

A hydrothermal time model explains the cardinal temperatures for seed germination

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ABSTRACT

Temperature (T) and water potential (ψ) are two primary environmental regulators of seed germination. Seeds exhibit a base or minimum T for germination (T_b), an optimum T at which germination is most rapid (T_o), and a maximum or ceiling T at which germination is prevented (T_c). Germination at suboptimal T can be characterized on the basis of thermal time, or the T in excess of T_b multiplied by the time to a given germination percentage (t_g). Similarly, germination at reduced ψ can be characterized on a hydro-time basis, or t_g multiplied by the ψ in excess of a base or threshold ψ that just prevents germination (ψ_b). Within a seed population, the variation in thermal times to germination among different seed fractions (g) is based on a normal distribution of ψ_b values among seeds ($\psi_b(g)$). Germination responses across a range of suboptimal T and ψ can be described by a general hydrothermal time model that combines the T and ψ components, but this model does not account for the decrease in germination rates and percentages when T exceeds T_o . We report here that supra-optimal temperatures shift the $\psi_b(g)$ distribution of a potato (*Solanum tuberosum* L.) seed population to more positive values, explaining why both germination rates and percentages are reduced as T increases above T_o . A modified hydrothermal time model incorporating changes in $\psi_b(g)$ at $T > T_o$ describes germination timing and percentage across all T and ψ at which germination can occur and provides physiologically relevant indices of seed behaviour.

Key-words: *Solanum tuberosum*; mathematical model; potato; water potential.

INTRODUCTION

Seed germination is a complex physiological process that is responsive to many environmental signals, including temperature (T), water potential (ψ), light, nitrate, smoke, and other factors (Bewley & Black 1994; Baskin & Baskin 1998). Temperature has a primary influence on seed dormancy and germination, affecting both the capacity for germination by regulating dormancy and the rate or speed of germination in non-dormant seeds. It has been recognized

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since at least 1860 that three cardinal temperatures (minimum, optimum and maximum) describe the range of T over which seeds of a particular species can germinate (Bewley & Black 1994). The minimum or base temperature (T_b) is the lowest T at which germination can occur, the optimum temperature (T_o) is the T at which germination is most rapid, and the maximum or ceiling temperature (T_c) is the highest T at which seeds can germinate. The temperature range between T_b and T_c is sensitive to the dormancy status of the seeds, often being narrow in dormant seeds and widening as dormancy is lost (Vegis 1964). In particular, low T_c values are often associated with seed dormancy, as in relative dormancy or thermo-inhibition exhibited by seeds whose germination is prevented at warm temperatures (Bradford & Somasco 1994). The cardinal temperatures for germination are generally related to the environmental range of adaptation of a given species and serve to match germination timing to favourable conditions for subsequent seedling growth and development.

Mathematical models that describe germination patterns in response to T have been developed (e.g. Garcia-Huidobro, Monteith & Squire 1982; Covell *et al.* 1986; Ellis & Butcher 1988). For suboptimal temperatures (from T_b to T_o), germination timing can be described on the basis of thermal time or heat units (Bierhuizen & Wagenvoort 1974). That is, the T in excess of T_b multiplied by the time to a given germination percentage (t_g), is a constant for that percentage (the thermal time constant, $\theta_T(g)$):

$$\theta_T(g) = (T - T_b)t_g \text{ or} \quad (1)$$

$$GR_g = 1/t_g = (T - T_b)/\theta_T(g) \quad (2)$$

This model predicts that the germination rate for a given seed fraction or percentage g (GR_g , or $1/t_g$) is a linear function of T above T_b , with a slope of $1/\theta_T(g)$ and an intercept on the T axis of T_b . In many cases, T_b varies relatively little among seeds in a population within a given species, as predicted by Eqn 1 (Garcia-Huidobro *et al.* 1982; Covell *et al.* 1986; Dahal, Bradford & Jones 1990; Kebreab & Murdoch 1999), although there are exceptions to this, particularly when dormancy is present (Labouriau & Osborn 1984; Fyfield & Gregory 1989; Grundy *et al.* 2000; Kebreab & Murdoch 2000). Nonetheless, the thermal time model (Eqns 1 & 2) has been extensively and successfully applied to describe seed germination timing at suboptimal T .

Similar models have been proposed to describe germination rates at supra-optimal temperatures (from T_o to T_c). In many cases, GR_g declines linearly with an increase in T

between T_0 and T_c (Labouriau 1970; Garcia-Huidobro *et al.* 1982; Covell *et al.* 1986). However, it is generally observed that different fractions of the seed population have different T_c values. To account for this variation in T_c values, Ellis and coworkers (Covell *et al.* 1986; Ellis *et al.* 1986; Ellis & Butcher 1988) proposed the following model:

$$\theta_2 = (T_c(g) - T)t_g \quad (3)$$

$$GR_g = 1/t_g = (T_c(g) - T)/\theta_2 \quad (4)$$

where θ_2 is a thermal time constant at supra-optimal T and $T_c(g)$ indicates that T_c values vary among fractions (g) in the seed population. In this model, differences in GR_g for the different seed fractions were a consequence of variation among seeds in their ceiling temperatures ($T_c(g)$), and the total thermal time remained constant in the supra-optimal range of T .

