Journal of Experimental Botany, Vol. 60, No. 12, pp. 3397–3406, 2009
doi:10.1093/jxb/erp178 Advance Access publication 2 June, 2009
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Quantitative description of the effect of stratification on dormancy release of grape seeds in response to various temperatures and water contents

W. Q. Wang[1,2], S. Q. Song[1], S. H. Li[1], Y. Y. Gan[1], J. H. Wu[1,2] and H. Y. Cheng[1,*]

[1] Institute of Botany, the Chinese Academy of Sciences, Beijing, China
[2] Graduate University of the Chinese Academy of Sciences, Beijing, China

Received 20 January 2009; Revised 6 May 2009; Accepted 8 May 2009

Abstract

The effect of stratification on dormancy release of grape seeds crossing from the sub- to the supraoptimal range of temperatures and water contents was analysed by modified threshold models. The stratification impacted on dormancy release in three different ways: (i) dormancy was consistently released with prolonged stratification time when stratified at temperatures of <15 °C; (ii) at 15 °C and 20 °C, the stratification effect initially increased, and then decreased with extended time; and (iii) stratification at 25 °C only reduced germinable seeds. These behaviours indicated that stratification could not only release primary dormancy but also induce secondary dormancy in grape seed. The rate of dormancy release changed linearly in two phases, while induction increased exponentially with increasing temperature. The thermal time approaches effectively quantified dormancy release only at suboptimal temperature, but a quantitative method to integrate the occurrence of dormancy release and induction at the same time could describe it well at either sub- or supraoptimal temperatures. The regression with the percentage of germinable seeds versus stratification temperature or water content within both the sub- and supraoptimal range revealed how the optimal temperature (T_{so}) and water content (W_{so}) for stratification changed. The T_{so} moved from 10.6 °C to 5.3 °C with prolonged time, while W_{so} declined from >0.40 g H_2O g DW^{-1} at 5 °C to ~0.23 g H_2O g DW^{-1} at 30 °C. Dormancy release in grape seeds can occur across a very wide range of conditions, which has important implications for their ability to adapt to a changeable environment in the wild.

Key words: 'Ceiling' temperature, dormancy induction, optimum temperature, optimum water content, physiological dormancy, thermal time, *Vitis*.

Introduction

Grape (*Vitis* spp.) is one of the most economically important fruit species in the world, with ~71% used for wine making, 27% as fresh fruit, and 2% as dried fruit (FAO, 2007). The dormancy characteristics of grape seeds have been known for many years (Flemion, 1937), and some research has been conducted on seed dormancy release (Singh, 1961; Manivel and Weaver, 1974; Ellis *et al.*, 1983; Spiegel-Roy *et al.*, 1987). Most research indicates that cold stratification is the most successful treatment for dormancy release of grape seed (Flemion, 1937; Singh, 1961; Ellis *et al.*, 1983), and the excised embryo can produce a normal seedling (Faure *et al.*, 1998; Gan *et al.*, 2008). Thus, dormancy of grape seeds may belong to the physiological dormancy (PD) type (Baskin and Baskin, 2004). Though cold stratification is the common method used to release the PD of grape seeds, the stratification temperature and period vary greatly among these studies. This may be due to inter- or intraspecies variation in dormancy, or lack of a detailed model to describe the effects of temperature and water content on dormancy release of grape seeds.

Dormancy release is tightly related to environment temperature and seed water content, and mathematical

* To whom correspondence should be addressed. E-mail: hycheng@ibcas.ac.cn

models can clearly describe how these factors impact on this process (Bradford, 2002; Finch-Savage and Leubner-Metzger, 2006; Batlla and Benech-Arnold, 2007). The population-based threshold model is the most useful mathematical model and has been successfully applied to describe dormancy release in many species (Christensen *et al.*, 1996; Bauer *et al.*, 1998; Kebreab and Murdoch, 1999; Steadman *et al.*, 2003*a*, *b*; Steadman, 2004; Steadman and Pritchard, 2004; Alvarado and Bradford, 2005; Bair *et al.*, 2006). Most of the models show that dormancy release not only increases the percentage of germinable seeds, but also changes some physiological parameters, such as base temperature (T_b), mean lower limit temperature [$T_{l(50)}$], and mean base water potential [$\Psi_b(50)$] for germination, which widen the range of temperature and water content permissive for germination (Bradford, 2002; Finch-Savage and Leubner-Metzger, 2006; Batlla and Benech-Arnold, 2007). For example, dormancy release of true (botanical) potato seeds decreases the $\Psi_b(50)$ for germination, and allows germination to proceed at more negative water potentials (Alvarado and Bradford, 2005). These indices are also indirectly related to temperature (Steadman and Pritchard, 2004), accumulated thermal time (Batlla and Benech-Arnold, 2003, 2004), and water potential (Alvarado and Bradford, 2005; Bair *et al.*, 2006) for stratification to quantify the effect of temperature, water potential, and period of time on seed dormancy status.

