary history (Allen et al., 2000a; Steinmaus et al., 2000; Trudgill et al., 2000), and seed treatments (e.g., Hardegree and Van Vactor, 2000). Priming that advances physiological and biochemical processes of germination can also lower the θ_T requirement and T_b (Hardegree and Van Vactor, 2000). Parameters, such as T_b, have been reported as stable traits within crop cultivars (Bradford, 1995), but they are not constant for non-crop species due to distinct genetic variability among seeds within the population (Wang et al., 1995; Squire et al., 1997). Therefore we accept the hypothesis that variation in model parameters in seed sizes is related to physiological mechanisms of seeds.

The physiological determinant of T_b was associated with physiological mechanisms of cold tolerance. In previous studies, the physiological relevance and determinants of $\psi_{b(g)}$ are well documented (Bradford, 1995; 2002). The loss of seed dormancy during after-ripening is associated with $\psi_{b(g)}$ shifting to more negative values (Christensen et al., 1996; Meyer et al., 2000). The physiological adjustment of $\psi_{b(g)}$ may also occur when seeds are incubated at low ψ (Dahal and Bradford, 1994; Cheng and Bradford, 1999) and highly stressed temperatures (Alvarado and Bradford, 2002; Rowse and Finch-Savage, 2003). Hormones, such as ABA and GA can

increase the $\psi_{b(g)}$ and interact with ψ to control germination (Corbineau and Côme, 2000; Toorop et al., 2000). Investigations on biochemical and molecular aspects are promising in identifying the establishment of $\psi_{b(g)}$ threshold in seeds (Chen and Bradford, 2000; Chen et al., 2002).

7.2.2 Ecological significance of threshold models

Model parameters reflect adaptations to local habitats in winterfat (Chapter 3). Seeds collected from the site with lower spring temperatures had a significantly lower T_b than that from the site with higher spring temperatures. On the other hand, the requirement of $\theta_{H(50)}$ and θ_{HT} was lower in the collection from the drier and colder site compared to the warmer and wetter site (Chapter 4). Steinmaus et al. (2000) studied the relationship between model parameters and germination responses in two winter annuals and six summer annuals and found that T_b was lower in winter annuals than in summer annuals and model parameters were associated with the patterns of plant establishment. Similar patterns were also found in 31 wild species growing in UK and the higher the T_b, the less the θ_T required within species (Trudgill et al., 2000). These studies indicate that early germinators start early but slowly in the growing season, while later germinators germinate fast when T is warmer, balancing the strategies between competition and seedling establishment. Spring germinating seeds tend to germinate early for competition of resources and to ensure the completion of life cycle (Angus et al., 1981). These studies demonstrated that θ_{HT} models for germination have ecological and biological relevance to plant survival and growth strategies. The common parameters in threshold models can be used to characterize germination to environments in different species (Allen et al., 2000a; Steinmaus et al., 2000; Trudgill et al., 2000), includes cardinal temperatures, germination T range, and maximum germination rates. Recently, seasonal changes in the germination behavior of plants in natural ecosystems can be predicted by the threshold models that are combined with modeling environmental conditions (Hardegree et al., 2003).

7.3 Modeling applications and seedbed ecology of winterfat

7.3.1 Microclimate and safesites

Winterfat seeds germinate readily and rapidly when T and ψ are favorable. Seed germination can reach >50% within 24 h in distilled water (Chapter 3) and 10% of the seedlings can emerge within 3 to 4 days after seeding under optimal field conditions (Chapter 5). Winterfat seeds can utilize relatively short periods of favorable temperature and water conditions to germinate and emerge from the soil surface before drought and desiccation affect seedling survival and establishment (Hou et al., 1999). Seeds can germinate at subzero temperatures with extraordinary cold tolerance to capture the earliest high water when snow melts. Conversely, the reduced ψ greatly decreased germination rate and percentage in all tested temperature (Chapter 4). Although winterfat is a cold desert species of North America, it is sensitive to low ψ at the early developmental stages such as germination based on this study. Therefore, snowmelt and precipitation in the spring will likely promote the seedling establishment of winterfat. Rapid germination when water is available may be drought avoidance mechanism for this species.

The interaction of ψ and T affects seed germination in winterfat (Chapter 4). Water availability can change seed responses to temperature during germination as reflected by θ_T model parameters, T_b and T_o. Therefore, GR and sensitivities to T stress may vary according to seedbed water status. When water is limiting in a seedbed, three temperature-related responses simultaneously affect seed germination. That is, GR is slower at a given T, less θ_T can be accumulated, and more θ_T is required to achieve the same germination percentage because T_b and θ_T increase linearly with decreasing ψ (Chapter 4). On the other hand, seeds can germinate at low temperatures or in cold seedbeds only when water is plentiful since θ_H and $\psi_{b(50)}$ increases largely when T is lower than 2^oC (Chapter 4). This is in agreement with the observation that winterfat seeds germinate under snow in early spring (Hilton, 1941; Romo, 2004), and provides physiological explanations of the importance of the water

status in seedbeds. Therefore, seedbeds that trap snow, or that maintain soil water, such as with litter cover, can promote winterfat germination in early spring.

Poor seedling emergence was observed when winterfat was seeded in late May and early June even with sufficient soil water (Chapter 5). Winterfat seeds consistently germinate in late March and early April in southern Saskatchewan (Romo unpub. data). This study showed that higher temperatures of the seedbed were the major cause for the poor seedling emergence, with the possibility of pathogenic activities (Garvin et al., 2004). The study supports previous reports that the best sowing dates for winterfat are in late fall, winter or early spring (Booth, 1992; Romo, 2004).

7.3.2 Seed size and ecotypic variation

Large seeds in this study have higher germination percentage and GR than that of small seeds (Chapter 3 and 6). Large seeds germinate faster than small seeds especially at lower temperatures because of lower T_b for germination (Chapter 3), less θ_H requirement (Chapter 4), higher cold tolerance, and more efficient respirational conversion at low T imbibition (Chapter 6). Seed size and seedling vigor are positively correlated in winterfat (Springfield, 1973; Hou and Romo, 1997; 1998; Hou et al., 1999). That large seeds germinated faster at low seedbed temperatures enable early emergence in the spring. Early emerging seedlings of winterfat have more freezing and desiccation tolerance than those emerging later (Hou and Romo, 1997; Hou et al., 1999), therefore higher chances of survival and establishing in seedlings. This study confirms the recommendation of heavier seeds for seeding winterfat (Booth, 1992; Hou and Romo, 1998) and it provides a physiological basis for this difference among different size of seeds.

The two seed collections used in this study were different in their germination responses to the environment associated with their original climatic characteristics (Chapter 3). Winterfat is widely spread in the Great Plains of North American (see Introduction). Ecotypic or population variation existed considerably in seed water content and metabolic activities during imbibition (Bai et al., 1999; Thygerson et al., 2002). Therefore, suitable ecotypes or populations may be important for specific

conditions for the best establishment of its restoration. The ecotypic variation should also provide guidelines for ecovar development and propagation in winterfat.

7.4 Future research

7.4.1 Germination and seedling elongation rate in response to environmental factors

The linear relations between $GR_{(g)}$ and T were changed as T approached T_o at reduced water potentials (Chapter 4), which disagree with the assumption that $T_{o(g)}$ is generally constant across ψ and germination subpopulations (Gummerson, 1986; Bradford, 1995; 2002). The GR as a function of T can be observed in a U-shape with a wider T_o range (e.g., Rowse and Finch-Savage, 2003). The width of this plateau can differ among seed lots (Ellis and Butcher, 1988). Further investigations on changes in T_o with ψ and differences among subpopulations or seed lots may lead to more accurate estimation of model parameters that are estimated based on the linear relations of GR and T, such as T_o, θ_T and T_b. In addition, the alteration of T_b and T_o at reduced water potential may narrow the temperature range for germination, and seeds will be more sensitive to T. This may help explain low seedling emergence from late sowing dates (Chapter 5), because the optimal T may shift down at reduced water potentials. The θ_{HT} model predicted inaccurately germination time courses at subzero T (Chapter 6). The quantitative relationships and physiological adjustments of seeds at T near T_b and T_o, and their interactions with ψ remain an area for further study.