Although this model or subsequent modifications of it have been relatively successful in describing germination timing at supra-optimal T , they do not offer a physiological explanation for this response (i.e. for the decrease in GR_g and variation in T_c). We propose that seed germination behaviour at supra-optimal T is a consequence of the sensitivity of germination to ψ . The hydrotime model describes the relationship between ψ and seed germination rates in analogy to the thermal time model. Gummerson (1986) defined the hydrotime constant (θ_H) as:

$$\theta_H = (\psi - \psi_b(g))t_g \quad (5)$$

where $\psi_b(g)$ is the base or threshold ψ that will just prevent germination of fraction g of the seed population. In this model, $\psi_b(g)$ represents the variation in threshold (ψ_b) values among seeds in the population, which often can be described by a normal distribution. Thus, since θ_H is a constant, variation in ψ_b values is reflected in a proportional variation in t_g values among seeds. A normal distribution of $\psi_b(g)$ values results in a right-skewed sigmoid cumulative time course of germination events, as is generally observed for seed populations (Bradford 1997). This model can accurately describe germination timing at reduced ψ , simultaneously accounting for reductions in both germination rates and percentages as ψ decreases (Gummerson 1986; Bradford 1990, 1995; Dahal & Bradford 1994).

The hydrotime and thermal time models have been combined into a hydrothermal time model that can describe seed germination patterns across suboptimal T and reduced ψ :

$$\theta_{HT} = (\psi - \psi_b(g))(T - T_b)t_g \quad (6)$$

where θ_{HT} is the hydrothermal time constant (Gummerson 1986; Bradford 1995). Using this model, seed germination times across the range of suboptimal T and ψ can be described with good accuracy (e.g. Dahal & Bradford 1994). However, the hydrothermal time model (Eqn 6) does not predict a decrease in germination rates as T increases above T_0 . Interactions have been observed between T and ψ in the supra-optimal range of T , such as for lettuce (*Lactuca sativa* L.) seeds (Bradford & Somasco

1994), where $\psi_b(g)$ values increased (became more positive) with increasing T . Similarly, Kebreab & Murdoch (1999, 2000) found that low ψ restricts the T range for germination in *Orobanche* seeds. These data suggest that changes in $\psi_b(g)$ could be responsible for the delay and inhibition of seed germination in the supra-optimal range of T , as hypothesized previously (Bradford 1996).

Here we report experimental tests of this hypothesis demonstrating that the decrease in germination rates and percentages at supra-optimal T is due to an increase in the $\psi_b(g)$ thresholds for germination in a seed population. When modified to account for this effect of supra-optimal T on $\psi_b(g)$, the hydrothermal time model can describe seed germination timing and percentages at temperatures from T_b to T_c and at all ψ at which germination can occur. This model provides both a mathematical description and a physiological rationale for the cardinal temperatures for seed germination.

MATERIALS AND METHODS

True (or botanical) potato seeds (*Solanum tuberosum* L.), which germinate over a range of T after dormancy is overcome and exhibit clear sub- and supra-optimal ranges of T (Pallais 1995), were used in these studies. Hybrid true potato seeds were produced in Chacas, Perú in 1996 by hand pollination of parental lines Yungay and 104.12LB. After harvest, the seeds were transported to the International Potato Center in Lima, Perú, stored at 15 °C until the seed moisture content was reduced to ~4.5% (fresh weight basis) and subsequently stored at 0 °C in sealed containers. After transfer to the University of California, Davis, the seeds were stored at -20 °C in sealed containers. To control ψ of the germination medium, solutions of polyethylene glycol 8000 were prepared according to Michel (1983). The ψ -values of the solutions were measured using a vapour pressure osmometer (Model 5100C; Wescor Inc., Logan, UT, USA) and corrected for the effect of temperature (Michel 1983). Five replicates of 25 seeds each were placed in 5-cm-diameter Petri dishes on two germination blotters saturated with water ($\psi = 0$ MPa) or solutions of polyethylene glycol 8000 that maintained specific water potentials ($\psi = -0.2$ and -0.4 MPa) at 14, 16, 18, 20, 22, 24, 27 and 28 °C. The replicates were randomized within isothermal lanes on a temperature gradient table. Germination was recorded at radicle protrusion to 2 mm, and germinated seeds were removed.

Germination time course data were analysed and the parameters were determined for the thermal time, hydrotime and hydrothermal time models using repeated probit regression analysis as described previously (Bradford 1990; Dahal & Bradford 1990, 1994). Germination rates were calculated as the inverses of the times to radicle emergence, and germination times for specific percentiles of the seed population were calculated by interpolation using curves fit to the time course data. Results for the 16th, 50th and 84th percentiles are reported to represent the median of the

population and one standard deviation (σ) above and below it.

