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Germination of winterfat (*Eurotia lanata* (Pursh) Moq.) seeds at reduced water potentials: testing assumptions of hydrothermal time model

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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 (*Eurotia lanata*) with two seed size classes. Winterfat is a native shrub with superior forage quality. Germination rates (GR) 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. Results indicate that 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 intermediate temperatures between 10 and 15 °C, while T_b decreased linearly with increasing water availability. The estimated shift rates of $T_{b(50)}$ with ψ were between 2.18 and 3.81 °C MPa^{-1} for the two collections and large seeds had a greater shift 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 ($\theta_{HT(50)}$), which was lower at intermediate temperatures than at either lower or higher temperatures. 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 variations in θ_{HT}.

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Keywords: Base water potential; Germination; Hydrothermal time; Seed collection; Seed size; Winterfat (*Eurotia lanata*)

1. Introduction

Hydrothermal time (θ_{HT}) was first proposed by Gummerson (1986) based on the linear relationship between germination rate and water potential at con-

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stant temperature in sugar beet (*Beta vulgaris* L.). The hydrothermal time 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 (θ_{HT}) quantifies the combined effects of temperature and water potential on the progress of seed germination, which includes both the thermal time accumulation above the thermal threshold (base temperature (T_b)) and the hydro time accumulation above the hydro threshold (base water potential (ψ_b)) towards seed germination. Base temperature varies little among individual seeds or subpopulations and the thermal time (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 (*g*), while hydro time (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 (i.e., 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 the entire seed population. Because of the involvement of both 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 both native and introduced species (Christensen et al., 1996; Kebreab and Murdoch, 1999; Grundy et al., 2000; Roman et al., 1999, 2000; Allen et al., 2000) 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., 2000) in the hydrothermal time model are ecologically relevant and the hydrothermal time concept is also useful for understanding physiological variations 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 range (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 θ_{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 (*B. vulgaris* L.). Kebreab and Murdoch (1999) tested the parallelism of $GR_{(g)}$ on water potential in *O. 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 paper, we used two seed size classes and two collections of winterfat (*Eurotia lanata*) to test assumptions in the hydrothermal time model and the influence of these assumptions on model predictability. Winterfat is a small, native shrub in the Great Plains of North America (Coupland, 1950; Romo et al., 1995) with superior forage quality (Smoliak and Bezeau, 1967). 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 and 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 variations in parameters of the model between seed size classes and seed collections.

2. Materials and methods

2.1. Seed sources and characteristics

Two collections of winterfat seed (diaspores), Cela-01 and #63, were purchased from Wind River Seed (Manderson, Wyoming, USA). Both collections originated from Utah. 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 1 week after purchase, and then cleaned by rubbing, fanning and passing serial sieves and blowers. Cleaned seeds were separated into two classes using a seed blower based on seed mass and hereafter were referred to seed mass classes as seed size classes (large and small). Seed moisture content was similar between large and small classes within each collection. Cleaned and classified seeds were then sealed in plastic bags and stored at $-18\,^{\circ}C$ until use. Please see Wang et al. (in press) for details regarding characteristics of the two seed collections.

2.2. 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 and Emmerich, 1990). Designated water potentials of PEG solution 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 and Emmerich (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 °C, were randomly allocated to each incubator. Only the middle three shelves of each incubator, 15 cm apart, were used for the germination test to minimize temperature variation among shelves within 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 randomized complete block design (RCBD) with five replicates was used and there was a 7-day interval between replicates. Seeds, 50 per unit, were carefully

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 milliliters 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 replication. Seed surface was 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-01, respectively.

