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Hydrothermal time analysis of seed dormancy in true (botanical) potato seeds

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Abstract

As seed dormancy is released within a seed population, both the rate and percentage of germination increase progressively with increasing dose of a dormancy-breaking treatment or condition. Population-based models can account for this behaviour on the basis of shifting response thresholds as dormancy is alleviated. In particular, hydrothermal time analysis of germination sensitivity to water potential (Ψ) and temperature (T) can describe these features of seed behaviour. We used the hydrothermal time model to analyse the effects of dormancy-breaking treatments on germination of dormant true (botanical) potato (*Solanum tuberosum* L.) seeds (TPS). After-ripening (37°C and 4% seed moisture content) of TPS for 7 or 30 days partially or fully alleviated primary dormancy. The median base water potential required to prevent germination [$\Psi_b(50)$] decreased from −0.25 MPa in control seeds to −0.87 MPa and −1.83 MPa after 7 and 30 days of after-ripening, respectively. In contrast, the base temperature for germination (T_b) was relatively unaffected (0–3.3°C). Fluridone (50 μM), an inhibitor of abscisic acid (ABA) biosynthesis, also promoted germination of dormant TPS and lowered $\Psi_b(50)$, indicating a role for *de novo* synthesis of ABA during dormancy maintenance. Moist chilling (3 days at 4°C) or gibberellin (100 μM) alleviated secondary dormancy and lowered $\Psi_b(50)$ values from −0.08 MPa to −0.36 and −0.87 MPa, respectively. The hydrothermal time model allows quantification of dormancy levels and explains why changes in germination speed and percentage are closely correlated during dormancy alleviation.

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Introduction

Dormancy is the lack of the capacity for a seed 'to germinate in a specified period of time under any combination of normal physical environmental factors (temperature, light/dark, etc.) that otherwise is favourable for its germination' (Baskin and Baskin, 2004). While studies on seed dormancy often consider only the final germination percentage as an indicator of dormancy status, the definition above indicates that completion of germination within a specified period of time is also relevant. That is, dormancy may also be manifested as a delay in completion of germination, even among seeds that will eventually germinate. In many species, dormancy loss is accompanied by an increased germination rate (i.e. the inverse of the time to completion of germination) in the fraction of seeds that is capable of completing germination (Gordon, 1973; Allen *et al.*, 1995; Bradford, 1996). For example, Favier (1995) showed that the mean time to germination of dormant barley (*Hordeum vulgare*) seeds continued to decrease as dormancy was lost due to after-ripening, even after all seeds were able to complete germination. Thus, germination rates as well as percentages should be considered in characterizing seed dormancy status.

The hydrothermal time model is a population-based threshold model that accounts for both germination percentages and rates simultaneously (Gummerson, 1986; Bradford, 1995, 1996, 2002; Finch-Savage, 2004). This model can describe germination time courses across water potentials (Ψ) and temperatures (T) in the sub-optimal range (between the minimum and optimum temperatures) and, with minor modification, also in the supra-optimal range (between the optimum and maximum temperatures) (Alvarado and Bradford, 2002; Rowse

and Finch-Savage, 2003). For the sub-optimal range of T, the hydrothermal time model can be written as:

$$\theta_{HT} = [\Psi - \Psi_b(g)](T - T_b)t_g \quad (1)$$

where θ_{HT} is the hydrothermal time constant, $\Psi_b(g)$ is the base water potential threshold for germination of fraction, g, of the seed population, T_b is the base or minimum temperature at which seeds can germinate, and t_g is the time required for fraction or percentage, g, of the seeds to germinate. The $\Psi_b(g)$ value varies among seeds in the population (generally, but not necessarily, in a normal distribution), while T_b is assumed here to be the same for all seeds, which is not always the case (e.g. Kebreab and Murdoch, 2000; Batlla and Benech-Arnold, 2003; Steadman and Pritchard, 2004). θ_{HT} is a constant for all seeds in the population, so as Ψ approaches the threshold value for a specific seed fraction [$\Psi_b(g)$], or T approaches T_b, the time to completion of germination (t_g) increases proportionately. Conversely, increasing differences between Ψ and $\Psi_b(g)$ or between T and T_b result in smaller t_g values or faster germination rates. When Ψ equals or is less than $\Psi_b(g)$ or T equals or is less than T_b, t_g essentially becomes infinite and germination does not occur. Thus, this simple model can account for both germination rates and percentages, and for the consistent relationship between them, as well as for differences among seeds within the population. In addition, the parameters of the hydrothermal time model provide quantitative indices of seed physiological status and ecological adaptation (Allen and Meyer, 1998; Allen *et al.*, 2000; Bradford, 2002; Allen, 2003).

A number of studies have applied the hydrotime or hydrothermal time model to analyse the effects of dormancy or environmental conditions on seed germination (reviewed in Bradford, 2002; Finch-Savage, 2004). Bradford and Somasco (1994) showed that as T increased to the point where lettuce (*Lactuca sativa*) seeds (achenes) were thermoinhibited, the $\Psi_b(g)$ thresholds of the seed population became more positive, making them more sensitive to inhibition at reduced Ψ and eventually preventing germination even in water. Ethylene, which allowed germination at higher temperatures, caused $\Psi_b(g)$ thresholds to remain lower as T increased (Dutta and Bradford, 1994). Similar positive shifts in $\Psi_b(g)$ distributions with increasing temperatures above the optimum for germination were reported for potato (*Solanum tuberosum*), carrot (*Daucus carota*), onion (*Allium cepa*), red fescue (*Festuca rubra*) and Kentucky bluegrass (*Poa pratensis*) (Alvarado and Bradford, 2002; Rowse and Finch-Savage, 2003; Larsen *et al.*, 2004). Negative shifts in the $\Psi_b(g)$ threshold distributions during after-ripening of dormant seeds of *Bromus tectorum* and *Elymus elymoides* accounted well for the seasonal changes in germination capacity due to after-ripening (Christensen *et al.*, 1996; Bauer *et al.*, 1998; Meyer *et al.*, 2000). Batlla and Benech-Arnold (2004) showed that the effects of imbibed chilling in alleviating dormancy can be modelled on the basis that $\Psi_b(g)$ distributions move toward lower values in proportion to the accumulation of thermal stratification time below a threshold temperature. Bradford (1996, 2002) has proposed that shifts in $\Psi_b(g)$ distributions due to environmental or hormonal signals may be a general physiological mechanism underlying dormancy and its relief.