Although the θ_T requirements of seed germination and seedling emergence can be well correlated by an exponential function, the effect of T and ψ on seedling elongation was not studied directly in winterfat. Because of the involvement of seedling growth in seedling emergence, understanding the elongation rate as affected by T, ψ, and other related factors, such as mechanical impedance, will improve modeling seedling emergence in the field. Seedling elongation prior to emergence is largely missing from the literature in spite of being a critical aspect in modeling emergence (Forcella et al., 2000). Parameters such as T_b and T_o were found different

not only between seed germination and seedling elongation, but also between the elongation of the shoot and radicle in *Chenopodium album* L. (Roman et al., 1999). In addition, temperature is recognized as a major driving force for seedling elongation (Fyfield and Gregory, 1989; Oryokot et al., 1997; Roman et al., 1999; 2000), whereas rainfall also influences the timing of field emergence (e.g., Roberts and Potter, 1980). Therefore, quantifying seedling elongation rate as affected by these interacting environmental factors is important for seedling emergence models.

7.4.2 Modeling seedling emergence under field conditions

Although various equipments are available to measure meteorological variables, accurate and continuous monitoring of several environmental variables for modeling purposes is still unsatisfactory. One of the environmental variables that is difficult to measure and make meaningful relevance to seed germination is soil water. Methods for direct and continuous monitoring of soil water potential are still unavailable or lack of accuracy (Forcella et al., 2000). Thermal time models based on germination at constant temperatures well fit under variable field temperature regimes (Hardegree and Van Vactor, 1999) even though more studies are needed on the physiological mechanisms. Hydrothermal time models developed under constant ψ have poor fit under field conditions and the effects of soil water fluctuation on germination have not been well investigated except for priming effects. Furthermore, when soil water fluctuates in the field, seed water content is arguably not the same as the surroundings (Finch-Savage et al., 2000). The endosperm of barley (*Hordeum* L.) seeds can be a temporary water reservoir during seedling elongation under reduced soil water (Allen et al., 2000b) and seed water may not change as rapidly as soil water (Allen et al., 2000a; Finch-Savage et al., 2000). Winterfat seeds germinate better in the field with diaspores attached than naked seeds (Booth, 1987). Removing the bracts of winterfat seeds significantly reduces seedling establishment (Booth and Schuman, 1983). The hairy structure and enclosing bracts of winterfat diaspores may help conserve seed hydration when ambient humidity decreases (Booth and Schuman, 1983).

Several growth models for crops used soil ψ in the surface layers that were estimated using a complex array of measurements including soil surface albedo, soil litter residues, soil bulk density, infiltration, above ground humidity, radiation, temperature, and wind speed (Forcella et al., 2000). Only a few simple and reliable models for estimating soil seed-zone ψ are available for modeling seedling emergence (Forcella, 1993). More models are needed for different soil types, microclimates and specific seedbed conditions. Accurate and reliable measurements of environmental variables as input parameters should be the first step for improving the accuracy of models in the field. The relation between the surrounding water environment and seed water status should also be considered, especially under the highly fluctuating conditions of soil surface layers.

Interactions among environmental variables are common and cannot be neglected in many cases (Vleeshouwers and Kropff, 2000; Whalley et al., 1999; Forcella et al., 2000; Romo, 2004). Soil water clearly affects soil temperature (Chapter 5) and may also affect soil mechanical impedance, soil air composition and air quantity. Soil texture affects hydraulic properties more than temperature, causing significant differences of priming effects in four grass species (Hardegree and Van Vactor, 2000). Quantifying these interactions among environmental variables will lead to better understanding of seedling emergence under different seedbed conditions and landscape positions.

Additional studies are needed before the variability in seedling emergence can be predicted under natural seedbed conditions, sub-models for seed-zone microclimate nay be a critical component of emergence models. The correlation between input parameters of microclimate, seedbed conditions and meteorological variables can be another approach for modeling seasonal and annual patterns of emergence in the field. Flerchinger and Hardegree (2004) used the Simultaneous Heat and Water (SHAW) model to simulate near-surface soil temperature and water in different soil types for predicting potential seed germination in post-wildfire revegetation. The SHAW model can be used for long-term simulations of seedbed microclimate that is necessary for evaluating potential germination responses of species regeneration (Hardegree et al., 2003, Flerchinger and Hardegree, 2004).

Incorporating these approaches into the seedling emergence model is promising and may provide reliable predictions of seedling emergence in the field over a large time and space scale.

7.4.3 Seed deterioration and seedling mortality

One assumption of germination modeling is that seed viability remains constant across germination conditions. As a result, the variable and continuous process of seed deterioration is generally overlooked in these models. Seed deterioration affects GR and germination percentage (Bradford et al., 1993). The deterioration occurs during prolonged germination processes caused by low T and/or ψ, especially for small seeded species with limited seed reserves like winterfat. The favorable T and ψ conditions for seed germination can be also optimal for pathogens, increasing the chance to be attacked for seeds. Winterfat seed deterioration during germination tests was associated with high T (25^{o}C) and ψ (0 MPa). Seed deterioration during seed storage can be quantified using T, seed water content or relative humidity, and accelerated aging treatments (Ellis and Roberts, 1981; Bradford et al., 1993). Although seed mortality during imbibition is recognized in some theoretical framework of germination modeling (Allen, 2003), not much attention has been paid to the deterioration and the mortality during the processes of germination. Bradford et al. (1993) studied the relationship between GR and seed deterioration and found that the continuous and progression nature of deteriorative processes in seeds increases the time requirement of germination in a seed lot. Their data also confirmed a critical imbibition time after which no additional normal seedlings were produced.

The final percentages between seed germination and seedling emergence diverged largely at the same thermal and water conditions in winterfat (Chapter 5), seed and seedling mortality in the soil is highly suspected. It is unclear how environmental conditions, especially T, ψ and their interactions with imbibition time, affect seed deterioration. Seedling mortality may be affected by soil water, soil temperature, mechanical impedance of soil, soil aerobic conditions, and seed reserves (Finch-Savage et al., 1998; 2001; Whalley et al., 1999). Longevity of moist seeds

(water content > 15%) continuously declined in anaerobic environment, but increased when oxygen was present (Roberts and Ellis, 1989). Although seed vigor or seed deterioration are recognized as being closely related to seed germination and seedling growth, they have not been extensively studied during germination and emergence. Further clarification and quantification of seed deterioration in soil and seedling mortality during penetration from soil would enhance predictability of models for field seedling emergence.

7.4.4 Cold and freezing tolerance of winterfat

Winterfat seeds have high cold and freezing tolerance during seed hydration and germination, which agrees with previous studies (Hilton, 1941; Booth, 1987; Bai et al., 1998). How winterfat seeds develop cold tolerance during germination remains unknown. For example, it is not clear whether the tolerance is acquired from acclimation during imbibition or from parental inherit and maturation. Metabolic rate and respiration measurements showed no evidence of cold acclimation during germination of winterfat seeds (Thygerson et al., 2002). Identifying physiological mechanisms from diaspore structure, tissue, to cellular structure and at biochemical and molecular levels for this cold tolerance would be valuable in understanding cold and freezing tolerance at early development stages, local ecotype adaptations, and crop improvement. Selecting early germinating sub-populations may produce genetically distinct populations with lower T_b for germination (Squire et al., 1997). Germination at low temperature is likely under genetic control and needs to be further investigated. Research on the genetic basis of GR (Squire et al., 1997; Foolad and Lin, 1999) would also be useful for understanding physiological determinants of model parameters.

In conclusion, thermal time and hydrothermal time models can quantify seed germination in winterfat, but model parameters varied with seed size and germination conditions. The interactive effects of T and ψ exist, altering germination responses and model parameters. Germination models can be the framework for seedling emergence and further studies on environmental factors and their effects on seedling growth would lead to the improvement of prediction of seedling emergence. The

model parameters are associated with physiological mechanisms of environmental regulation and ecotypic adaptations for seed germination.

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Appendix A. Water Relations during Seed Germination Using an Aquaporin Inhibitor $HgCl_2$

Abstract Water relations play a critical role in regulating seed germination and seedling growth. The function of aquaporins in intact seeds was investigated by $HgCl_2$ inhibition during germination and seedling growth of winterfat (*Krascheninnikovia lanata*). A serial concentration of $HgCl_2$, 0, 30, 35, 40, 50, 100 μM, was used during seed imbibition and seedling growth. Water uptake, water distribution among seed tissues, and water mobility were affected by the inhibition observed by NMR spectroscopy. Water uptake pattern in a winterfat seed was from the embryo to the perisperm and mercury significantly restricted water flux to the perisperm during seed imbibition. With higher water content, there was lower proton intensity, that is, less mobile water or high water viscosity, in the embryo by $HgCl_2$ inhibition. The results showed aquaporins functioned in the initial seed water uptake and aquaporin inhibition reduced significantly germination rate and final germination percentage. Seedling water content was reduced largely by $HgCl_2$ in both root and cotyledons. The root length was 2 to 4 times shorter with mercury inhibition. Dry matter allocated more to cotyledons than to root during seedling growth with the inhibition, showing higher impact on root growth than cotyledon growth.