2.3. Parameter estimation for the hydrothermal time model

In order to estimate the germination rate of subpopulations ($GR_{(g)}$), germination time courses of each temperature, water potential, and replicate were fitted separately using probit analysis procedures as described in Wang et al. (in press). 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, which is 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. (1) (Bradford, 1990, 1995) at each temperature, and estimated $\theta_{H(50)}$ was used in the equation:

$$\text{Probit}(g) = \frac{\psi - (\theta_H/t_g) - \psi_{b(50)}}{\sigma_{\psi b}} \qquad (1)$$

2.4. 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):

$$\theta_{HT} = (T - T_b)(\psi - \psi_{b(g)})t_g \qquad (2)$$

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 (Dahal and Bradford, 1994; Cheng and Bradford, 1999):

$$\text{Probit}(g) = \frac{\psi - (\theta_{HT}/(T - T_b)t_g) - \psi_{b(50)}}{\sigma_{\psi b}} \qquad (3)$$

The hydrothermal time for 50% subpopulation ($\theta_{HT(50)}$) was used in the hydrothermal time model for simplification even though variations existed among subpopulations. The predictability of the hydrothermal time model (Eq. (3)) 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 po-
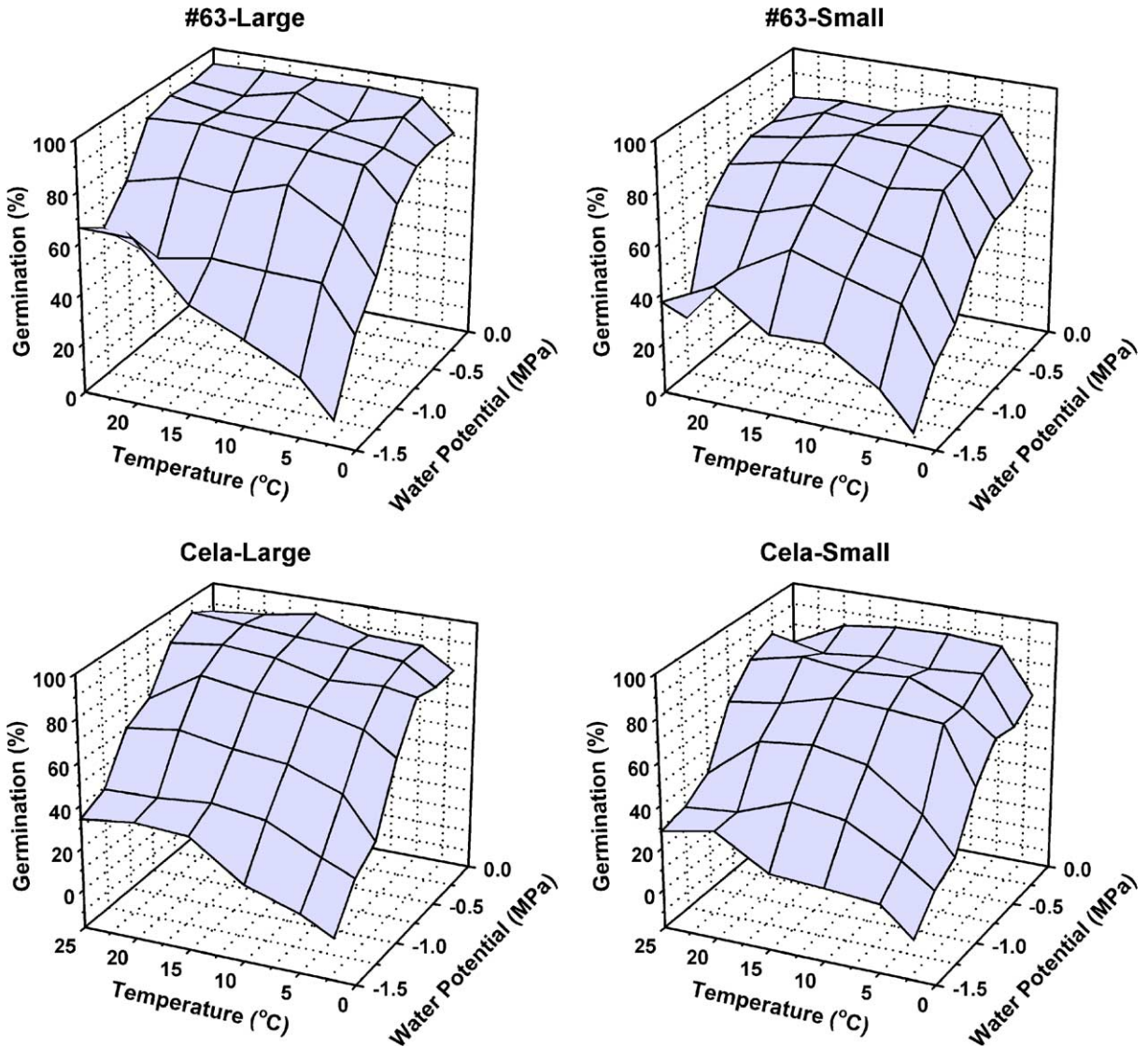
Fig. 1. Effects of water potential and temperature on the final germination percentage of two seed size classes and two collections of winterfat.