True (botanical) potato seeds (TPS) show a strong primary dormancy that is temperature dependent; dormant TPS may germinate at 17°C but not at 27°C (Pallais, 1995a). Dormancy in TPS can be broken by dry after-ripening, which expands the upper temperature range for germination (Pallais, 1995a, b). This dormancy pattern is classified as Type 1 non-deep physiological dormancy (Baskin and Baskin, 2004). Gibberellin (GA) also promotes germination in freshly harvested TPS (Spicer and Dionne, 1961; Bamberg and Hanneman, 1984). Blocking abscisic acid (ABA) synthesis using fluridone (an inhibitor of carotenoid biosynthesis) extended the upper temperature range for germination in TPS (Alvarado *et al.*, 2000), as it did in lettuce (Yoshioka *et al.*, 1998; Gonai *et al.*, 2004). Thus, GA and ABA may be involved in determining the depth of dormancy of TPS, as in other seeds (e.g. Grappin *et al.*, 2000; Koornneef *et al.*, 2002; Benech-Arnold *et al.*, 2003).

We applied the hydrothermal time model to quantify and analyse dormancy and the effects of various dormancy-breaking treatments in TPS. We showed previously that the reduction in germination of non-dormant TPS at supra-optimal temperatures was associated with a linear increase in $\Psi_b(g)$ as T increased above the optimum temperature (Alvarado and Bradford, 2002). Here, we tested whether alleviation of dormancy of TPS by various methods, which improve germination at sub-optimal temperatures and expand the upper temperature limit for germination, is consistently associated with a decrease in $\Psi_b(g)$ distributions or with changes in other parameters of the hydrothermal time model.

Materials and methods

Hybrid TPS were produced in Ancash, Perú (3500 m altitude). The parental lines were Yungay and 104.12LB, which is resistant to late blight (*Phytophthora infestans*). The seed lots were harvested in Chacas in 1996 and Jambon in 1997. After harvest, the seeds were transported to Lima, Perú and stored at 15°C and low relative humidity until the seed moisture content (dry weight basis) was reduced to *c.* 4.5%, and then stored at 0°C in sealed aluminium containers. Upon arrival at

UC Davis in 1998, the seeds were stored at −20°C. The Jambon seed lot retained primary dormancy, while the Chacas seed lot had lost primary dormancy by that time.

Several treatments were employed to alleviate dormancy. For after-ripening, the seed moisture content was reduced to 4% by holding the seeds for 10 days at 20°C and 44% relative humidity over a saturated solution of K_2CO_3. Seeds were then transferred to 37°C in sealed vials for 7 or 30 days. For a chilling treatment, TPS seeds imbibed in water were held at 4°C for 3 days before transfer to 18°C for germination. Gibberellin treatment (GA_{4+7}, 100 μM; Abbott Labs, Chicago, Illinois, USA) was as an aqueous solution and with the addition of polyethylene glycol (PEG) 8000 to make solutions of −0.2 and −0.4 MPa at 18°C. Fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl-(phenyl)]-4-(1H)-pyridinone) solutions (50 μM; SePRO, Carmel, Indiana, USA), with or without PEG 8000, were prepared from a stock solution (100 μM) according to the method of Ober and Sharp (1994), to obtain solutions of 0, −0.2 and −0.4 MPa at 13 and 18°C.

For germination tests, solutions of PEG 8000 were prepared according to Michel (1983) to give equal Ψ at the experimental temperatures; Ψ values were confirmed by measurement with a vapour pressure osmometer. Five replicates of 25 seeds were placed in 5 cm diameter Petri dishes on two germination blotters saturated with water ($\Psi = 0$) or solutions of PEG 8000 of −0.2 and −0.4 MPa at constant temperatures between 12 and 24°C. The replicates were located in different positions within the temperature lanes on a thermogradient table, or randomly within constant temperature chambers. Temperatures were monitored, and the actual mean temperatures are reported for each experiment. Germination was recorded as radicle protrusion to 2 mm, and germinated seeds were removed.

Germination time-course data were analysed by repeated probit regression using the thermal time, hydrotime and hydrothermal time models, as described previously (Bradford, 1990, 1995, 2002; Dahal and Bradford, 1994).

Results

Primary dormancy

The Jambon seed lot exhibited a germination pattern typical of temperature-dependent primary dormancy in TPS (Pallais, 1995a, b). Germination in water was slow and incomplete, and was reduced further as T exceeded the optimum of 18°C (Fig. 1A–E). Germination was completely inhibited at 27°C (data not shown; Alvarado *et al.*, 2000). Even slight reductions in Ψ (−0.2 MPa) delayed or prevented germination, and little or no germination occurred at −0.4 MPa at any temperature (Fig. 1A–E). The hydrotime model [i.e. equation (1) without the $T - T_b$ term and using θ_H instead of θ_{HT}] was fit to germination data at each constant temperature (12, 14, 16, 18 and 20°C). The $\Psi_b(g)$ distributions of the dormant seeds have relatively high median [$\Psi_b(50)$] values (−0.18 to −0.36 MPa) with standard deviations (σ_{ψ_b}) of 0.19–0.29 MPa (Table 1A). The hydrotime constants (θ_H) varied between 48 and 96 MPa h, but in no consistent relationship with temperature. Predicted time courses at each T and Ψ, using the parameter values in Table 1A, are shown in Fig. 1A–E, illustrating the close match to the actual data ($r^2 = 0.92$–0.98).