RESULTS

Germination responses to temperature and water potential

Germination of true potato seeds in water (0 MPa) progressed more rapidly as T increased in the suboptimal range (Fig. 1a–c). In contrast, germination in water was progressively delayed and final percentages were reduced as T increased above 20 °C (Fig. 2a–e). Plotting the germination rates ($1/t_g$) for the 16th, 50th and 84th percentiles versus T revealed the characteristic linear increases and decreases in GR_g below and above T_o (Fig. 3a). The lines drawn through these points in Fig. 3a for different germination fractions (based on the model to be described subsequently) converged to a common T_b at suboptimal temperatures, but extrapolated to different T_c values in the supra-optimal range of T .

When seeds were placed to germinate at reduced ψ in any constant T , a delay in germination was observed relative to the time course in water (Figs 1a–c & 2a–e). At each

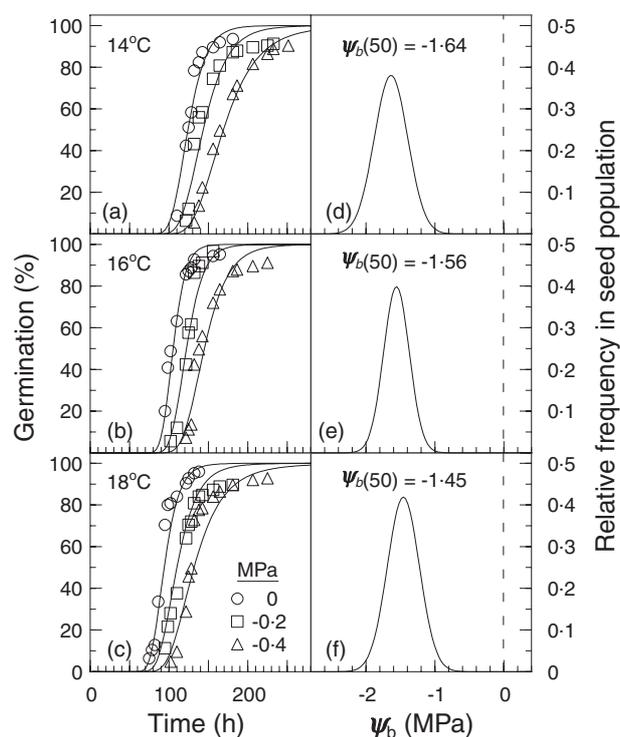


Figure 1. Germination time courses of true potato seeds at suboptimal temperatures. (a–c) Germination time courses at 14, 16 and 18 °C and 0, –0.2 and –0.4 MPa. The symbols are the actual data, and the lines are the time courses predicted by the hydrotime model using the values shown in Table 1A. (d–f) Normal distributions showing the relative frequencies of $\psi_b(g)$ values at each temperature. The median or $\psi_b(50)$ values are shown (see Table 1A).

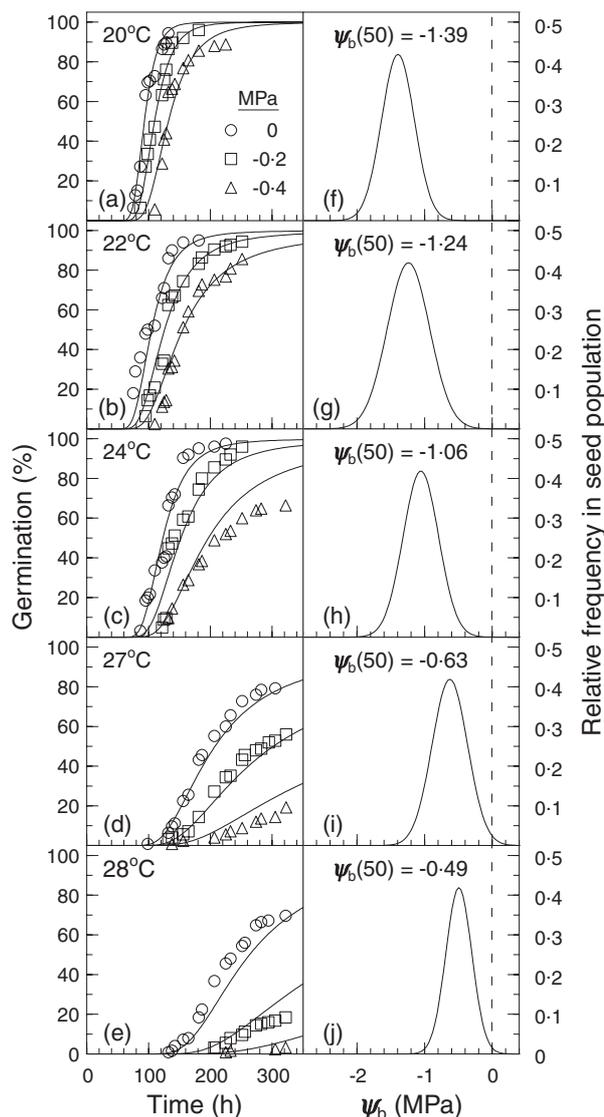


Figure 2. Germination time courses of true potato seeds at supra-optimal temperatures. (a–e) Germination time courses at 20–28 °C and 0, –0.2 and –0.4 MPa. The symbols are the actual data, and the lines are the time courses predicted by the hydrotime model using the values shown in Table 1C. (f–j) Normal distributions showing the relative frequencies of $\psi_b(g)$ values at each temperature. The median or $\psi_b(50)$ values are shown (see Table 1C).