tential (Gummerson, 1986), the common θ_{HT} was estimated as the inverse slope of the linear regression of $1/\theta_{T(50)}$ on ψ:

$$\frac{1}{\theta_{T(50)}} = \frac{\psi - \psi_{b(50)}}{\theta_{HT(50)}} \tag{4}$$

(2) $\theta_{HT(50)}$ was allowed to change with temperature and estimated using Eq. (2).

(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.

The fitness of models was tested using modified R^2, which was 1 minus the ratio of the sum square of resid-
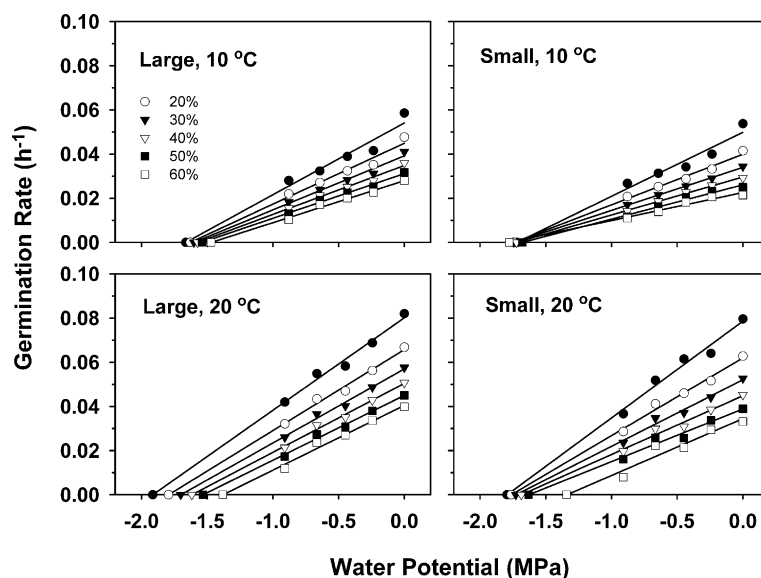
Fig. 2. Germination rates (h^{-1}) as a linear function of water potential at various temperatures in two seed size classes in Collection #63 of winterfat. Data points at *x*-interceptions were calculated using linear equations.

uals, the difference between observed and predicted values, and the sum of squares of observed values and observed means (Wang et al., in press).

3. Results

3.1. Germination percentage as affected by water potential and temperature

Both water potential and temperature affected the final germination percentage of winterfat, which was reduced especially when $\psi < -0.50$ MPa and $T < 5\,°C$ (Fig. 1). The final germination percentage was more sensitive to changes in water potential than temperature. Both water potential and temperature and their interactions significantly influenced the final germination percentage ($P < 0.001$–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, $2\,°C$; only 37% for the small seeds under the same condition of collection #63; and 31 and 24% germinated for the large and small seeds of collection Cela-01, respectively.