The data for germination at sub-optimal temperatures (12–18°C) could be combined using hydrothermal time, calculated as in equation (1), to also account for the effect of temperature on germination rates. According to this analysis, T_b of the dormant seeds (0 days after-ripening) was 0°C and the combined estimate of $\Psi_b(50)$ was −0.25 MPa, with σ_{ψ_b} of 0.21 MPa (Table 1B), resulting in a fraction of the seed population having $\Psi_b(g)$ values greater than 0 MPa (Fig. 2B). Seeds in that fraction were unable to germinate in water, and the remaining seeds were slow to germinate and sensitive to small reductions in Ψ due to their high Ψ_b values (Fig. 1A–E). Using the values in Table 1B, data from all sub-optimal temperatures and Ψ conditions could be plotted together on a normalized thermal time scale (Fig. 2A; Bradford, 1990). The close match of the data to the modelled prediction ($r^2 = 0.96$) indicates that the hydrothermal time model can account well for the germination behaviour of the seeds, and that the hydrothermal time parameters are relatively constant across the range of sub-optimal T and Ψ tested.

After-ripening

After-ripening (AR) at 37°C and 4% moisture content rapidly alleviated dormancy in TPS. Seven days of AR were sufficient to improve germination to almost 100% at sub-optimal T, although germination was still reduced at 23°C (Fig. 1F–J). The germination percentages and rates also became less sensitive to reduced Ψ. The hydrotime model accurately described seed germination time courses across Ψ at each constant T (solid and dashed lines in Fig. 1F–J; parameters in Table 1A). The $\Psi_b(g)$ distributions shifted to lower median values, except at 23°C, where dormancy was still expressed (Table 1A). After 30 d of AR, dormancy was essentially eliminated at temperatures below 18°C, and a Ψ of −0.4 MPa only slightly delayed germination at the optimal temperature (Fig. 1K–M), compared to preventing germination in dormant seeds (Fig. 1A–D). Germination at supra-optimal temperatures also

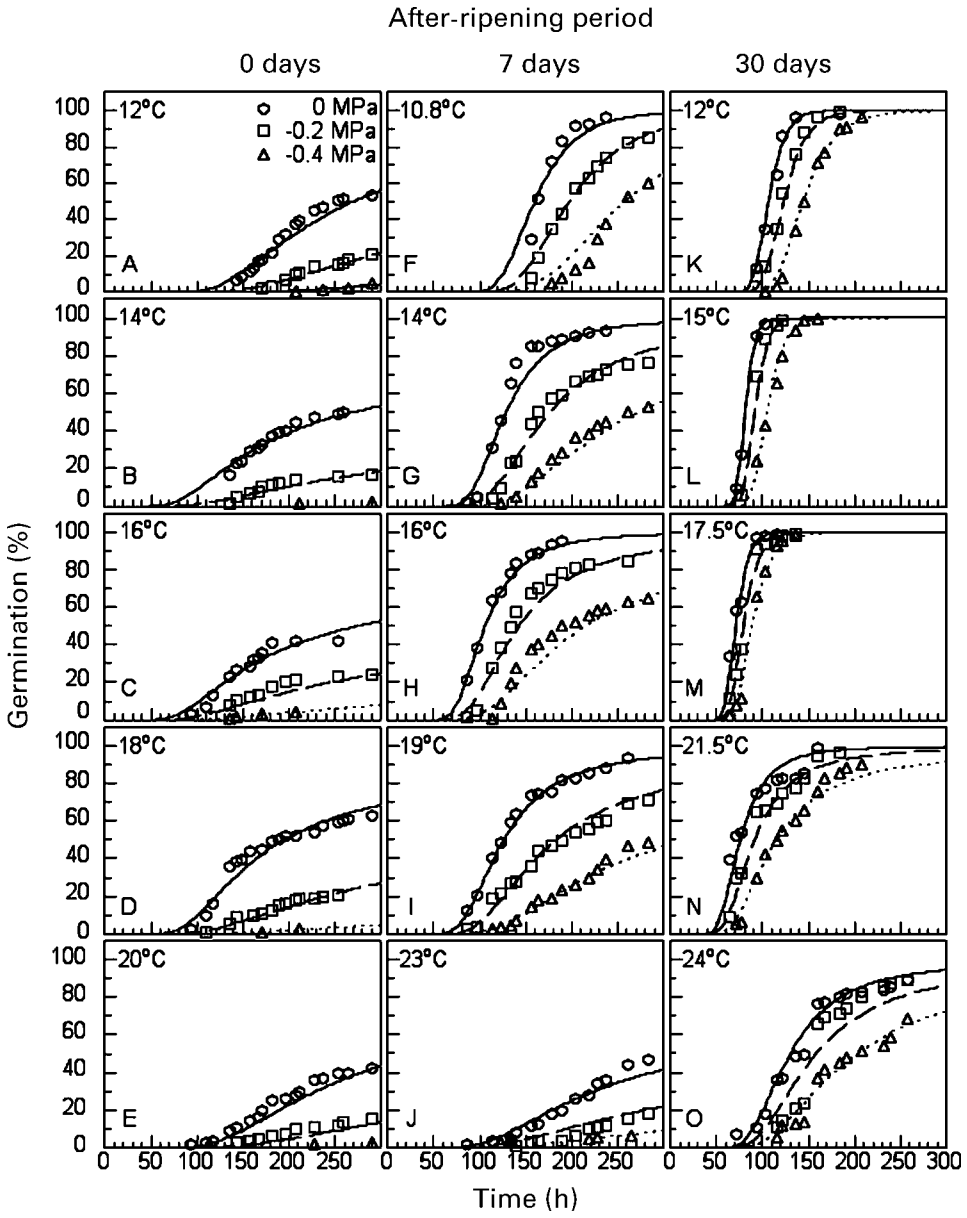

Figure 1. Germination of true potato seeds (Jambon) at different temperatures and water potentials, as affected by after-ripening period at 4% seed moisture content and 37°C. The temperatures of each test are indicated in the individual panels. In all panels, the symbols are the actual data and the solid and dashed curves are predicted by the hydrotime model, using the values in Table 1A.

increased after 30 d of AR, although some dormancy was still evident by the delay in germination and sensitivity to Ψ as T increased above 18°C (Fig. 1N, O). This was due primarily to the effect of T on the $\Psi_b(g)$ of the seeds, although θ_H also increased at 24°C (Table 1A; Alvarado and Bradford, 2002).