A.1 Introduction

Water relations play a key role in regulating seed germination processes (Bradford, 1995). Water flowing across tissues has three routes: through cell walls (apoplastic path), from cell to cell across either the plasmodesmata (symplastic path), or traversing the cell membranes (transcellular path) through aquaporins, a water selective channel (Preston et al., 1992; Agre et al., 1998). Aquaporins are expressed prominently in various membrane compartments including plasma (Kammerloher et al., 1994; Johansson et al., 1998) and vacuolar membranes, tonoplast (Maurel et al.,

1993). Once proteins are involved in water relations, the cell has the ability to regulate its abundance and to modulate its activities (Maurel and Chrispeels, 2001; Jovot and Maurel, 2002, and references in). The cell-to-cell pathway plays a major role in overall root water uptake of tomato (80-90% of total) (Maggio and Joly, 1995) and is important in whole-plant water relations of tobacco (Siefritz et al., 2002). Aquaporins are enriched in zones of fast cell growth and expansion that are undergoing vacuolization (Ludevid et al., 1992; Schäffner, 1998), or in areas where high water flow or solute flux density is expected (Ludevid et al., 1992; Sarda et al., 1997; Barrieu et al., 1998) such as cells of vascular and young tissues (Barrieu et al., 1998; Frangne et al., 2001). These observations suggest that aquaporins are involved in cell elongation and growth, osmotic regulation and resistance to drought and salinity stress (reviewed in Schäffner, 1998; Johansson et al., 2000). However, amplification of the aquaporin function in intact plant tissue has lagged behind molecular and cellular investigations. Aquaporins are also abundant in seeds; isoform α-TIP was estimated to constitute 2% of total extractable protein from bean cotyledons (Johnson et al., 1989). Its role remains unclear, however, after the mobilization and conversion of large storage molecules during seed germination, α-TIP is successively substituted by the vegetative γ-TIP isoform (Maurel et al., 1997). As a result, various investigations have concluded that aquaporins mediate storage nutrient metabolism during seed germination and regulate cell growth in radicle protrusion (Maurel et al., 1997; Gao et al., 1999). There is, however, little research examining the functions of aquaporins in water relations during seed germination, radicle elongation, and subsequently, metabolic activity, germination rate, and seedling growth.

Inhibition of water transport by mercury ($HgCl_2$) was reported in cell membranes isolated from higher plants (Maurel, 1997; Niemietz and Tyerman, 1997) and in whole root systems (Maggio and Joly, 1995; Carvajal et al., 1996; Barrowclough et al., 2000; Jovot and Maurel, 2002). The activity of water channels in the membrane of the cortex cell of wheat roots exhibited more than 75% inhibition by mercurials (Niemietz and Tyerman, 1997; Zhang and Tyerman, 1999). Mercury acts as an efficient blocker of many aquaporins (Daniels et al., 1994; Jovot and

Maurel, 2002). Mercurials bind to the sulfhydryl group of cysteine residues located in the vicinity of the aqueous pore and induce a conformational change (Barone et al., 1997), thereby blocking water permeability (Agre et al., 1998). As long as plant materials with genetically altered aquaporin expression are not available, mercurials are the only general blockers known to date and will remain as a useful tool. Mercuric chloride, $HgCl_2$ is able to attack intra-membraneous sites because of the permeability across membrane (Schütz and Tyerman, 1997). Unfortunately, these compounds have side effects and target other proteins that have Cys residues as well (Maggio and Joly, 1995; Carvajal et al., 1996). The concentration of the mercury should be as low as possible to reduce indirect metabolic inhibitions and the results should be interpreted with caution. The proper dose for aquaporin inhibition may also be tissue, organ and species-specific, and proper use of $HgCl_2$ can obtain reversible changes and reliable results (Maggio and Joly, 1995; Zhang and Tyerman, 1999; Wan and Zwiazek; 1999).

The seed germination process has been described by a tri-phasic water uptake curve (Bewley, 1997). Recently, Nuclear Magnetic Resonance (NMR) technology has been recruited to examine seed water (Gruwel et al., 2001; 2002). NMR is a powerful, non-destructive tool for visualizing water mobility, characterizing its physical states and quantifying water distribution within living tissues (reviewed by Ishida et al., 2000). Viable and nonviable seeds can be distinguished by their water uptake patterns within seed tissues soon after the initiation of imbibition using NMR techniques (Gruwel et al., 2002). Phases of seed water uptake can be studied using ^1H relaxation time measurements with NMR (Gruwel et al., 2001). In this paper, we studied the water relations of seed germination at low-temperature with two collections of winterfat (*Krascheninnikovia lanata* (Pursh) A.D.J. Meeuse & Smit) using ^1H NMR spectroscopy. Winterfat seeds are capable of germinating and accumulating heat unit, thermal time, towards germination at sub-zero temperatures (Wang et al., 2004). Fully hydrated seeds and young seedlings of winterfat are tolerant to freezing stress (Bai et al., 1998). The specific objectives of this study are to determine: 1) the role of aquaporins in seed water uptake and early seedling growth of winterfat at low temperatures; 2) the effect of $HgCl_2$ concentration on seed water

uptake, seed viability, germination and seedling growth; and 3) water distribution and biophysical status within a germinating seed. We hypothesize that water relations of winterfat are largely associated with aquaporins from seed imbibition to early seedling growth, which is critical for radicle and root elongation.

A.2 Materials and methods

Inhibition treatments using $HgCl_2$ Five replicates of twenty seeds were imbibed at 5 ^{o}C in darkness. The imbibing solution for each treatment replicate (2.5mL of 0, 30, 35, 40, 50 and 100 μM $HgCl_2$ (Sigma, USA)) was soaked into two layers of filter papers (Whatman No.1) in 5-cm Petri dishes. Germination was checked and recorded daily for seven days. Germinated seeds, radicle > 1 mm, were transferred to the same concentration of $HgCl_2$ or distilled water after rinsing at 20 ^{o}C. The viability of non-geminated seeds was determined using 0.5%, 2,3,5-triphenyltetrazolium chloride (TTC) at the end of the germination test. The length of seedling radicle and hypocotyl (hereafter root), and cotyledons was measured after 4 days of growth. Water content and dry weight of seedlings was determined using oven drying method (48 hours at 80 ^{o}C).

Measurement of seed water uptake Twenty seeds in five replicates were imbibed in distilled water or 30 μM $HgCl_2$ solution at 5^{o}C. Preliminary tests indicated that the lethal dose of $HgCl_2$ for 50% reduction (LD_{50}) in viability of winterfat was usually higher than this concentration. Seed water content was measured at 2, 4, 8, 12, 24, 48 and 72 h intervals. Seeds were blotted using paper tissues to quickly remove surface water and seeds were weighed with a microbalance (± 1μg). Water content was determined using the oven drying method (48 hours at 80^{o}C). The same seeds were measured repeatedly for whole seed water content, while a different seed was used for embryo isolation and weighed at each interval.

NMR measurement during seed imbibition Four replicates of five seeds from collection #63 were imbibed at 5^{o}C with distilled water or 30 μM $HgCl_2$ solution for 2, 14 or 48 h before radicle protrusion. NMR spectroscopy was used to determine the distribution of mobile water and its biophysical status among seed tissues for the three time intervals. NMR spectroscopy was performed on a Bruker

Advance DRX 360 WB system (Bruker BioSpin Ltd., Milton, ON, Canada), operated at 360.13 MHz. ^{1}H microimaging was performed at room temperature (20^{o}C) on a single seed mounted in a shortened pipette tip inserted into a plexiglass foot and then placed in a 10-mm NMR tube. A Bruker Micro-2.5 cm microimaging probe head and gradient system was used for the measurement. Relative humidity in the tube was maintained by damp cotton wadding at the top and bottom of the tube. Each image took 17 minutes for 4 scans and used a Field of View (FOV) of 1.5 cm and a slice thickness of 1.0 mm. Spin intensity was normalized using the highest signal intensity at 48 h for comparisons among intervals. Spin-lattice relaxation rates, R_1 ($1/T_1$), and spin-spin relaxation rate, R_2 ($1/T_2$), were also measured at each interval. The NMR spectrometer was calibrated prior to each measurement using the solution of 0.01M $CuSO_4$ and four-point calibration was used covering the expected range of spectral intensities. The mean proton intensity was analyzed for cotyledons, hypocotyl, radicle and perisperm by defining regions-of-interest. The defined regions was processed within the same area (40 pixel) using the software ImageJ 1.31V (http://rsb.info.nih.gov/ij/, Wayne Rasband National Institutes of Health, USA) from NMR raw data.