3.2. Effects of water potential and temperature on $GR_{(g)}$, $\psi_{b(g)}$, and T_b

Germination rates of subpopulations were linearly related to water potential at each temperature ($P < 0.05$, Fig. 2). However, these regression lines were not always parallel. Parallelism was found at $20\,°C$, and the regression lines became unparallel or crossed to a common point at the *x*-intercept at low temperatures (i.e., at $10\,°C$ for small seeds of collection #63, Fig. 2). Similar patterns were observed in both collections (data not shown for Cela-01). Consequently, $\psi_{b(g)}$ estimated from these *x*-intercepts varied among subpopulations, temperatures, seed size classes, and seed collections (Fig. 3). Variations of ψ_b among subpopulations were generally greater at higher temperatures (i.e., 20 or $25\,°C$) than at lower to intermediate temperature (i.e., 2–$15\,°C$). 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. The estimated $\psi_{b(50)}$ was curvilinearly correlated to temperature in both collections and seed size classes except for small seeds of Cela-01 which exhibited large variability among temperatures (Fig. 4). The value of $\psi_{b(50)}$ was lowest at intermediate temperatures (10–$15\,°C$) and the differ-
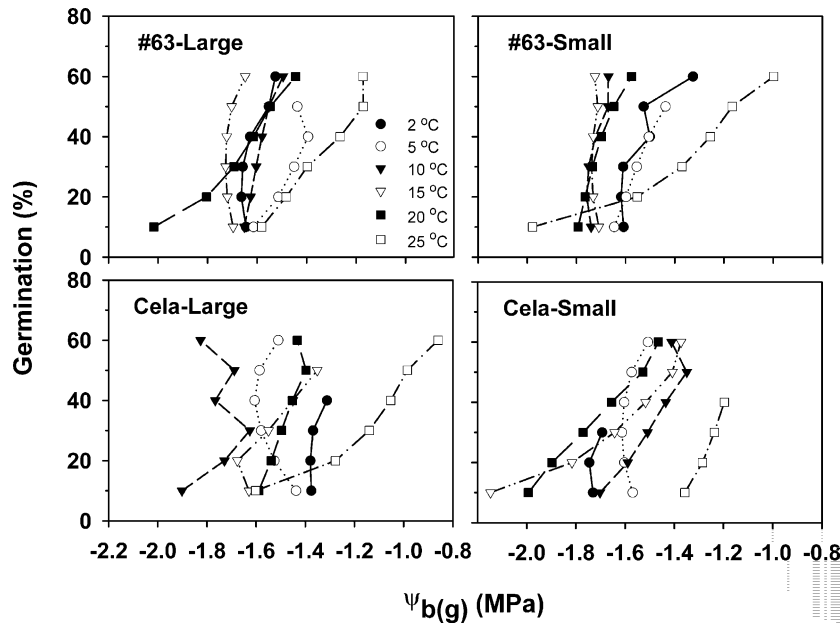
Fig. 3. Base water potentials ($\psi_{b(g)}$) among germination subpopulations at temperatures from 2 to 25 °C in two seed size classes and two collections of winterfat. The value of $\psi_{b(g)}$ was estimated from the *x*-intercept of the linear relation of $GR_{(g)}$ on water potential.

ence in $\psi_{b(50)}$ between temperatures can be more than 0.5 MPa.

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 both seed size classes and collections
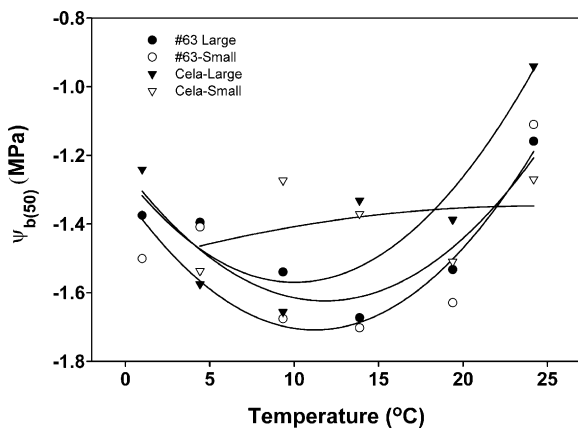

Fig. 4. Variations of the base water potential of the 50% subpopulation ($\psi_{b(50)}$) with temperature in two seed size classes and two collections of winterfat. The value of $\psi_{b(50)}$ was estimated by the linear function of $GR_{(50)}$ on water potential at each temperature.

(Fig. 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.

Base temperature was assumed constant among subpopulations for a given water potential. The estimated $T_{b(50)}$ decreased linearly with increasing water potential (Fig. 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 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 1).