Hydrothermal time analysis at sub-optimal temperatures showed that T_b increased slightly due to AR, from 0°C (0 and 7 d AR) to 3.3°C (30 d AR) (Table 1B). The $\Psi_b(50)$ values at sub-optimal temperatures decreased with increasing AR from -0.25 MPa (0 days AR) to -0.87 and -1.83 MPa after 7 and 30 d AR (Fig. 2B), while θ_{HT} approximately doubled during AR, and σ_{ψ_b} remained essentially constant (Table 1B). Plotting the data from all Ψ and sub-optimal temperatures on a normalized thermal time scale illustrates the extent to which the hydrothermal time model could describe the improvement in seed

Table 1. Hydrotime (A) and hydrothermal time (B) parameters characterizing germination of primary dormant true potato seeds (Jambon) exposed to different after-ripening (AR) times (0, 7 and 30 days) at 37°C and 4% seed moisture content

(A) Hydrotime

AR (days)	T (°C)	θ_H (MPa h)	$\Psi_b(50)$ (MPa)	σ_{ψ_b} (MPa)	r^2
0	12	96	−0.36	0.24	0.97
	14	48	−0.18	0.23	0.97
	16	60	−0.22	0.29	0.92
	18	52	−0.27	0.19	0.97
	20	77	−0.22	0.26	0.98
7	10.8	168	−1.05	0.23	0.95
	14	105	−0.81	0.24	0.94
	16	88.5	−0.84	0.25	0.92
	19	86	−0.68	0.26	0.99
	23	99.4	−0.26	0.36	0.88
30	12.5	180	−1.69	0.23	0.95
	15	153	−1.89	0.20	0.98
	17.5	150	−2.11	0.32	0.98
	21.5	90	−1.19	0.35	0.91
	24	145	−1.11	0.39	0.89

(B) Hydrothermal time model[a]

AR (days)	θ_{HT} (MPa °h)	T_b (°C)	$\Psi_b(50)$ (MPa)	$\sigma_{\psi b}$ (MPa)	r^2
0	828	0	−0.25	0.21	0.96
7	1500	0	−0.87	0.24	0.89
30	1800	3.3	−1.83	0.24	0.93

[a] The hydrothermal time model has been calculated only for the sub-optimal range of temperatures: 0 days AR, 12–18°C; 7 days AR, 10.8–16°C; 30 days AR, 12.5–17.5°C.

germination behaviour in response to AR (Fig. 2A), which was primarily due to the negative shifts in $\Psi_b(g)$ distributions (Fig. 2B).

Fluridone treatments

Fluridone (50 μM) improved germination of primary dormant Jambon seeds at three values of Ψ and two sub-optimal values of T (Fig. 3). The hydrothermal time model described 91–94% of the variation in seed germination times in treated and non-treated seeds (Table 2). A shift in estimated T_b from 3.7°C to 8.0°C was observed when seeds were treated with fluridone (Table 2), and a shift in the $\Psi_b(g)$ distribution to lower values also occurred (Fig. 4B). The effects of fluridone on $\Psi_b(g)$ and on germination were similar to the changes observed following 7 days of AR (compare Figs 2 and 4), although fluridone had a greater effect on T_b.

Secondary dormancy

The Chacas seed lot developed secondary dormancy after more than 1 year of storage at −20°C. Seeds that had previously germinated fully in water at 18°C (data not shown; Alvarado and Bradford, 2002) germinated poorly in this condition after storage (Fig. 5A). The $\Psi_b(50)$ was −0.08 MPa (Table 3; Fig. 5D), so only *c.* 50% of the population would be able to germinate in water, and they were very sensitive to reduced Ψ (Fig. 5A), similar to the primary dormant seeds (Fig. 1C).

Chilling treatment (imbibed for 3 d at 4°C) lowered the $\Psi_b(50)$ to −0.36 MPa, allowing more seeds to germinate in shorter times (Fig. 5B; Table 3). The increased germination rate due to a reduction in $\Psi_b(50)$ was partially offset by a threefold increase in θ_H (Table 3). GA_{4+7} (100 μM) was even more effective in breaking dormancy, as the $\Psi_b(50)$ shifted to −0.87 MPa (Fig. 5F; Table 3), germination rate increased and germination at reduced Ψ improved (Fig. 5C).

Discussion

Freshly harvested TPS exhibit Type 1 non-deep physiological dormancy that lasts for months if the seeds are not after-ripened or subjected to other dormancy-breaking treatments (Pallais, 1995a, b; Baskin and Baskin, 2004). This dormancy represents a problem for the potato growers in tropical countries, where propagation from TPS is being adopted due to the advantages over seed tubers in these areas (Pallais, 1987; CIP, 2002). Although some seeds in dormant lots are able to complete germination at low temperatures, both percentages and rates are low, and decrease further at higher temperatures (Fig. 1A–E). Primary dormancy of TPS is readily alleviated by AR using warm temperatures and low seed moisture contents (Pallais, 1995a). After-ripening at 37°C and 4% seed moisture content resulted in considerable improvement in germination after 7 d and complete loss of dormancy after 30 d (Fig. 1). Thus, TPS provide an experimental system for studying physiological seed dormancy that is closely related genetically to tomato (*Lycopersicon esculentum* Mill.), which has served as a model for molecular and biochemical studies of germination, but which has largely lost dormancy through domestication (Alvarado *et al.*, 2000; Bradford *et al.*, 2000; Koornneef *et al.*, 2002). Until recently, potatoes were propagated exclusively through tubers and have retained seed dormancy in their sexual reproductive cycle (Pallais, 1987).