Data analysis Data were analyzed by SAS package (The SAS® System for Windows, V8.2), ANOVA and GLM procedures were used at 0.05 significant level.

A.3 Results

A.3.1 Effect of $HgCl_2$ concentration on seed germination and viability

Both seed germination and viability significantly declined with increasing concentration of $HgCl_2$ at 5^{o}C incubation (Fig. A.1). More significant inhibition (p value: 0.021~0.000) by $HgCl_2$ on seed germination and viability was found in small seeds than large seeds in both collections. For example, 55% of large seeds and 25% small seeds of collection Cela germinated in the presence of 30 μM $HgCl_2$. In addition, 87% of large seeds and 57% of small seeds of collection #63 were viable at 30 μM $HgCl_2$ imbibition for seven days. The LD_{50} of $HgCl_2$, according to viability tests after seven days exposure at 5^{o}C for large and small seeds were 65 and 35 in

collection #63, and 56 and 32 μM in collection Cela, respectively. Germination rates were significantly decreased by $HgCl_2$ inhibition (Table A.1), especially in the small seeds of both collections.

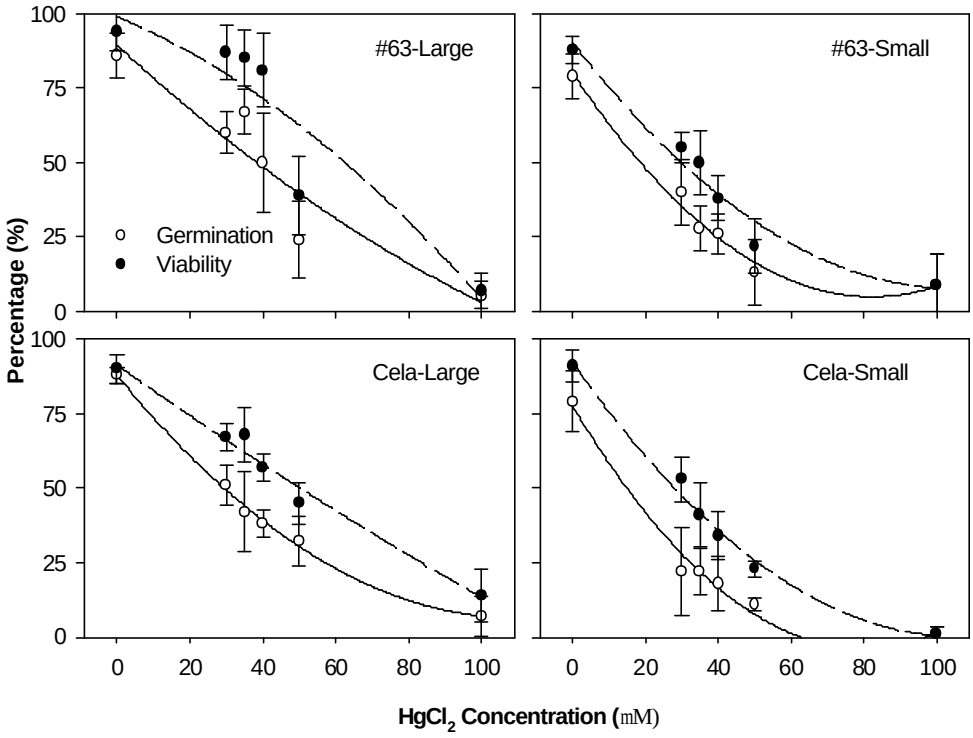

Fig. A.1 Seed germination and seed viability at 5°C after seven days exposure to different concentrations of $HgCl_2$ in large and small seeds of two collections of winterfat (*Krascheninnikovia lanata*). Fitted lines are a least square fit quadratic function.

Table A.1. The effect of mercury ($HgCl_2$) and its concentration on germination rate index[1] (GRI) of large and small seeds of winterfat in two collections at 5°C.

$HgCl_2$ (μM)	#63-Large		#63-Small		Cela-Large		Cela-Small	
0	3.88	a[2]	3.25	a	3.99	a	2.82	a
30	1.93	b	0.85	b	1.29	b	0.31	b
35	2.34	bc	0.44	bc	0.95	bc	0.24	b
40	1.41	c	0.37	c	0.81	c	0.20	b
50	0.31	d	0.13	c	0.58	c	0.07	b
100	0.02	d	0.08	c	0.05	d	0.00	b

[1] GRI=g/p *Σgi /ti, where *g* is the number of germinated seed, *p* the total number of seeds, and *ti* the time to germination *gi* in days.

[2] Values followed by the same letter are not significantly different at 0.05 significant level.

A.3.2 Effects of $HgCl_2$ on seed water uptake

The water uptake of an individual seed was reduced by the $HgCl_2$, while that of embryo was increased in both seed size classes of both collections (Fig. A.2). The differences of water contents of seeds and embryos between the imbibition in distilled water and in $HgCl_2$ were not significant, but the trend was consistent in both collections. The individual seed water content was 5 to 20% lower in $HgCl_2$ solution than in distilled water after 72 h imbibition. By contrast, embryo water content was 3 to 35% higher in $HgCl_2$ solution than in distilled water after 72 h imbibition. Therefore, $HgCl_2$ reduced total water uptake of seeds, and water distribution between the embryo and the rest of the seed tissue was altered.

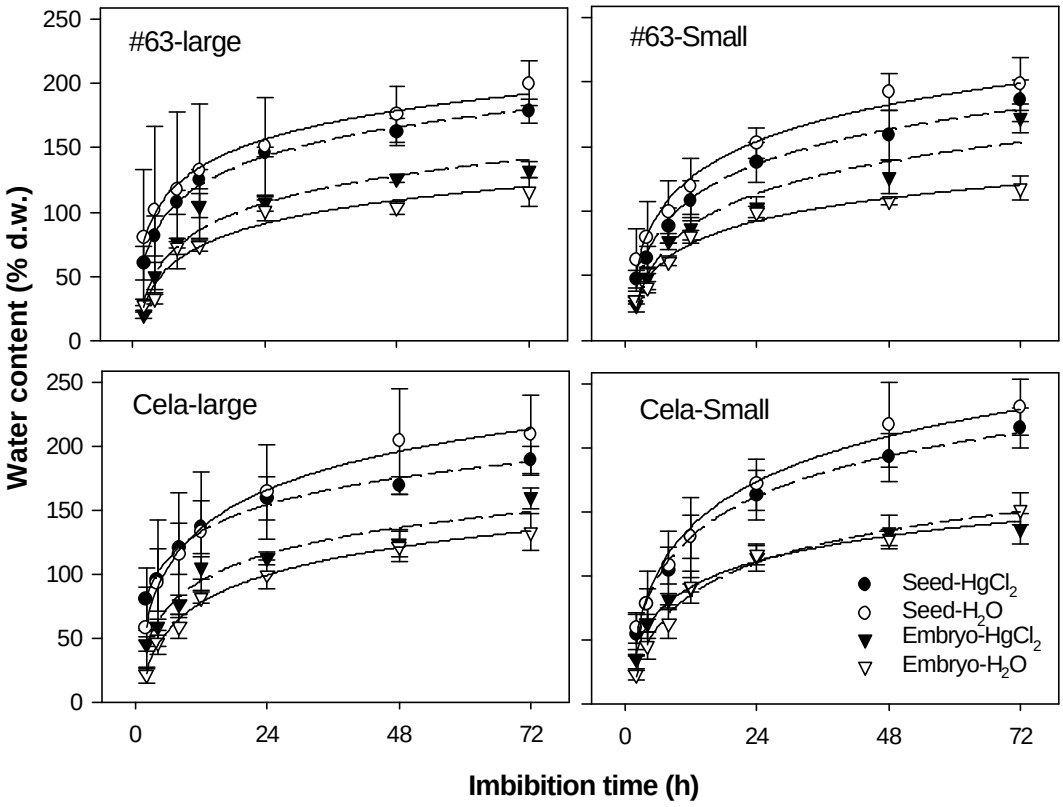

Fig. A.2. The effects of aquaporin inhibition by 30 μM $HgCl_2$ on water uptake during seed imbibition at 5°C in large and small seeds of two collections of winterfat (*Krascheninnikovia lanata*). Fitted lines are a least square fit quadratic function.