3.3. Constancy of hydro time (θ_H), thermal time (θ_T), and hydrothermal time (θ_{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 poten-
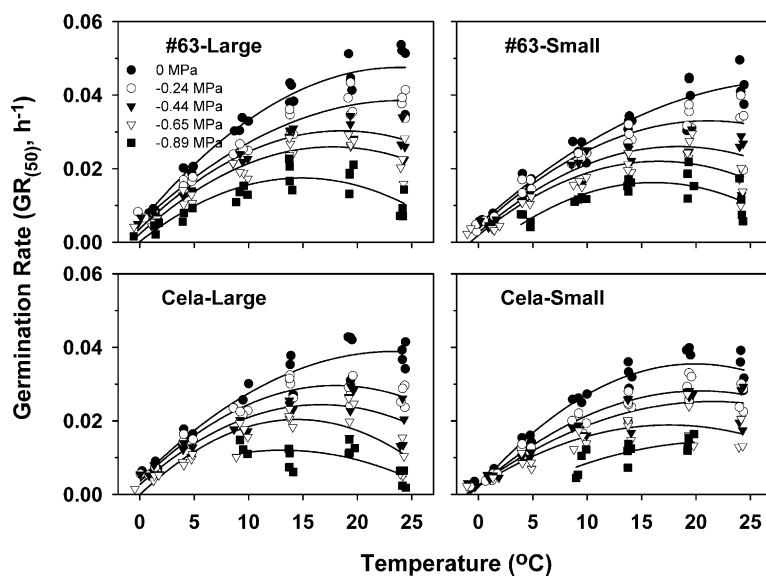
Fig. 5. Relationship between $GR_{(50)}$ and temperature as affected by water potential in two seed size classes and two collections of winterfat.

tial, was not always constant among subpopulations, and the constancy varied with temperature (Fig. 7). Hydro time was relatively constant among subpopulations when temperatures were above $10\,^{\circ}\text{C}$, but it increased with increasing germination fractions or sub-

population when temperature was lower than $10\,^{\circ}\text{C}$, especially at $2\,^{\circ}\text{C}$. 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
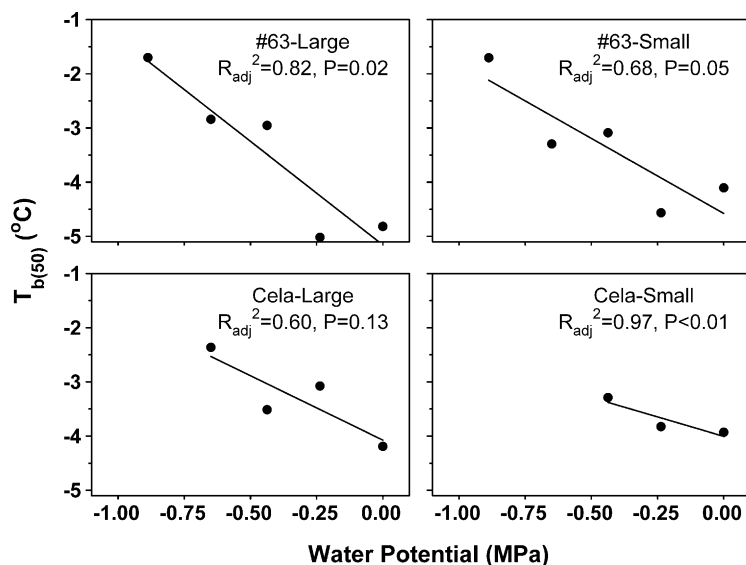

Fig. 6. Base temperatures of the 50% subpopulation ($T_{b(50)}$) as affected by water potential in two seed size classes and two collections of winterfat. The value of $T_{b(50)}$ was estimated by the linear function of $GR_{(50)}$ on temperature at each water potential within the suboptimal temperature range.