T , the hydrotime model closely matched actual true potato seed germination time courses at different ψ , as can be seen from comparison of the predicted germination times (curves) and the actual data (symbols). The predicted curves were based upon the $\psi_b(g)$ threshold distributions (Figs 1d–f & 2f–j) and the parameter values in Table 1. In the suboptimal range of T , $\psi_b(50)$ and σ_{ψ_b} values were relatively constant, with differences in germination rates being primarily reflected in decreasing θ_H values (Table 1A). That is, the time required for germination decreased as T increased, in accordance with the thermal time model, and the seeds' sensitivity to ψ remained relatively unchanged.

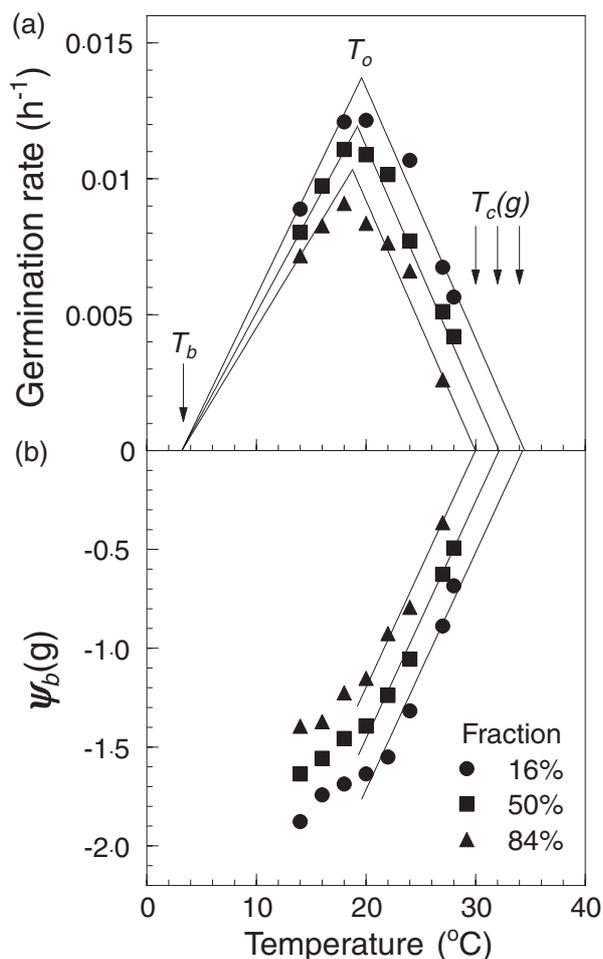


Figure 3. (a) The cardinal temperatures for germination of true potato seeds. Germination rates at different temperatures show the minimum or base temperature (T_b), optimal temperature (T_o) and the maximum or ceiling temperatures (T_c). For this seed lot, T_b is 3.2 °C, T_o is 19.3 °C and T_c varies with the seed fraction, being 34.3, 32.1 and 29.9 °C for the 16th, 50th and 84th percentiles, respectively. The symbols are the actual data derived from the germination time courses of Figs 1 and 2. The lines are based upon the parameters found after fitting the hydrotime model at sub- and supra-optimal T (Tables 1B & D). (b) $\psi_b(g)$ increases linearly as T increases in the supra-optimal range. The $\psi_b(g)$ values calculated from the hydrotime model at supra-optimal temperatures (Fig. 2; Table 1C) are plotted (symbols) with the linear increases (lines) predicted by the model (Table 1D). The projected lines for different seed fractions (16, 50 and 84%) intercept the $\psi_b(g) = 0$ MPa axis at the T_c values for these fractions.

However, in the supra-optimal range of T , $\psi_b(50)$ values increased (became more positive) with T , rising from -1.39 MPa at 20 °C to only -0.49 MPa at 28 °C (Fig. 2f-j; Table 1C). This was evident in the greater effect of reduced ψ on germination as T increased (Fig. 2a-e). The variation in ψ_b among seeds ($\sigma_{\psi b}$) was relatively constant with T (Table 1C), indicating that the threshold values of all seeds in the population increased by approximately equal amounts as T increased. When the $\psi_b(g)$ values estimated by the hydrotime model were plotted versus T for the 16th,

50th and 84th percentiles (Fig. 3b), points in the supra-optimal range showed linear increases that intercepted $\psi = 0$ MPa at the T_c values extrapolated from the GR_g data (Fig. 3a). This would be expected if the increase in $\psi_b(g)$ is responsible for the decrease in GR_g ; when the ψ_b value of a given seed increases to 0 MPa, the seed would be unable to germinate in water at that T , which is also the definition of T_c .