We tested whether changes in primary and secondary dormancy in TPS due to AR, chilling, GA or fluridone were associated with corresponding shifts in the $\Psi_b(g)$ distributions or other parameters characterized by the hydrothermal time model. Overall, the hydrotime and hydrothermal time models described both the timing and extent of germination

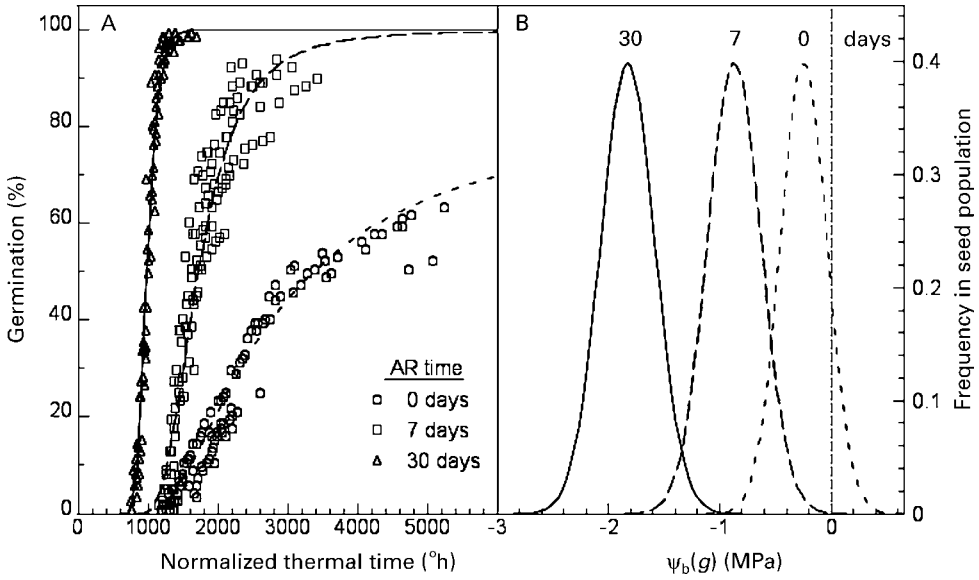

Figure 2. Time courses of germination on a normalized thermal time scale and $\Psi_b(g)$ distributions of true potato seeds (Jambon) after different periods of dry after-ripening. (A) The effects of T and Ψ on germination before or following after-ripening (AR) (from Fig. 1) were combined into single time courses on a normalized thermal time scale by multiplying the times to germination at each Ψ by $1 - [\Psi/\Psi_b(g)](T - T_b)$ for each fraction g (Bradford, 1990). Only data in the sub-optimal range of temperatures were used (see Table 1B). The symbols are the actual data and the solid and dashed curves are predicted by the hydrothermal time model, using the values in Table 1B. (B) The $\Psi_b(g)$ distributions obtained with the hydrothermal time model for sub-optimal temperatures (Table 1B) are plotted as frequency distributions.

very well across a range of T and Ψ conditions and dormancy states. Improvements in germination due to all of these dormancy-breaking factors were consistently associated with more negative $\Psi_b(50)$ values, while changes in θ_H or θ_{HT} were more variable (Tables 1–3). Negative shifts in a normal distribution of thresholds [$\Psi_b(g)$; Figs 2B, 4B] would result in a sigmoid increase in total germination percentages as dormancy is released during AR, which has been well documented in rice (*Oryza sativa*), barley and other species (Roberts, 1961; Favier and Woods, 1993). In addition, germination rates increased, and sensitivity to Ψ decreased, between 7 and 30 d AR, after essentially all seeds were capable of completing germination in water (Figs 1F–H versus K–M). Thus, dormancy can delay germination and increase sensitivity to Ψ even in seeds that eventually complete germination, suggesting that germination rates and hydrotime parameters are more sensitive indicators of dormancy status than are germination percentages alone.

Changes in both θ_H and $\Psi_b(g)$ can affect germination rates, and θ_H values generally decrease in non-dormant seeds as germination rates increase with increasing temperature (Dahal and Bradford, 1994). In the present case, there was no consistent change in θ_H or $\Psi_b(50)$ with temperature in dormant seeds (0 d AR) (Table 1A), and germination patterns and timing were similar between 12 and 18°C (Fig. 1A–D). Perhaps the germination rates of partially dormant seeds are limited more by internal physiological factors than by temperature, making them relatively insensitive to the latter. Germination rates of after-ripened seeds increased as temperature increased, and then decreased at supra-optimal temperatures (Fig. 1F–O). This was evident in decreasing θ_H values as temperature increased in the sub-optimal range, then an increase in both θ_H and $\Psi_b(50)$ values at warmer temperatures (Table 1A). Increases in θ_H and $\Psi_b(50)$ at warmer temperatures would both tend to delay or prevent germination relative to lower temperatures. Increasing $\Psi_b(50)$ values at supra-optimal temperatures have been reported previously for TPS (Alvarado and Bradford, 2002) and other species (Rowse and Finch-Savage, 2003).