The quantity of mobile water (water that is available for transport) in a seed during seed imbibition can be visualized by ^{1}H NMR images (Fig. A.3). No apparent differences in mobile water between $HgCl_2$ and H_2O imbibition were detected after 2 h imbibition. After 14 h imbibition, however, $HgCl_2$ reduced mobile water in the embryo, and significantly (P values, 0.013) in the perisperm (Figs A.3, A.4).

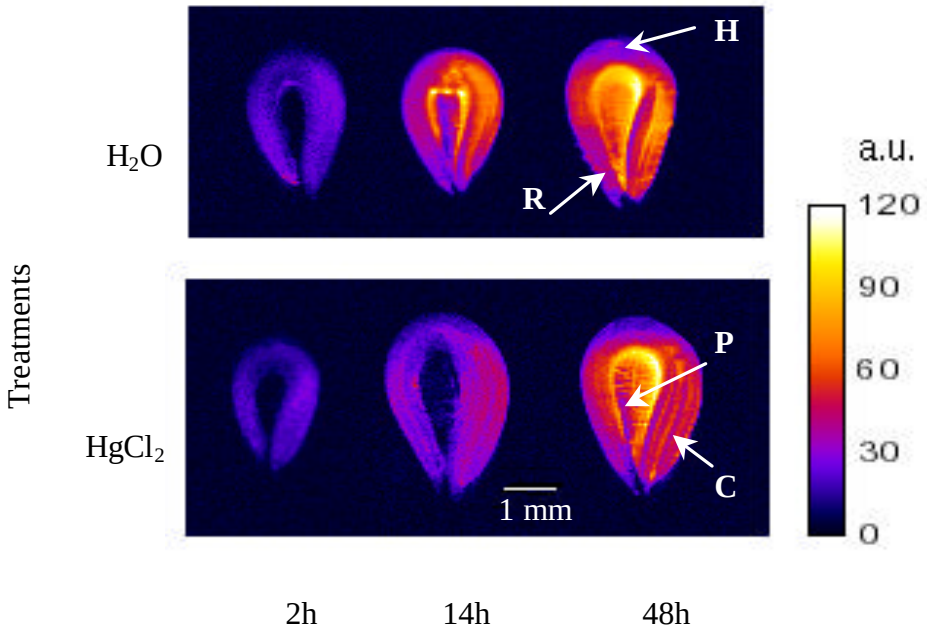

Fig. A.3 ^{1}H NMR images of winterfat (*Krascheninnikovia lanata*) seeds of collection #63 at three imbibition time intervals with distilled water and 30 µM $HgCl_2$ at 5°C with Field of View (FOV) of 1.5 cm and a slice thickness of 1.0 mm. C: Cotyledons, P: perisperm, R: Radicle, H: Hypocotyl, a.u.: arbitrary unit.

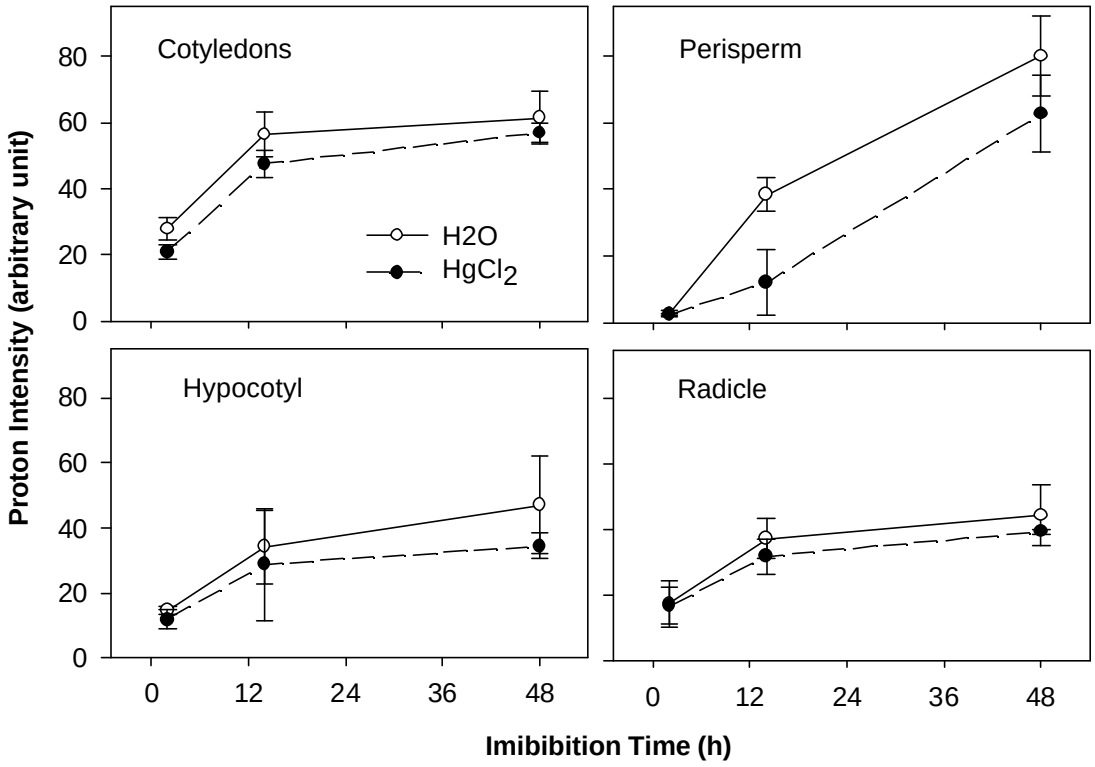

Fig. A.4. Mean proton intensity of regions of interest from four replicated ^{1}H NMR images at three imbibition time intervals with distilled water and 30 μM $HgCl_2$ at 5°C in winterfat (*Krascheninnikovia lanata*) of collection #63.

A similar trend was noted at 48 h imbibition but the increase in the amount of mobile water from 14 to 48 h was highest for the perisperm. No statistically significant (P value: 0.074) changes were observed in the hypocotyl; however this is attributed, in part, to high variation among individual seeds. Figs. 3 and 4 indicate water first enters the embryo then the perisperm. Proton spin-lattice relaxation rate of whole seeds, R_1, was affected by $HgCl_2$ at the initial water uptake stage (<24 h). However, R_2 was similar between the two imbibition treatments (P value: 0.91) (Fig. A.5).

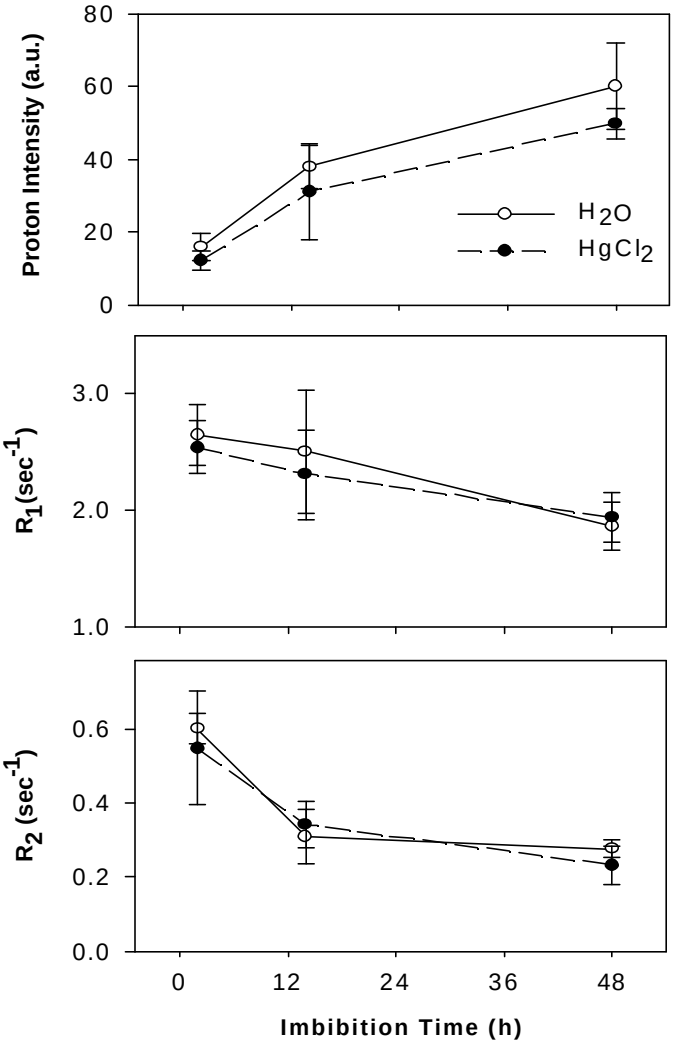

Fig. A.5. Seed mobile water measured by proton intensity (a.u.: arbitrary unit), and water mobility measured by proton spin-lattice relaxation rate, R_1 ($1/T_1$), and spin-spin relaxation rate, R_2 ($1/T_2$), during seed imbibition at 5°C in winterfat (*Krascheninnikovia lanata*) of collection #63.