Table 1
Estimated parameters for the construction of the hydrothermal time model in two seed size classes and two collections of winterfat

Collection	Size	$\theta_{HT(50)}$ (MPa °C h)[a]	$\psi_{b(50)}$ (MPa)	$\sigma_{\psi b}$ (MPa)	$T_{b(50)}$ (°C)[b]	$mT_{b(50)}$ (°C MPa^{-1})[c]
#63	Large	805	−1.56	0.81	−4.82	3.81
	Small	979	−1.60	1.05	−4.12	2.76
Cela-01	Large	856	−1.51	0.97	−4.19	2.38
	Small	985	−1.46	0.86	−3.93	2.12

[a] Estimated from the slopes of linear relations of Eq. (4).
[b] Based on germination at 0 MPa.
[c] Estimated using the linear regression of $T_{b(50)}$ on water potential.

was greater at the low temperature range (2–10 °C) than the moderate to high temperature range (Fig. 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 °C. Collection #63, which originated from a dryer site, required less θ_H than collection Cela-01, especially at low temperatures.

Thermal time ($\theta_{T(g)}$) increased linearly with decreasing water potential ($P < 0.05$ except for the large seeds of Cela-01 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 300–400 °C h per MPa (data not shown). The linear regression lines between $1/\theta_{T(g)}$ and water potential among subpopulations were generally paralleled (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 seed class had smaller $\theta_{HT(50)}$ than the small seed class in both collections (Tables 1 and 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 3).
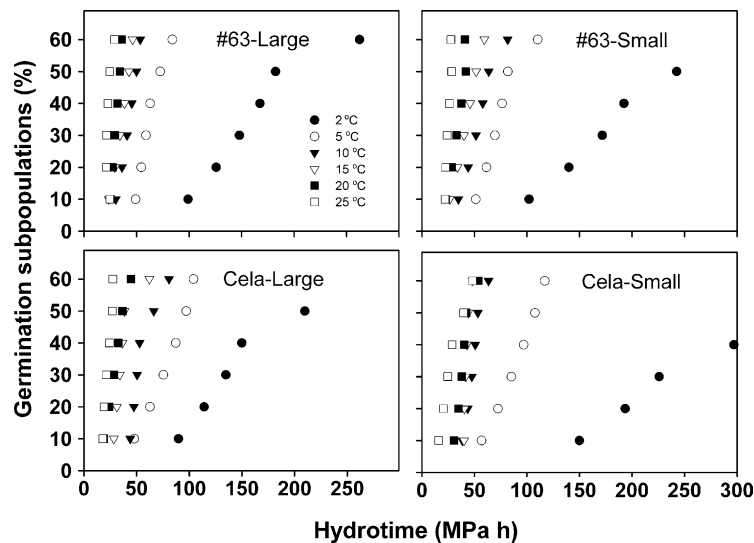

Fig. 7. Variations of hydro time (θ_H) among subpopulations at various temperatures in two seed size classes and two collections of winterfat. Hydro time was estimated from the slope ($\theta_H = 1/\text{slope}$) of the linear function of $GR_{(g)}$ on water potential.
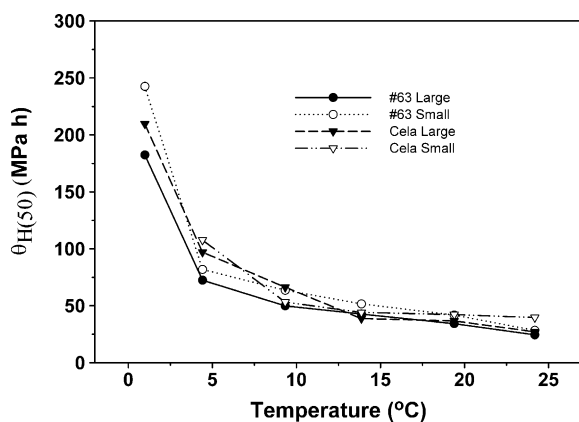
Fig. 8. Effect of temperature on hydro time of the 50% sub-population ($\theta_{H(50)}$) of two seed size classes and two collections of winterfat. The value of $\theta_{H(50)}$ was estimated from the slope ($\theta_H = 1/\text{slope}$) of linear function of $GR_{(50)}$ on water potential.

Table 2
Variations in $\theta_{HT(50)}$ with temperature in two seed size classes and two collections of winterfat

Temperature (°C)	#63		Cela-01	
	Large	Small	Large	Small
2	1070 a[a]	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[b]	871 A	843 B	922 A

Values were calculated using Eq. (2) using $T_{b(50)}$ and $\psi_{b(50)}$ as listed in Table 1.