There was relatively little change in σ_{ψ_b} due to AR (Table 1A), indicating that the variation among seeds in their threshold Ψ values for germination was not greatly affected by dormancy or its alleviation. An exception to this was GA_{4+7}, which caused σ_{ψ_b} to approximately double (Fig. 5F; Table 3). This may reflect variation among seeds in their sensitivity or responsiveness to GA, which would exacerbate variation within the seed population in response to applied GA (Ni and Bradford, 1993).

A small increase in T_b (from 0 to 3.3°C) was observed with extended AR (Table 1B) and a somewhat

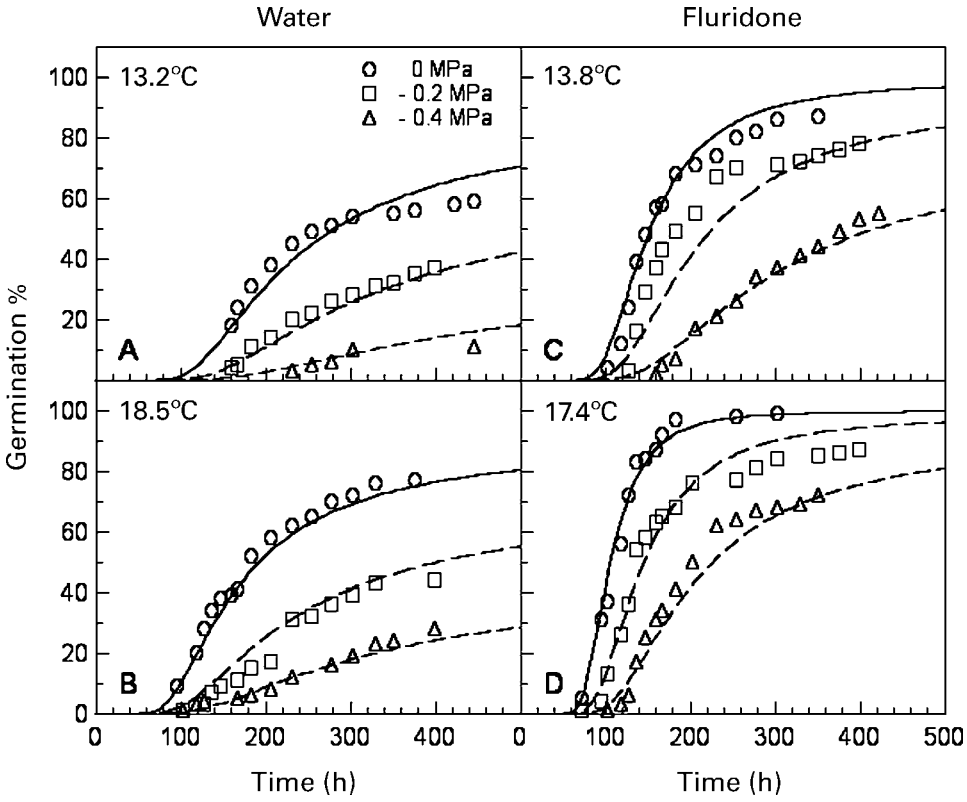

Figure 3. Influence of fluridone on germination of primary dormant true potato seeds (Jambon). Seeds were imbibed in water (A, B) or 50 μM fluridone (C, D) at sub-optimal temperatures and three water potentials. The symbols are the actual data, and the lines are the germination time courses predicted by the hydrotime model at each temperature (Table 2).

greater increase (from 3.7 to 8°C) occurred due to fluridone (Table 2), although the latter is based upon data at only two temperatures and may be less reliable. In contrast, reductions in T_b occurred during dormancy-breaking conditions in some species (e.g. Batlla and Benech-Arnold, 2003; Steadman and Pritchard, 2004). As T_b was 0 to 3.7°C in dormant TPS, T_b was not the limiting factor for germination at the test temperatures, and is not a likely mechanism for dormancy in this species. More extensive data would be required to determine the significance of the increases in T_b reported here due to AR and fluridone. On the other hand, shifts in $\Psi_b(g)$ distributions could contribute to apparent changes in T_b by delaying or accelerating germination, particularly in tests for relatively short times at low temperatures. In *Polygonum aviculare*, for example, improved germination due to stratification involved thermal (chilling) time-dependent shifts in both the lower temperature limits for germination and in $\Psi_b(g)$ distributions (Batlla and Benech-Arnold, 2003, 2004).

Table 2. Parameters of the hydrothermal time model across two temperatures (13.2, 18.5°C) and three water potentials (0, −0.2, −0.4 MPa) for dormant true potato seeds (Jambon) imbibed in water or in 50 μM fluridone

Treatment	θ_{HT} (MPa°h)	T_b (°C)	$\Psi_b(50)$ (MPa)	σ_{ψ_b} (MPa)	r^2
Water	1020	3.7	−0.37	0.29	0.94
Fluridone	715	8.0	−0.75	0.25	0.91

It is rather surprising that the hydrothermal time constant (θ_{HT}) approximately doubled with AR, while $\Psi_b(50)$ decreased markedly (Table 1B). In general, one might expect that θ_{HT} would either be constant or decrease as germination rates increased. This discrepancy may be due to the time required for imbibition and activation of germination-related processes, which may not be shortened in direct proportion to the changes in Ψ_b thresholds. The germination parameters of the hydrothermal time model are calculated based on the time from the start of imbibition to radicle emergence, and some of this time will be required for water uptake and metabolic activation (i.e. phase I, imbibition), which may be relatively insensitive to temperature or dormancy status. This time period would contribute increasingly more to the total time