A hydrothermal time model of germination across all temperatures

Bradford (1990) derived a factor allowing the normalization of germination time courses across a range of ψ . This factor, $[1 - (\psi/\psi_b(g))] t_g$, normalizes the germination time of a seed fraction at any ψ to the corresponding germination time that would occur in water, given the ψ_b value for that seed fraction. In essence, this factor removes the effect of reduced ψ on the germination time course. If application of this factor normalizes time courses at different ψ to a common predicted time course in water, it indicates that the model parameters have accurately described the sensitivity

Table 1. Parameters of the hydrothermal time model characterizing germination of true potato seeds imbibed at three water potentials at sub- and supra-optimal temperatures

A						
T (°C)	θ_H (MPa h)	$\psi_b(50)$ (MPa)	$\sigma_{\psi b}$ (MPa)	r^2		
14	203	-1.64	0.24	0.92		
16	164	-1.56	0.19	0.92		
18	137	-1.46	0.23	0.89		
B						
T (°C)	θ_{HT} (MPa°h)	$\psi_b(50)$ (MPa)	$\sigma_{\psi b}$ (MPa)	T_b (°C)	r^2	
14-18	2090	-1.54	0.23	3.2	0.87	
C						
T (°C)	θ_H (MPa h)	$\psi_b(50)$ (MPa)	$\sigma_{\psi b}$ (MPa)	r^2		
20	130	-1.39	0.24	0.94		
22	130	-1.24	0.31	0.90		
24	130	-1.06	0.26	0.89		
27	130	-0.63	0.26	0.96		
28	130	-0.49	0.19	0.92		
D						
T (°C)	θ_H (MPa h)	$\psi_b(50)$ (MPa)	$\sigma_{\psi b}$ (MPa)	T_o (°C)	k_T (MPa °C ⁻¹)	r^2
20-28	130	-1.54	0.26	19.3	0.12	0.88

(A) At suboptimal T (14-18 °C), the hydrotime model was fitted to data from three ψ 's (0, -0.2, -0.4 MPa) at each T . (B) The hydrothermal time model was fitted across all three T 's and ψ 's. (C) In the supra-optimal range of T (20-28 °C), a single value of θ_H was used to fit germination data across ψ 's at each T , and $\psi_b(50)$ and $\sigma_{\psi b}$ were calculated. (D) The supra-optimal hydrotime model was fit to all data across 20-28 °C and 0, -0.2, -0.4 MPa. Coefficients of determination (r^2) are shown in each case.

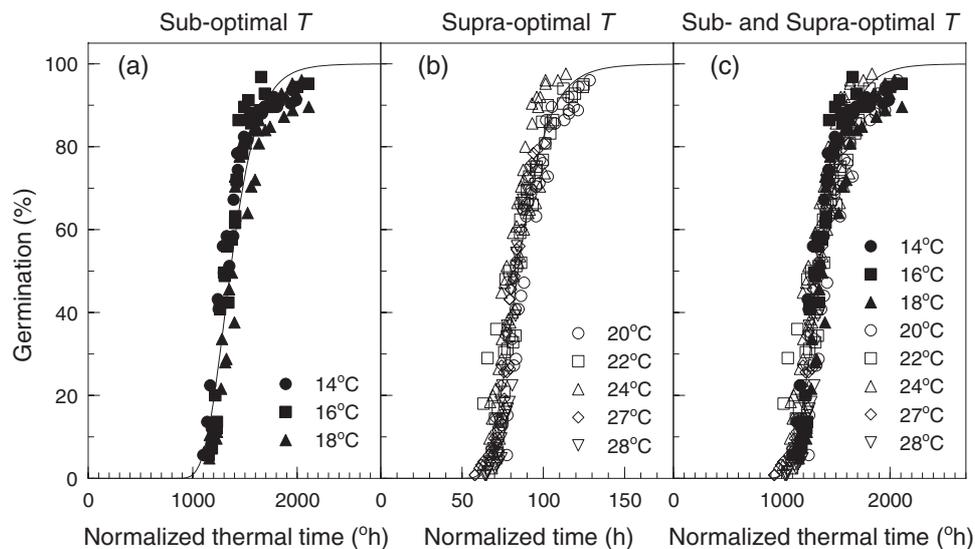


Figure 4. Normalized suboptimal and supra-optimal hydrotime models. (a) Germination time course data from Fig. 1a–c are plotted on a normalized thermal time scale showing the predicted time courses in water, based upon the parameters shown in Table 1B. (b) Germination time course data from Fig. 2a–e are plotted on a normalized time scale showing the predicted time courses in water based upon the parameters shown in Table 1D. A linear increase in $\psi_b(g)$ of 0.12 MPa °C⁻¹ accounts for the effect of supra-optimal T on germination and allows germination time courses to be normalized to a single time course equivalent to that in water. (c) Germination time courses at all T and ψ shown in Figs 1 and 2 can be combined on a single normalized thermal time scale. The normalized values from the supra-optimal model (Fig. 4B) were multiplied by $T_o - T_b = 16.1$ °C in order to convert to thermal time units (°h).