A.3.3 Effects of aquaporin inhibition by $HgCl_2$ on seedling water uptake

The water content of both roots (radicle and hypocotyl) and cotyledons of young seedlings was greatly decreased by $HgCl_2$ during seedling growth (Figs A.6, A.7). Root water content was reduced more than double by 30 μM $HgCl_2$ (Fig. A.6).

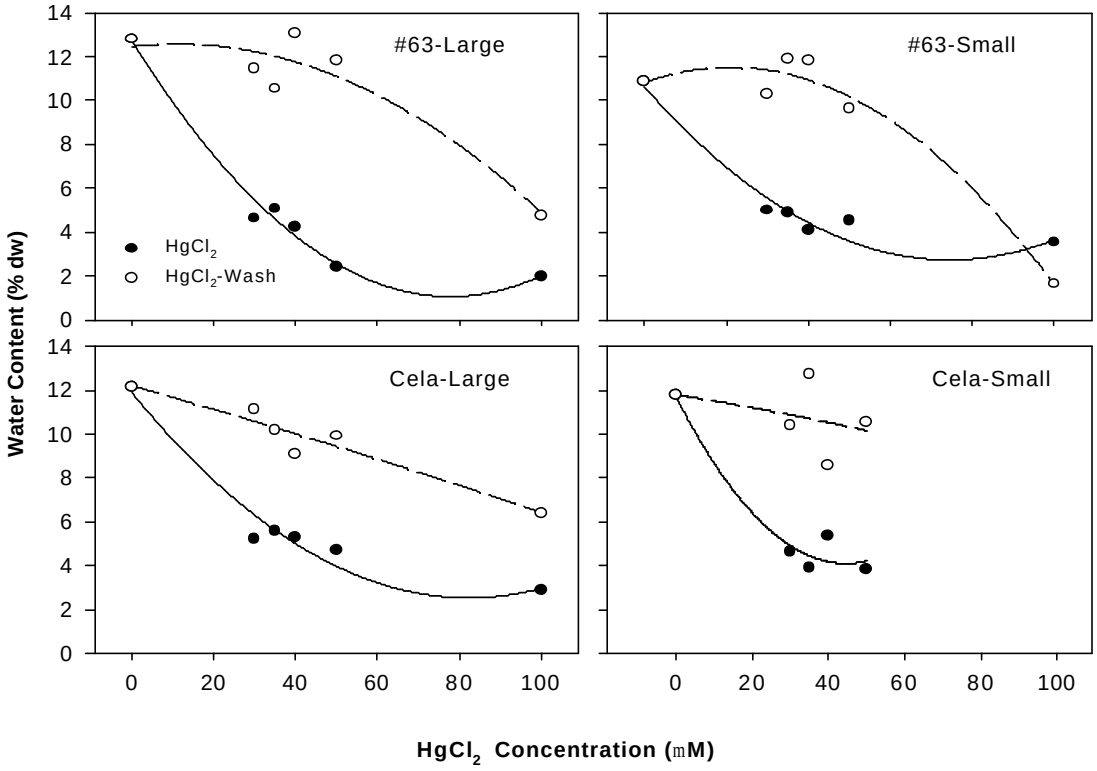

Fig. A.6. The water content of seedling root (radicle and hypocotyl) four days after germination at 20°C with the same concentration of $HgCl_2$ or with distilled water after rinsing in large and small seeds of two collections in winterfat (*Krascheninnikovia lanata*). Seeds were imbibed at 5°C and were transferred to 20°C when radicle protrusion was visible.

Rinsing after seed germination effectively reduced the effect of $HgCl_2$ on seedling water content until the concentration of 50 μM. A similar trend was observed in the water content of cotyledons but to a lesser degree (Fig. A.7).

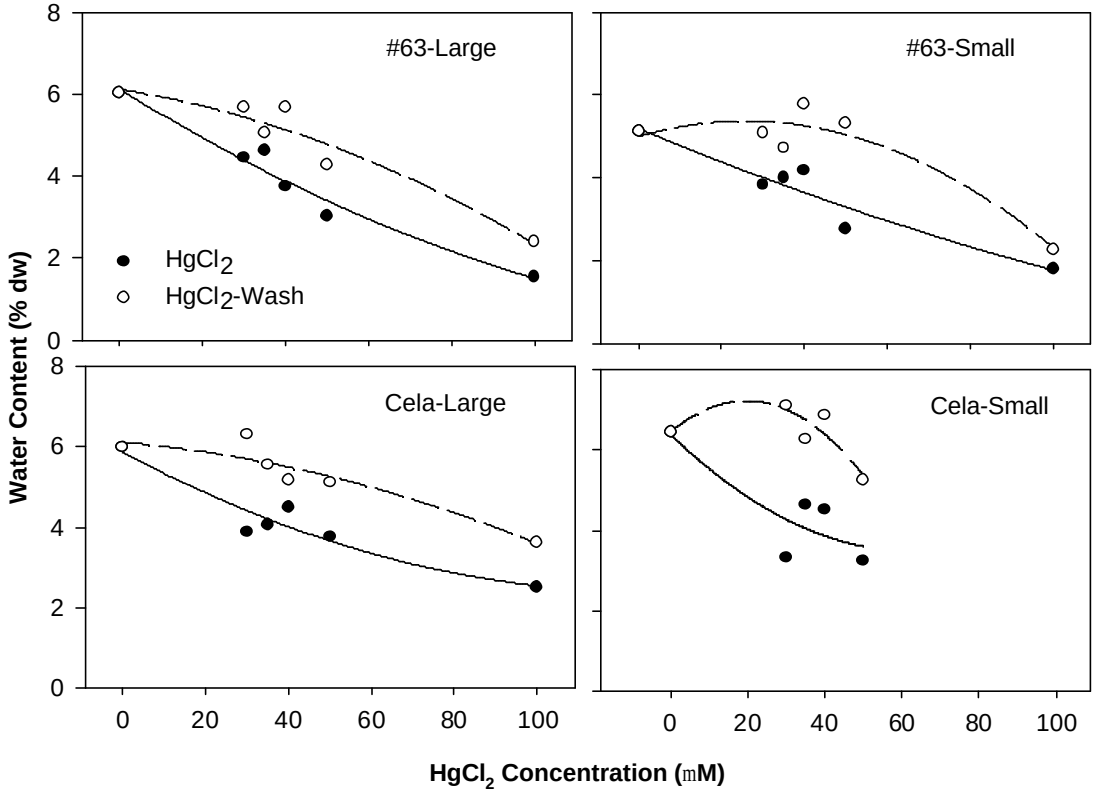

Fig. A.7. The water content of cotyledons of the seedling four days after germination at 20°C with the same concentration of $HgCl_2$ or with distilled water after rinsing in large and small seeds of two collections in winterfat (*Krascheninnikovia lanata*). Seeds were imbibed at 5°C and were transferred to 20°C when radicle protrusion was visible.

A.3.4 Effects of $HgCl_2$ on seedling growth

Root length after four days of growth was reduced greatly by $HgCl_2$ during the post germination period even when germinated seeds were rinsed and incubated in distilled water (Fig. A.8).