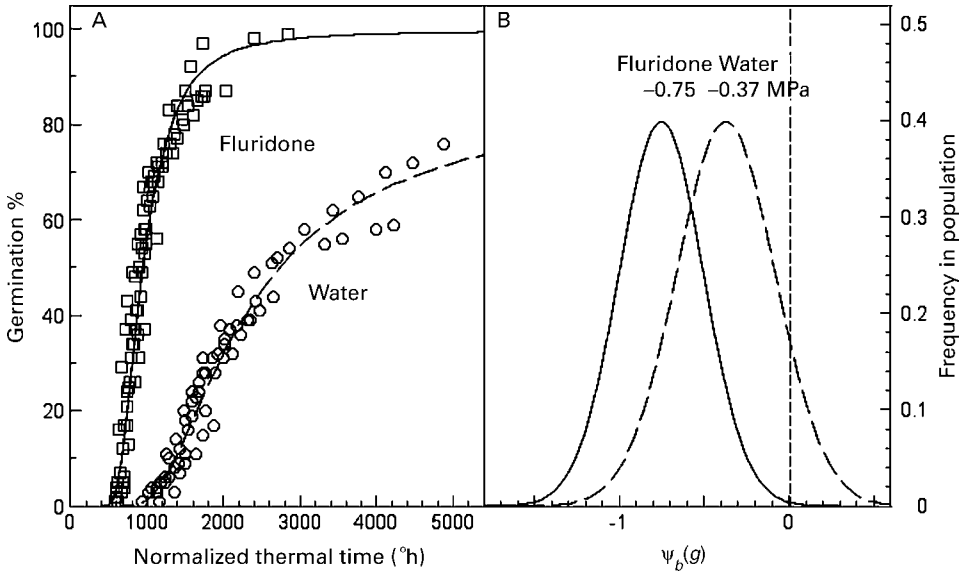

Figure 4. Time courses of germination on a normalized thermal time scale and Ψ_b distributions of true potato seeds (Jambon) as affected by 50 μM fluridone. (A) Germination on a normalized thermal time scale (as in Fig. 2A) across temperatures and water potentials (from Fig. 3) for seeds germinated in the presence or absence of 50 μM fluridone. The symbols are the actual data, and the solid and dashed curves are predicted by the hydrothermal time model, using the values in Table 2. (B) The $\Psi_b(g)$ distributions obtained with the hydrothermal time model are plotted as frequency distributions.

to radicle emergence as germination rates increase following AR (i.e. as phase II post-imbibition decreases). Thus, the primary effect of AR on dormancy release is through a reduction in $\Psi_b(g)$, with all other factors influencing germination rates being combined in the θ_{HT} term of the hydrothermal time equation. The decrease in θ_{HT} in response to fluridone can be attributed largely to the apparent increase in T_b (Table 2).

Fluridone is able to improve germination in dormant TPS (Fig. 3; Alvarado *et al.*, 2000), as has been shown previously in lettuce (Yoshioka *et al.*, 1998; Gonai *et al.*, 2004), *Nicotiana plumbaginifolia* (Grappin *et al.*, 2000), sorghum (*Sorghum bicolor*) (Benech-Arnold *et al.*, 2003) and other species. The effectiveness of fluridone in breaking dormancy and lowering $\Psi_b(g)$ in TPS (Table 2) is consistent with the ability of ABA to increase Ψ_b thresholds for germination (Ni and Bradford, 1992; Toorop *et al.*, 2000), and implies that *de novo* synthesis of ABA following imbibition plays a role in the maintenance of dormancy. However, Gonai *et al.* (2004) found in lettuce that while fluridone could increase the upper temperature limit for germination, the addition of GA with fluridone increased the limit even further. The sensitivity of germination to ABA increased with temperature, while GA increased ABA catabolism and reduced seed ABA content. Together, they argued, fluridone and GA reduced the ABA content sufficiently to compensate for the increased ABA sensitivity at high temperature and permit germination. Similar processes may be at work in TPS, as fluridone mimicked a short AR treatment, but did not fully overcome dormancy (Figs 3, 4). GA induces early germinative processes in TPS and tomato seeds, such as the expression of a germinative endo-β-mannanase that is not inhibited by ABA (Alvarado *et al.*, 2000; Nonogaki *et al.*, 2000). Fluridone was less effective in inducing this gene in TPS than was GA, although fluridone was more effective than GA in promoting germination at high temperature (Alvarado *et al.*, 2000). Changes in sensitivity to GA and/or ABA with AR or temperature may also be involved, as in other species (Bianco *et al.*, 1994; Benech-Arnold *et al.*, 2003; Gonai *et al.*, 2004). Thus, while the balance of ABA and GA contents and sensitivities are involved in regulating dormancy and germination, the hormones do not always have opposing effects on their molecular targets; each hormone may regulate a different subset of germination-related pathways (Alvarado *et al.*, 2000; Bradford *et al.*, 2000). The molecular and biochemical mechanisms by which $\Psi_b(g)$ values are affected by dormancy, hormones and other factors remain to be discovered.

The hydrothermal time model provides a way to normalize germination time-course data on a common scale with respect to both T and Ψ (Figs 2A, 4A). It should be noted that this normalized thermal time scale $\{[1 - \Psi/\Psi_b(g)]\ [T - T_b]t_g\}$ is not the same as a 'hydrothermal' time scale calculated as $[\Psi - \Psi_b(50)]\ (T - T_b)t$, where t is accumulated time since