to ψ of the seed population. Using the hydrothermal time model (Eqn 6) and this normalization factor, all of the germination time courses at suboptimal T (Fig. 1a–c) were plotted on a common normalized thermal time scale (Fig. 4a). That is, the time courses at different ψ at each T were normalized to the equivalent time course in water at that T , and then these were plotted on a thermal time scale. These data at suboptimal T and reduced ψ are described well ($r^2 = 0.87$) by a common set of hydrothermal time parameters (Table 1B).

This approach clearly would not work at supra-optimal T , as the $\psi_b(g)$ distributions shifted positively as T increased (Fig. 2f–j), precluding a common set of model parameters. However, the value of $\psi_b(g)$ can be easily modified as follows to account for its linear increase as a function of T above T_o :

$$\psi_b(g)_{T>T_o} = \psi_b(g)_{T_o} + k_T(T - T_o) \quad (7)$$

where $\psi_b(g)_{T>T_o}$ is the $\psi_b(g)$ threshold distribution at T above T_o , $\psi_b(g)_{T_o}$ is the $\psi_b(g)$ distribution at T_o , and k_T is the slope of the relationship between $\psi_b(g)$ and T in the supra-optimal range of T (Fig. 3b). The value of $\psi_b(g)$ is simply increased linearly as T increases above T_o . This modified value of $\psi_b(g)_{T>T_o}$ can then be used in the hydrotime model (Eqn 5) to predict germination timing. It should therefore be possible to combine together all of the data shown in Fig. 2(a–e) and determine the parameter values that describe germination in this range of T .

To facilitate fitting this model by the repeated probit regression method (Bradford 1990), we used the fact that the model responds only to the difference between ψ and

$\psi_b(g)$, so a positive shift in $\psi_b(g)$ has the same effect as an equivalent negative shift in ψ . Thus for calculation purposes, ψ can be shifted negatively instead of shifting $\psi_b(g)$ positively in order to fit the model and determine the parameter values. The model for fitting was therefore:

$$\text{probit}(g) = [(\psi - k_T(T - T_o)) - (\theta_H/t_g) - \psi_b(50)] / \sigma_{\psi b} \quad (8)$$

where θ_H is the hydrotime value at T_o . We fit this model by changing systematically T_o , k_T and θ_H until the $\psi_b(50)$ for this model was similar to the $\psi_b(50)$ of the hydrothermal time model at suboptimal T (–1.54 MPa). The values that best fit the model are shown in Table 1D. Normalization of all the data of Fig. 2(a–e) using these parameters illustrated that the model worked well to predict germination timing and extent under these assumptions (Fig. 4b). Assuming further that thermal time accumulation is maximal at T_o and no additional thermal time accrues at $T > T_o$, the normalized data at supra-optimal T were multiplied by $T_o - T_b$ to put them on a thermal time basis (Fig. 4c). These normalized thermal time courses then coincided with the normalized data from the hydrothermal time model at suboptimal T (Fig. 4c). By accounting separately for germination behavior at sub- and supra-optimal ranges of T , the model could normalize all data from T_b to T_c and across ψ -values to a single common thermal time course.

The hydrotime parameters at each supra-optimal T mentioned previously in Fig. 2(f–j) and Table 1C were actually derived by fitting the data at each T using the common hydrotime constant (θ_H) predicted by the supra-optimal model across 20–28 °C (130 MPa h; Table 1D). The $\psi_b(g)$ distributions calculated at each T using this common θ_H

value are shown in Fig. 2(f–j) and were used to generate the predicted germination time courses at each T and ψ combination shown in Fig. 2(a–e). Similarly, the lines drawn in Fig. 3 are derived from the sub- and supra-optimal model parameters for the 16th, 50th and 84th germination percentiles (Tables 1B & D).

A modified hydrothermal time model can describe and predict both germination timing and percentage across all constant T and ψ at which germination can occur according to:

$$\theta_{HT} = \{\psi - \psi_b(g) - [k_T(T - T_o)]\}(T - T_b)t_g \quad (9)$$

where $[k_T(T - T_o)]$ applies only when $T > T_o$, and in this range of T the value of $\psi_b(g)$ is equal to $\psi_b(g)_{T_o}$ and $T - T_b$ is equal to $T_o - T_b$.