There are also a few models directly relating the temperature and water content to some physiological indices of dormancy release, such as base temperature for dormancy release and rate of loss (Prichard *et al.*, 1996; Kebreab and Murdoch, 1999; Steadman, 2004). The model establishes the relationship between dormancy release and treatment temperatures or seed water content, and describes how the rate of dormancy release changes with temperature (Pritchard *et al.*, 1996; Steadman *et al.*, 2003*b*; Steadman, 2004). With regard to the thermal time theory, Steadman *et al.* (2003*b*) and Steadman (2004) characterized the change of percentage of germinable seeds in relation to thermal time and showed that the change was dependent on temperature or water content in *Lolium rigidum* seed. These results revealed the varied mechanisms of dormancy release in different conditions of temperature and seed water content.

Most of the above models only characterize dormancy release at suboptimal temperature, but there have been few attempts to model the dormancy release across a wider range of temperatures or water contents. In the wild, the conditions for dormancy release are very complicated, and temperature and soil moisture vary greatly by season. In addition, release of seed dormancy may be simultaneously accompanied by induction of secondary dormancy within a wide range of temperatures (Totterdell and Roberts, 1979; Kebreab and Murdoch, 1999; Baskin and Baskin, 2004; Batlla and Benech-Arnold, 2007), which results in further complications in seed dormancy release. Thus, the present study aimed to develop a threshold model to describe and quantify grape seed dormancy release across a series of both sub- and supra-optimal temperatures and water contents while considering the possible occurrence of dormancy re-induction. It would be helpful to understand ecologically how grape seed responds and acclimates to the variable environmental conditions.

Materials and methods

Plant materials

Beichun grape (a cross-breed of *Vitis vinifera*×*V. amurensis*) fruits were collected after maturity in the germplasm resource nursery of grapes in the Institute of Botany, the Chinese Academy of Science, Beijing, on 10 September (sample 1) and 28 September (sample 2) 2007. The seeds were removed from the berry and the appendages of the outer seed coat were removed by abrading. The seeds were cleaned in water and dehydrated to a water content of 0.13 ± 0.02 g H_2O g^{-1} DW (g g^{-1}) at 25 °C and 60% relative humidity (RH). The seeds of sample 1 and sample 2 were used for model evaluation and establishment, respectively.

Water content determination

The water content of seeds was determined according to the methods of the International Seed Testing Association (1999) and expressed on a dry mass basis [g H_2O (g DW)$^{-1}$, g g^{-1}].

Germination testing

Four replicates of 50 seeds stratified for different periods of time were germinated on two layers of filter paper moistened with 5 ml of distilled water in 9 cm diameter Petri dishes at a fluctuating temperature of 30 °C/20 °C (daytime 12 h, 30 °C/night-time 12 h, 20 °C) in darkness for 30 d. Radicle protrusion of 2 mm was used as the criterion for germination.

Stratification treatments

In the first treatment, seeds were put into a loosely tied opaque plastic bag and mixed with moist perlite whose water content was ~2 g g^{-1} (seeds:perlite=1:5, v/v). Sample 1 was put into an incubator in the dark at 5, 10, 15, 20, and 25 °C, and sample 2 was placed in an incubator in the dark at 0, 3, 6, 10, 15, 20, 25, and 15 °C/5 °C (daytime 12 h, 15 °C/night-time 12 h, 5 °C). The seeds were taken out of the bag and germinated after stratification for different periods of time. The periods of time for sample 1 were 7, 14, 21, and 40 d, and for sample 2 were 0, 5, 10, 15, 20, 30, and 60 d.

For the second treatment, seeds placed in 9 cm diameter Petri dishes were moisture-equilibrated to different water contents at a controlled RH, and then stratified in an incubator in the dark for 90 d at 5 °C and at 7.5% (saturated NaOH solution), 43% (saturated K_2CO_3 solution), 76% (saturated NaCl solution), 96% (v/v, 20% glycerol), 98% (v/v, 12% glycerol), 99% (v/v, 6% glycerol), and 100% (water) RH; at 20 °C and at 33% (saturated $MgCl_2$ solution), 43% (saturated K_2CO_3 solution), 76% (saturated NaCl solution), 96% (v/v, 20% glycerol), 98% (v/v, 12% glycerol), 99% (v/v, 6% glycerol), and 100% (water)

RH; or at 30 °C and at 26% (saturated $CaCl_2$ solution), 75% (saturated NaCl solution), 84% (saturated KCl solution), 96% (v/v, 20% glycerol), 98% (v/v, 12% glycerol), 99% (v/v, 6% glycerol), and 100% (water) RH. In addition, some of the seeds were also embedded in moist perlite with water contents of 0.2 g g^{-1} and 2 g g^{-1} at 5 °C, and 2 g g^{-1} at 20 °C and 30 °C to obtain higher water contents of seeds for stratification. These seeds were subsequently sampled for germination and water content determination.