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

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

3.4. Model predictability as affected by parameter assumptions

Hydrothermal time models were constructed using parameters based on different assumptions as listed in Tables 1–3 and model predictability was measured by the modified R^2. When all parameters were assumed constant (M1), model predictability was higher ($R^2 > 0.70$) at moderate water potential range than at higher or lower water potential at 5 or 20 °C (Fig. 9). Model predictability was generally reduced when $\psi < -0.63$ MPa at 5 °C and when $\psi < -1.20$ MPa at 20 °C. The predictability was lower at low water potential range and low temperature (i.e., 2 °C, data not shown). When θ_{HT} was allowed to vary with temper-
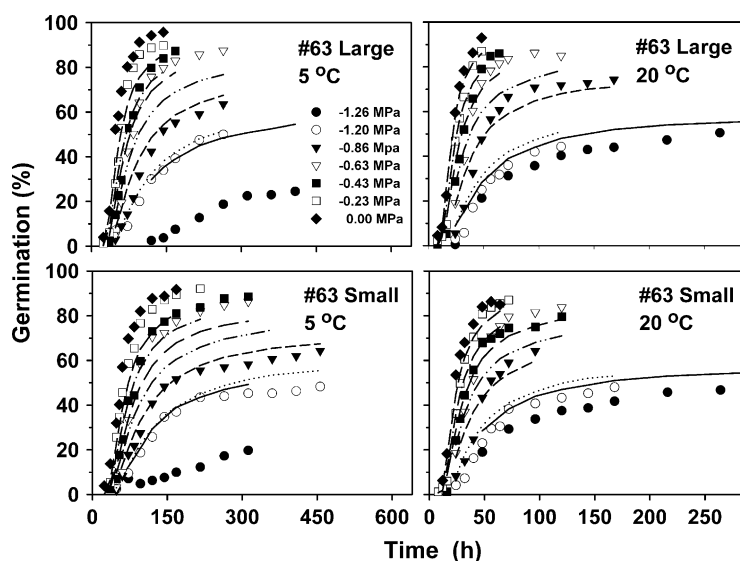

Fig. 9. The observed (symbols) and predicted (lines) germination time courses of collections #63 of winterfat at various water potentials at 5 and 20 °C when all parameters were assumed constant. Water potentials at 20 °C were 0.01–0.07 MPa lower than that of the same solutions at 5 °C.

Table 3
Variations in $\psi_{b(50)}$ (MPa) and $\sigma_{\psi b}$ (MPa) with temperature in two seed size classes and two collections of winterfat

Temperature (°C)	#63				Cela-01			
	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	−[a]	−
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

Values were estimated as *x*-intercepts of the linear regression of $GR_{(50)}$ on water potential at each temperature.

[a] Germination did not reach to 50%.

ature (M2), model predictability was improved at the low temperature and high water potential range, indicating that changes in θ_{HT} with temperature have an important impact on model predictability in winterfat (Fig. 10). When changes in T_b with ψ and changes in θ_{HT}, $\psi_{b(50)}$, and $\sigma_{\psi b}$ with temperature were allowed (M3), model predictability was improved from M1 but not from M2 (data not shown). Germination was highly sensitive to the reduced water potential and was highly correlated to temperature (Fig. 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.