DISCUSSION

It has long been recognized that seed germination is characterized by minimum (T_b), optimum (T_o) and maximum (T_c) temperatures (Bewley & Black 1994). It has also been known for many years that the timing of germination at suboptimal T conforms to a heat units or thermal time model (Labouriau 1970; Bierhuizen & Wagenvoort 1974). The thermal time approach is well understood and has wide applicability for modelling developmental rates of plants, insects and other poikilothermic organisms (Ritchie & NeSmith 1991). However, this model (Eqn 1) does not predict the decrease in germination rates and percentages that occurs at $T > T_o$. Empirical models have been proposed that can match the observed changes in germination in this temperature range (e.g. Garcia-Huidobro *et al.* 1982; Ellis *et al.* 1986; Orozco-Segovia *et al.* 1996; Kebreab & Murdoch 2000). For purposes of ecological modelling of seed germination and seedling emergence, an empirical approach may be satisfactory (Forcella *et al.* 2000). However, if the model is to be used to guide further biochemical investigation into mechanisms controlling germination responses to environmental factors, a physiologically based model is preferable to a purely empirical one (Vleeshouwers & Kropff 2000; Benech-Arnold *et al.* 2000).

The hydrotime and hydrothermal time models provide insight into how physiological and environmental factors interact to regulate the germination behavior of seed populations (Bradford 1995, 2002). These models have revealed that at a given T , the timing and percentage of germination in a seed population are controlled by the difference between a physiologically determined ψ threshold (which can vary among individual seeds in the population) and the ψ of the seed. Studies have found that seed dormancy is a reflection of high (more positive) values of the $\psi_b(g)$ threshold, and that conditions that break dormancy (after-ripening, hormones, etc.) shift the $\psi_b(g)$ distribution to lower (more negative) values (Ni & Bradford 1992, 1993; Bradford 1996; Christensen, Meyer & Allen 1996; Allen & Meyer 1998; Meyer, Debaene-Gill & Allen 2000; V. Alvarado and K.J. Bradford, unpublished results). $\psi_b(g)$ values

have also been reported to be at a minimum around the optimum T and to become more positive at supra-optimal T (Bradford & Somasco 1994; Dutta & Bradford 1994; Christensen *et al.* 1996; Kebreab & Murdoch 1999; Meyer *et al.* 2000).

We have demonstrated here that when T exceeds T_o , the $\psi_b(g)$ distribution of a true potato seed population shifts to higher values (Figs 2 & 3). This has the consequence of delaying germination for all seeds in the population, and of preventing germination in those seeds whose ψ_b thresholds now exceed the ψ of the environment. As T increases further above T_o , different fractions of the seed population will have different T_c values, or temperatures at which $\psi_b(g)$ for the particular fraction is equal to 0 MPa. This explains the common observations that GR_g values decrease as T exceeds T_o and that T_c values vary among seeds in a population, often in a normal distribution (e.g. Ellis *et al.* 1986; Ellis & Butcher 1988) that reflects the normal distribution of $\psi_b(g)$ values. Treatments such as seed priming or ethylene, which can expand the high T range for germination, do so by increasing the T at which $\psi_b(g)$ begins to be affected (Bradford & Somasco 1994; Dutta & Bradford 1994). Thus, T acts on seed germination in the supra-optimal range primarily by causing a positive shift in the $\psi_b(g)$ values of the seed population (Figs 2 & 3).

Kebreab & Murdoch (1999) proposed an alternative interpretation and model for interactions between T and ψ where reduced ψ modifies the upper and lower temperature limits and germination rate relationships within an essentially thermal time model. However, a limitation to this model is that it predicts that the seed population will eventually achieve 100% germination under all conditions within the temperature limits, which is not the case. A subsequent version of the model was developed to predict the final germination percentages at different T and ψ combinations, but these two models have to be combined sequentially in order to describe both germination rate and percentage (Kebreab & Murdoch 2000). The hydrotime model, however, predicts both germination rates and percentages and closely matches the actual time courses, which asymptote at different final percentages depending upon the values of ψ and $\psi_b(g)$ (Fig. 2; Bradford 1995). Shifting $\psi_b(g)$ distributions in response to T automatically adjusts both germination rates and percentages. The parameters of the hydrothermal time model also have clear physiological meaning, which is not always the case with other empirical models. Thus, we believe that the approach proposed here wherein $\psi_b(g)$ distributions shift in response to changes in T is a more accurate and parsimonious description of actual seed behavior than the alternative of modifying thermal coefficients in response to changes in ψ .