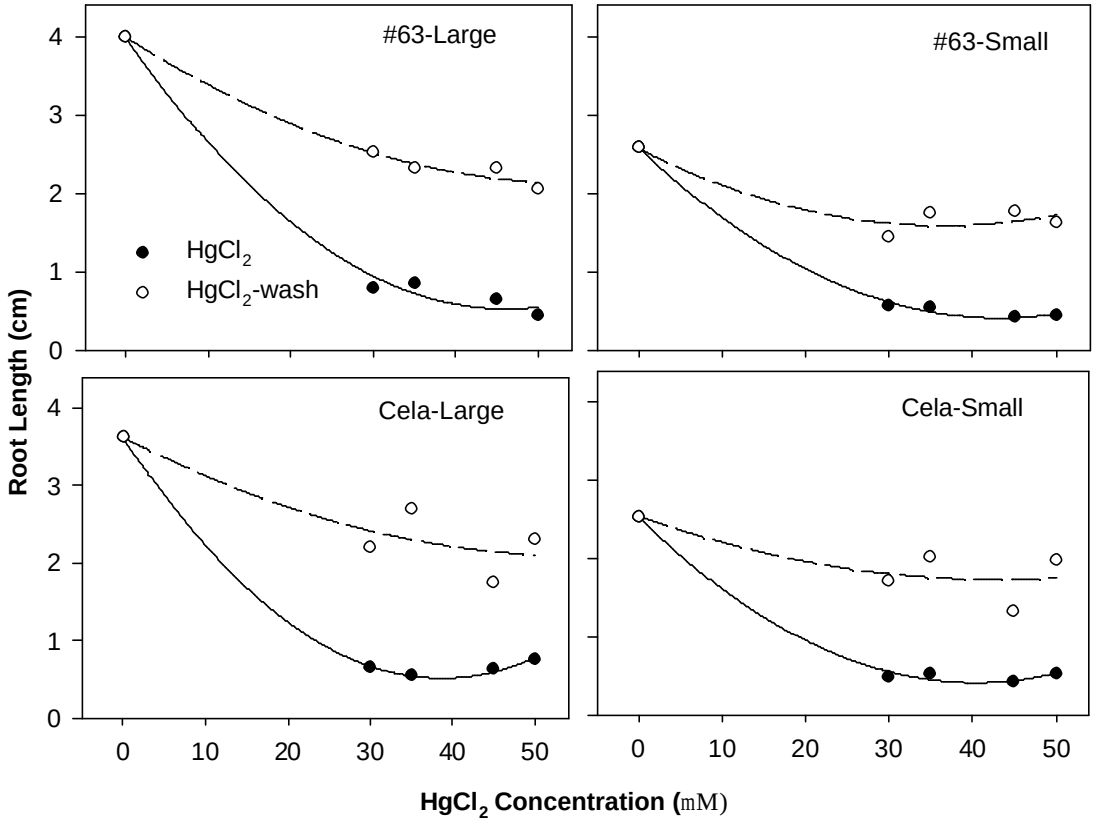

Fig. A.8. The root length (radicle and hypocotyl) of seedlings four days after germination at 20°C with the same concentration of $HgCl_2$ or with distilled water after rinsing in large and small seeds of two collections in winterfat (*Krascheninnikovia lanata*). Seeds were imbibed at 5°C and were transferred to 20°C when radicle protrusion was visible.

Post germination continuously incubating in $HgCl_2$ further reduced root growth. Cotyledon growth, conversely, was less affected by $HgCl_2$ solutions up to 30 µM, even though continuous incubation in $HgCl_2$ after germination also reduced cotyledon growth (Fig. A.9).

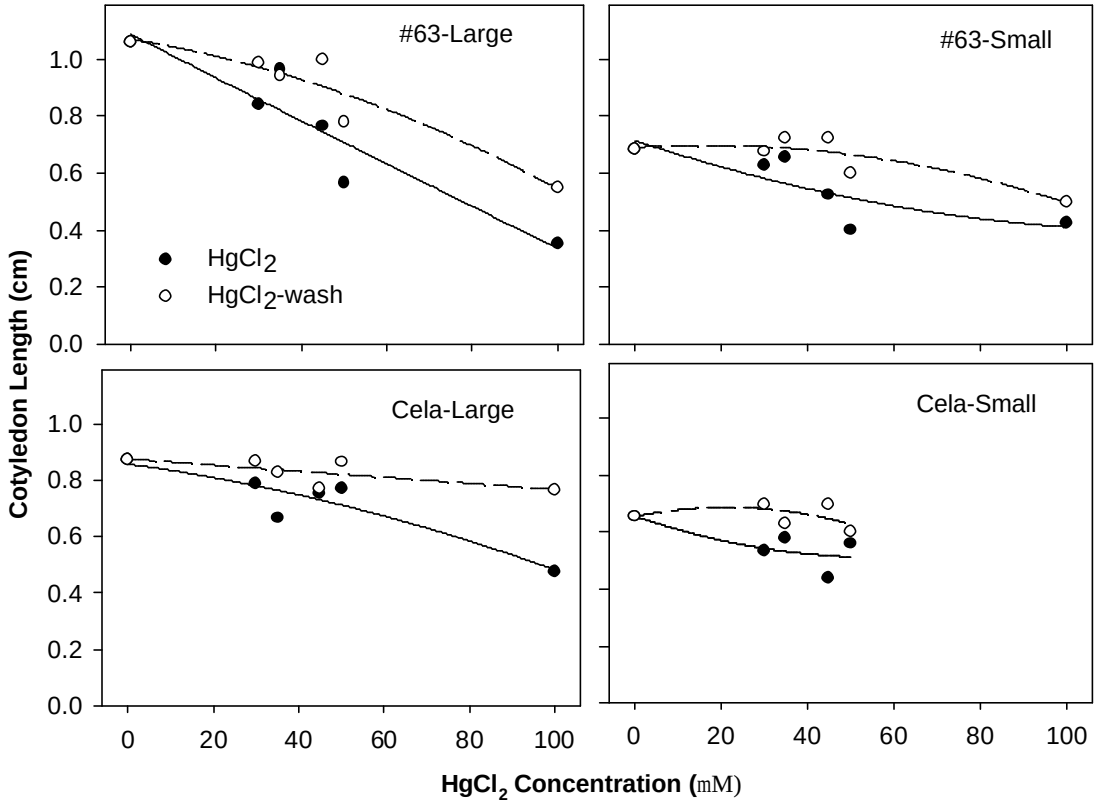

Fig. A.9. The length of cotyledons of seedlings four days after germination at 20°C with the same concentration of $HgCl_2$ or with distilled water after rinsing in large and small seeds of two collections in winterfat (*Krascheninnikovia lanata*). Seeds were imbibed at 5°C and were transferred to 20°C when radicle protrusion was visible.

The differential inhibition of $HgCl_2$ on root and cotyledon growth was further demonstrated by the increased ratio of cotyledon/root dry weight under $HgCl_2$, indicating that winterfat had more allocation to cotyledons by $HgCl_2$ inhibition (Fig. A.10). The cotyledon/root dry weight ratio increased from 1-1.5 in distilled water to more than 2.5 with the inhibition of $HgCl_2$.

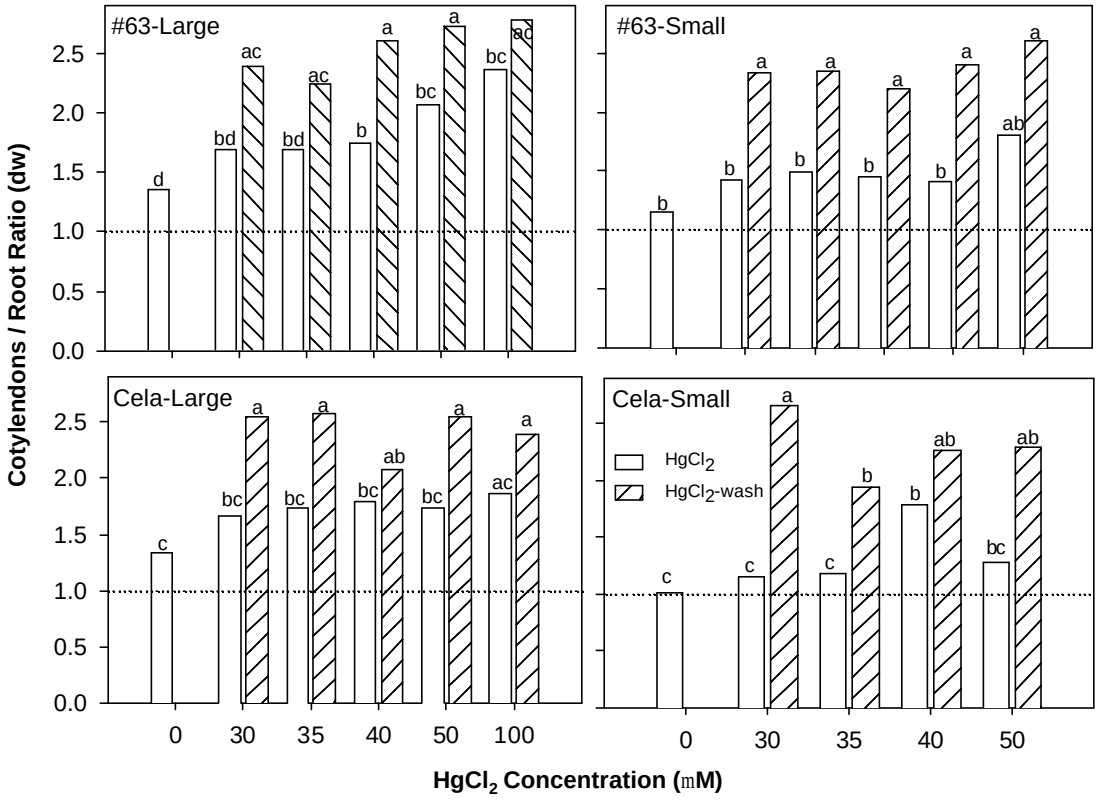

Fig. A.10. Dry weight ratio of cotyledons and root (radicle and hypocotyl) four days after germination at 20°C with the same concentration of $HgCl_2$ or with distilled water after rinsing in large and small seeds of two collections in winterfat (*Krascheninnikovia lanata*). Seeds were imbibed at 5°C and were transferred to 20°C when radicle protrusion was visible.