4. Discussion

Reduced water potential lowered both 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
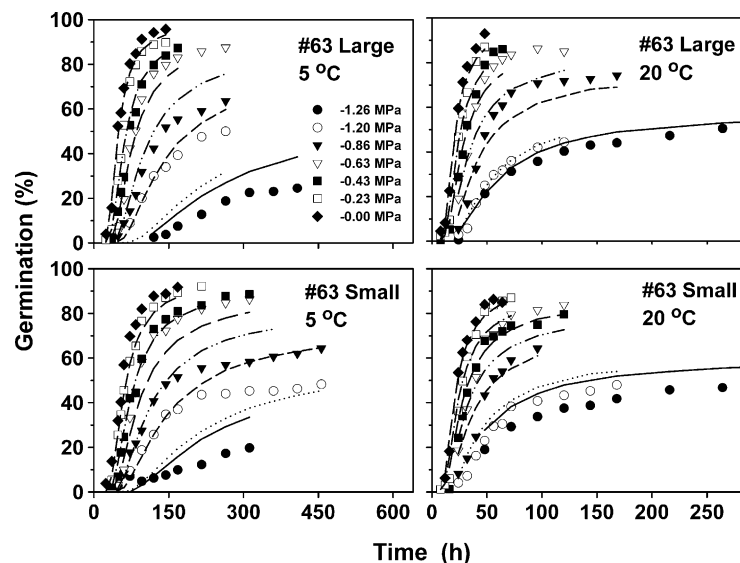

Fig. 10. The observed (symbols) and predicted (lines) germination time courses of collection #63 of winterfat at various water potentials at 5 and 20 °C when $\theta_{HT(50)}$ was allowed to vary with temperature. Water potentials at 20 °C were 0.01–0.07 MPa lower than that of the same solutions at 5 °C.

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 potential where germination percentage is low (i.e., Dahal and Bradford, 1994). Attempts have been made to include final germination percentage in the hydrothermal time model at reduced water potential (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 based on 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). Kebreab and Murdoch (1999) found optimum temperature for final germination percentage was lower than rate optimum in *O. aegyptiaca*.

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 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 other candidate cell wall hydrolases (Chen et al., 2002; Mo and Bewley, 2003). If seeds have adjusted or modified their physiological processes according to germination conditions, variations 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 found variable with germination conditions (i.e., 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 not only with germination conditions, but also 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).

Also θ_H was found not constant among subpopulations at low temperatures in winterfat, indicating the altering of water relations at low temperature. Hydro time determines germination rate at a given temperature, which is a temperature-specific parameter (Kebreab and Murdoch, 1999). Large increase in θ_H with decreasing temperature was 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 lead to the failure of achieving high germination percentage at low water potential and low temperature. 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. (1). 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 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 the large seed of winterfat, shows advantages of large seeds to germinate 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 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 θ_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), this may be an inappropriate approach as explained by Bradford (2002). Mathematically from Eq. (4), 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). It was significantly different among temperatures and seed size classes in both collections from the estimation of Eq. (2). 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 (Wang et al., in press), the difference in θ_{HT} likely resulted from the difference 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 potential 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 a 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 (i.e., cold desert) showed greater variations than that from more predictable, extreme environments (Allen and Meyer, 2002).

The general model (M1) 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 (M1) 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 wild and unpredictable conditions are more likely able to adjust germination response to their environment than crop species that are 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 (M3) did not further improve the fit under most conditions. As mentioned earlier, the inconsistency of θ_H at low temperature may cause the incorrect estimation of $\sigma_{\psi b}$. The increment of θ_H among subpopulations at low temperature gives an over estimation of the $\sigma_{\psi b}$ from Eq. (1). It was demonstrated that the variation of $\psi_{b(g)}$ in low temperature was smaller than higher temperature from the $\psi_{b(g)}$ estimation of *x*-intercepts (Fig. 3), but not in the estimation from Eq. (1) (Table 3). The parameter adjustment with subpopulations should further improve the model predictability, but over parameterization may lose the advantage of simplification of the hydrothermal time model.

5. Conclusion

Water potential influenced both germination rate and final germination percentage in winterfat. The hydrothermal time model detected the physiological adjustment during seed germination under reduced water potential 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 potential, temperature and the seed size within a seed collection. Both thermal and hydro threshold varied with germination conditions, and θ_{HT} was altered by low temperature. Hydro time was not just a temperature-specific parameter because variations among subpopulations at low temperature were 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 potential in winterfat. The general model (M1) had the least model fit among tested models due to the assumption of constant parameters. The adjustment of θ_{HT} according to temperature greatly improved the predictability of the hydrothermal time model, while the inclusion of T_b variation with water potential improved predictability at low water potential. The non-linearity relations of

$GR_{(g)}$ on temperature at reduced water potential may also account for reduced model fit at reduced water potential.

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