Application of the population-based hydrothermal time model also allows normalization of germination time courses across all T and ψ at which germination can occur. In the suboptimal range of T , a common T_b among seeds and essentially constant values of $\psi_b(50)$ and σ_{ψ_b} made the application of this model straightforward. The factor developed by Bradford (1990) was used with the hydrothermal time

model to normalize the effects of ψ on germination across suboptimal T (Fig. 4a; Dahal and Bradford, 1994). When the effect of supra-optimal T on $\psi_b(g)$ was taken into account, germination time courses at these T could be normalized onto a common scale (Fig. 4b). As shown here by our application of Eqn 8 to fit the model, and in the final formulation of the model (Eqn 9), the effect on germination of increasing T above T_o was equivalent to the effect of a reduction in ψ . The k_T value of $0.12 \text{ MPa } ^\circ\text{C}^{-1}$ indicates that for every degree that T increased above T_o , the effect on germination was as if the seed ψ was reduced by 0.12 MPa . As $\psi_b(g)$ increased further at higher T , germination became more and more sensitive to ψ and eventually was prevented even in water when $T = T_c(g)$, or the T at which $\psi_b(g)$ was equal to 0 MPa (Figs 2a–e & 3b). Furthermore, when the normalized t_g values in Fig. 4b were multiplied by $T_o - T_b$, they coincided with the normalized time courses at suboptimal temperatures (Fig. 4c). This demonstrates that thermal time accumulation per unit actual time increases as T exceeds T_b , but further accumulation stops when $T > T_o$. In the supra-optimal range of T , the purely thermal effect of T on germination rates is maximal, and germination behaviour is governed by the values of the $\psi_b(g)$ threshold distribution relative to the ψ of the environment.

Although we used a single distribution of $\psi_b(g)$ to describe germination at suboptimal T , there was a small increase in $\psi_b(50)$ values in this range also as T increased (Table 1A; Fig. 3b). This could potentially be accounted for with another slope constant to adjust $\psi_b(g)$ values relative to T in this suboptimal range. Since a common estimate of the hydrothermal time model parameters did an adequate job of accounting for the data in this example (e.g. Fig. 4a), we did not pursue this further. However, there are cases where variation in apparent T_b among seeds in a population or in germination sensitivity to ψ at low T were observed (Orozco-Segovia *et al.* 1996; Kebreab & Murdoch 1999, 2000; Grundy *et al.* 2000), and changes in both the upper and lower temperature limits for germination are often associated with the imposition and release of dormancy (e.g. Kruk & Benech-Arnold 2000). Thus, further modification of the hydrothermal time model to allow variation in $\psi_b(g)$ at both sub- and supra-optimal temperatures may be necessary to describe germination behaviour of other species (Bradford 2002).

The conclusion that shifts in $\psi_b(g)$ distributions determine the germination behaviour of seeds in the supra-optimal temperature range has a number of significant consequences. It further confirms that rather than being fixed values, $\psi_b(g)$ thresholds of seed populations are under physiological control in response to environmental and hormonal conditions (Ni & Bradford 1992, 1993; Bradford & Somasco 1994; Dahal & Bradford 1994; Christensen *et al.* 1996; Meyer *et al.* 2000). As noted above, the common observation that the temperature limits for germination widen as dormancy is released and narrow as dormancy is imposed (Vegis 1964) is likely to be due to corresponding shifts in the $\psi_b(g)$ distributions of the seed populations in response to dormancy-regulating factors. We can predict

that additional factors that regulate germination under natural conditions, such as after-ripening, light or nitrate (Hilhorst & Toorop 1997; Benech-Arnold *et al.* 2000), will act via this mechanism. Although variable $\psi_b(g)$ values complicate some applications of the hydrothermal time model for predicting seedling emergence in the field, this may also explain why models assuming fixed values of $\psi_b(g)$ could not adequately describe all aspects of seed behaviour across variable environmental conditions (e.g. Kebreab & Murdoch 1999; Finch-Savage *et al.* 2000; Grundy *et al.* 2000; Bradford 2002). Although the model does not identify the biochemical mechanism(s) by which $\psi_b(g)$ is itself regulated, it clearly points further biochemical and molecular investigations in this direction. A number of candidate genes and processes have been identified that could be involved in the initiation of germination (Bradford *et al.* 2000), at least some of which are regulated and expressed in a manner consistent with a role in establishing ψ_b thresholds (Chen & Bradford 2000; Nonogaki, Gee & Bradford 2000; Chen, Dahal & Bradford 2001, Chen, Nonogaki & Bradford 2002). Finally, we anticipate that this population-based threshold model can be applied to describe the responses of other biochemical or physiological processes in cell populations to changing T , ψ , hormonal, or developmental conditions (Bradford & Trewavas 1994).

In conclusion, we have extended the hydrothermal time model to describe germination timing and percentage across all T and ψ at which germination can occur. This comprehensive, physiologically based model accounts for all three of the cardinal temperatures for seed germination. The parameters of the model can be used to quantitatively characterize and compare the physiological status of seed populations under different environmental conditions or having different genetic backgrounds. In addition, the model targets the processes by which seed water potential thresholds are determined for further biochemical and molecular investigation.

ACKNOWLEDGMENTS

This project was supported in part by Western Regional Research Project W-168. We thank Dr Noel Pallais of the International Potato Center, Lima, Perú, for providing the true potato seeds and encouraging this investigation.

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Received 22 December 2001; received in revised form 1 April 2002; accepted for publication 1 April 2002