A.4 Discussion

There is strong evidence that aquaporins are central components in plant water relations (Tyerman et al., 2002; and citations in). This study demonstrates that aquaporins play a major role in water relations from initial seed water uptake to seedling elongation in winterfat. The ^{1}H NMR image showed that upon imbibition, water uptake of winterfat seeds began with the embryo and subsequently to the perisperm. This water uptake dynamic pattern in a seed has also been found in other species with different seed anatomical structures, e.g., in the seed of western white pine (*Pinus monticola* Dougl. ex D. Don) (Terskikh et al., 2004), and barley (*Hordeum vulgare* L.) (Gruwel et al., 2002). The endosperm of barley seeds later on is served as a water reservoir for embryo elongation (Allen et al., 2000). The results showed water content of winterfat seeds was lower in the whole seed, but higher in the embryo with $HgCl_2$, indicating a possible blockage of the major water pathway from the embryo to the rest of the seed tissues. The blocking of water flux to the perisperm, where nutrient and water may otherwise supplement to embryo in winterfat, may not only reduce seed water uptake, but the embryo elongation, thus seedling growth. Therefore, water diffusion from cell to cell could play a critical role for embryo growth, being a major water pathway within a lived seed. While rapid, uncontrolled water flux may enter and distribute within a non-viable seed through apoplastic pathway or damaged tissue (Gruwel et al., 2002; Terskikh et al., 2004).

Water content measured gravimetrically includes water in all physical status, e.g., mobile water, bounded water and less bounded water in a sample. The aquaporin inhibition by $HgCl_2$ affected not only water uptake and dynamics in seeds and seedlings, but also the biophysical status of water. The amount of mobile water (proton intensity) of the embryo (cotyledon, hypocotyl and radicle) when imbibed with $HgCl_2$ tended to be lower than that with distilled water. Aquaporins may be involved in the control of the rehydration of protein storage vacuoles and their breakdown and mobilization (Maurel et al., 1997) and of the membrane fluidity of vacuoles (Strzalka et al., 1995) during the early stages of germination. More water molecules would be bound if embryo cells have more macromolecules due to slow

breakdown and mobilization rate, reducing the amount of mobile water even when the water content is high. It has been reported that water redistribution induced by ethylene within the embryo permitted radicle growth during precocious germination (Fountain et al., 1998) and isolated radicles elongated immediately upon access to free water (Fountain et al., 1990). The results here demonstrated that aquaporin inhibition by $HgCl_2$ altered water content, its distribution and its biophysical status within the winterfat seed. As a consequence, germination was delayed and reduced.

Mobility of water can be quantified using NMR relaxation time measurements. The relaxation time indices are the coefficients (R_1 and R_2) of the equations that describe the rate of nuclear relaxation after excitation. Relaxation rates are influenced by the macroscopic (R_1) and nuclear (R_2) environments. Thus, changes in these indices reflect changes in water viscosity, temperature, ionic strength and may indicate the presence of specific chemical constituents (reviewed in Ishida et al., 2000). Previous reports show that R_2 is sensitive to vacuole size in mushroom (Donker et al., 1997), the size of parenchyma cells in ripe grape berries (Glidewell et al., 1997) and plasmalemma and tonoplast permeability and integrity (Chen and Gusta, 1978; Maheswari et al., 1999). An observed R_2 value increase in tulip bulb basal plate and scales during cool temperature storage was attributed to water redistribution. These changes coincided with budding and flowering in geophytes (Van der Toorn et al., 2000). R_1 and R_2 changes in winterfat seeds before 10 h were in opposite directions to barley seeds (Gruwel et al., 2001). However at 10 – 48 h imbibition times, R1 and R_2 decreased significantly in barley seeds and in this study. That NMR proved insensitive to changes in non-lethal membrane permeability changes induced by the mercury treatment is attributed to large inter seed variability. When the injury is severe, R_2 values correlated with stress injuries of freezing (Chen and Gusta, 1978) or high temperature (> 44^oC) (Maheswari et al., 1999).

The role of aquaporins in seed germination is not fully understood. One attractive hypothesis is that aquaporins help regulate cell osmotic potential during seed germination, specifically the fine control of cytoplasmic osmoregulation and vacuolar volume (Maurel et al., 1997). Aligning relaxation rates with this model provides the following testable model: initially, low water-proton intensity in the

presence of soluble macromolecules will result in higher R_1 compared to control; this is the so-called bound water fraction. However, as imbibition proceeds, but before substantial cell rehydration and activation, bulk water will be present in cavities. Relative to the bound fraction this will have a relatively lower R_1 value due to reduced water varicosity. Indeed, this has been observed in cereals. R_1 increased during early seed imbibition (< 7 h) in barley (Gruwel et al., 2001) and (< 5 h) in wheat (Gambhir et al., 1997). However, at still later imbibition times, the evolution of R_1 values is consistent with viscous phase dynamics. That is, as the matrix viscosity increases following cell activation, the rate of relaxation increases. Although additional observations at early imbibition time might have provided better detail, our measurements generally are consistent with this interpretation of the hydration phenomenon.

The mercury treatment alters root growth and physiology. Root elongation in winterfat seedlings was reduced (2.5 to 4 times) and an abnormal growth was observed. In maize, root elongation was reduced by around 75% after exposure to 20 µM $HgCl_2$ (Hukin et al., 2002). For winterfat at least, seedling water content was also reduced, especially in the root. This may account for the altered growth since turgor pressure and protoplasmic water supply are limiting factors for growth or cell elongation (Fricke and Peters, 2002). Furthermore, symplastic water accounts for up to 81% of the water needed for the growth of maize root cells (Bret-Harte and Silk, 1994), 50% in pea stem elongation (Schmalstig and Cosgrove, 1990) and 78% in barley roots (Pritchard et al., 2000). The reduction in root elongation was much greater in winterfat than in maize, possibly due to different growth stages, genetic differences and sensitivity to $HgCl_2$. Growth reduction varies within the root zone, being greatest in the older growing cells and least in the younger cells near the root tip (Hukin et al., 2002). Radicle and hypocotyl elongation in initial germination would be from cells that are not newly formed because cell division normally occurs later in germination (Bewley, 1997).

Despite the relatively low $HgCl_2$ concentration used here, seed viability was significantly reduced. Even though the use of the mercurial agents is well established, the effective concentration varies widely, ranging from 20 to 500 µM of

$HgCl_2$. The relative lower concentration of mercury generally had no significant metabolic inhibition and toxic effects, such as 100 μM in seedling root of aspen (*Populus tremuloides* Michx) (Wan and Zwiazek, 1999) and mesophyll cells of pea (*Pisum sativum* L.) (Willmer et al., 1999), and 50 μM in onion (*Allium cepa* L.) roots (Barrowclough et al., 2000). Unwanted metabolic effects, however, are suspected (Maggio and Joly, 1995; Carvajal et al., 1996; Zhang and Tyerman, 1999; Barrowclough et al., 2000). This study determined that winterfat seeds are more sensitive to mercury; germination and seedling growth were significantly reduced at 30 μM. Clearly, imbibition time is an important factor since a strong inhibitory response was noted at 7 days, but not at 3 days. These studies suggest that imbibition time is an important experimental design factor as are seed size and accession.

In summary, aquaporins were determined to play a critical role in germinative water dynamics. Water entered the embryo in early water uptake stage and then diffused to the perisperm reaching the maximum near embryo protrusion in winterfat. $HgCl_2$ mediated aquaporin inhibition tended to reduce seed water uptake, altered water distribution among seed tissues and modified the physical state of the imbibed water. These factors resulted in a reduction of the rate and the ultimate percentage of seed germination. Aquaporin inhibition reduced seedling root elongation even when the $HgCl_2$ was rinsed and removed at the elongation stage. Dry matter was preferentially allocated to cotyledons whose growth was less impacted by mercury.

A.5 Acknowledgements

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