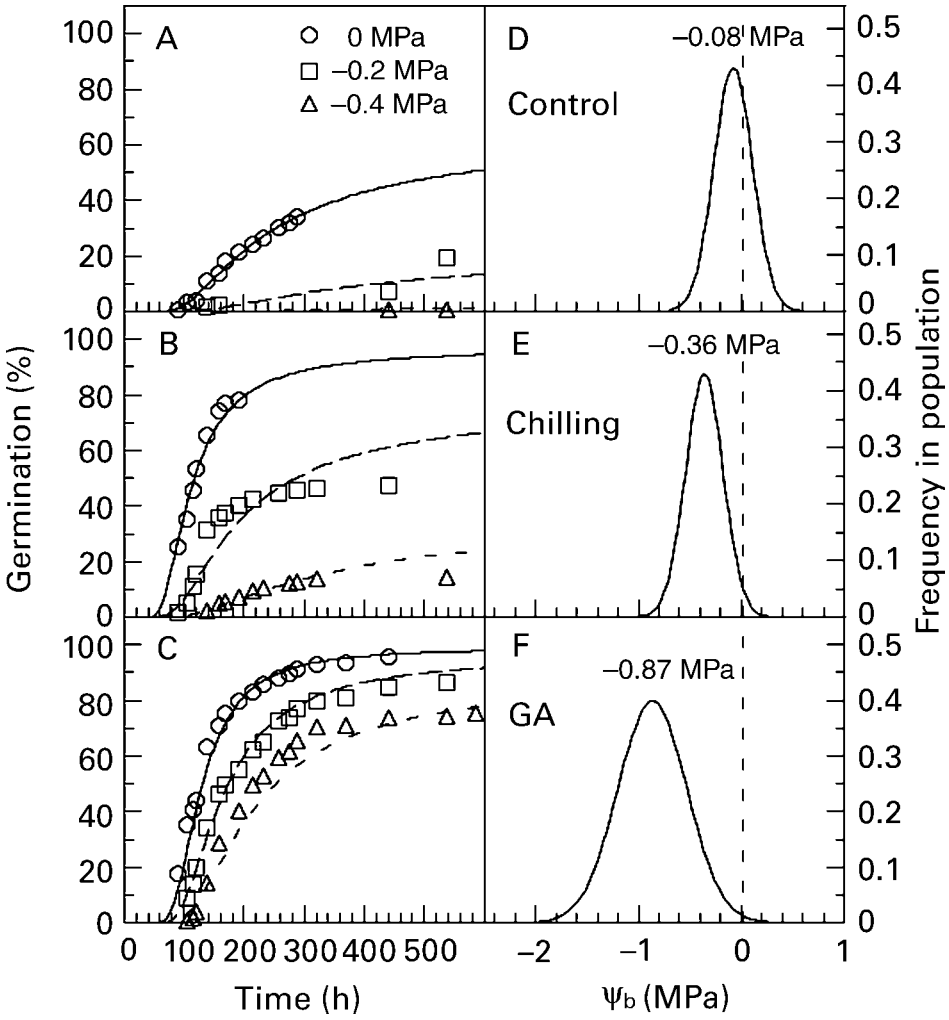

Figure 5. Influence of chilling (imbibed 3 d at 4°C) and GA_{4+7} (100 μM) on germination of secondary dormant true potato seeds (Chacas). Germination time courses at constant temperature (18°C) of (A) control, (B) chilled and (C) GA_{4+7}-treated seeds at three water potentials. The symbols represent the actual data, and the lines are the germination time courses predicted by the hydrotime model (Table 3). (D–F) The $\Psi_b(g)$ distributions obtained with the hydrothermal time model are plotted as frequency distributions.

imbibition or planting, which has been used by some investigators (e.g. Finch-Savage *et al.*, 1998; Roman *et al.*, 1999; Shrestha *et al.*, 1999; Grundy *et al.*, 2000; Rowse and Finch-Savage, 2003). Since all seeds in a population complete germination after accumulating the same hydrothermal time [i.e. θ_{HT} in equation (1) is a constant], but their threshold values vary, it is not correct to plot germination time courses at different ψ on a single hydrothermal time scale based on the $\Psi_b(50)$ of the population. Time courses at different ψ may appear to consolidate on such a time scale, but will be accurate only for the 50th percentile, and will deviate increasingly at other percentiles, particularly as the difference $\Psi - \Psi_b(g)$ decreases (for further discussion and examples, see Bradford, 2002). Instead, the times to germination of the different seed fractions at different Ψ can be normalized to what their germination times would be in water (0 MPa) using the normalization factor $\{1 - [\Psi/\Psi_b(g)]$; Bradford, 1990$\}$. This normalization factor compensates for the

Table 3. Parameters of the hydrotime model for true potato seeds (Chacas) germinated at 18°C when secondary dormancy had been induced (control) or broken by chilling (imbibed 3 d at 4°C) or GA_{4+7} (100 μM)

Treatment	θ_H (MPa °h)	$\Psi_b(50)$ (MPa)	σ_{ψ_b} (MPa)	r^2
Control	45	−0.08	0.19	0.96
Chilling	150	−0.36	0.18	0.95
GA_{4+7}	113	−0.87	0.34	0.96

effect of reduced Ψ on germination, and the effect of temperature can then be included by multiplying by $T - T_b$ to put the normalized time scale on a thermal time basis (Figs 2A, 4A).

The results with TPS reported here provide additional evidence that the induction and alleviation of seed dormancy are accompanied by shifts in $\Psi_b(g)$ distributions that can largely account for the changes in germination capacity (both rate and percentage). Thus, $\Psi_b(g)$ distributions are indicators of the physiological response of the seed population to the environmental and hormonal cues it is receiving and integrating. The hydrothermal time model provides a quantitative picture of the current physiological status of the seed population and its capacity for germination under a range of conditions. Shifting $\Psi_b(g)$ distributions in response to diverse environmental and hormonal inputs provides great flexibility in describing and quantifying the range of dormancy mechanisms exhibited by seeds. Other modelling approaches can be used to analyse the same types of data and have utility for specific applications (e.g. Hardegree *et al.*, 2003; Rowse and Finch-Savage, 2003; Finch-Savage, 2004). However, the hydrothermal time model requires the fewest parameters and is fundamentally a biological rather than an empirical mathematical approach to describing seed dormancy and germination behaviour. As modelling dormancy release is often a limiting factor for predicting seed germination and emergence in the field (Vleeshouwers and Kropff, 2000; Batlla *et al.*, 2004), the hydrothermal time model provides a method to incorporate changes in depth of dormancy directly into environmentally driven predictive models (Allen, 2003).

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