Viability test

The tetrazolium test could not detect the viability of seeds; therefore, after the experimental stratification treatment, part of the samples of seeds were transferred to 3 °C for a further stratification. After 30 d, the seeds were moved to Petri dishes for germination tests to determine viability.

Threshold model definition and statistical analysis

The thermal time [$\theta_T(g)$], ceiling temperature [$T_c(g)$], and base water potential values [$\Psi_b(g)$] for germination are normally distributed among seeds in a population (Bradford, 2002), and thus seed germination at the corresponding temperature or under the corresponding water potential will have the following relationship:

$$y = G_{max} \times \Phi[(x-\mu)/\sigma] \quad (1)$$

where y is the percentage of seed germination at the corresponding temperature or under the corresponding water potential of x, x represents the temperature or water potential for germination, G_{max} is the maximum percentage of seeds to germinate, Φ is the normal probability integral, μ is the mean, and σ is the standard deviation of the original distribution of y.

Similarly, dormancy release also follows a cumulative normal distribution, so Equation 1 can also be applied to predict the proportion of the seed population in which has been released dormancy, when x represents the temperature or water potential for dormancy release and y is the percentage of seeds in which dormancy is released at or under the corresponding x. However, dormancy induction may occur simultaneously during the process of dormancy release, and be independent of dormancy release (Totterdell and Roberts, 1979; Kebreab and Murdoch, 1999). If it is supposed that dormancy induction also follows a normal distribution (Kebreab and Murdoch, 1999), the percentage of seed in which dormancy is induced at a given temperature could also be estimated from Equation 1. If dormancy release and induction occurred independently at a certain temperature and the maximum numbers of seeds in which dormancy was released and induced were equal, the percentage of germinable seeds would be:

$$y = G_{max} \times \Phi(g1 + r1 \times t_{sg}) \times \{1 - [\Phi(g2 + r2 \times t_{sg}) - \Phi(g2)]\} \quad (2)$$

where G_{max} is the maximum percentage of seeds that can be released from dormancy in the experimental population, $g1$ is the initial probit percentage of non-dormant seeds in a population with 100% of seeds that can be released from dormancy, i.e.

$$g1 = \Phi^{-1}(g/G_{max}) \quad (3)$$

(g is the observed germination of fresh harvest seeds), $r1$ and $r2$ are the rate of dormancy release and induction respectively, t_{sg} is the stratification time, and $g2$ is the probit percentage of seeds that cannot be released from dormancy in the experimental population, i.e.

$$g2 = \Phi^{-1}(1 - G_{max}) \quad (4)$$

If the parameter $r1$ was related to temperature, the change of percentage of germinable seeds could be quantified according to the thermal time theory (Garcia-Huidobro *et al.*, 1982; Covell *et al.*, 1986; Ellis *et al.*, 1986, 1987; Bradford, 2002; Hardegree *et al.*, 2006; Chantre *et al.*, 2009), but if $r2$ was related to temperature synchronously, Equation 2 could be similarly used to describe and predict the dormancy release in relation to temperature and time (Kebreab and Murdoch, 1999).

For application of the thermal time approaches in describing dormancy of grape seeds, it is assumed that the base temperature for dormancy release (T_{sb}) was constant and the thermal time for stratification (S_{tt}) followed a log-normal distribution in the suboptimal range of stratification temperature, whereas in the supraoptimal range, the thermal time for stratification (S_{Tc}) was assumed to be constant and the 'ceiling' temperature for dormancy release (T_{sc}) was assumed to follow a log-normal distribution. Then S_{tt} exhibited

$$S_{tt} = (T_s - T_{sb}) \times t_{sg} \quad (5)$$

and T_{sc}

$$T_{sc} = S_{Tc}/t_{sg} + T_s \quad (6)$$

where T_s is the dormancy release temperature and t_{sg} is the time for dormancy release.

Then, the percentage of seeds in which dormancy is released in relation to time and suboptimal temperatures was:

$$y = b + G_{max} \times \Phi\{[\log(S_{tt}) - \mu]/\sigma\} \quad (7)$$

or to supraoptimal temperatures was:

$$y = b + G_{max} \times \Phi\{[\log(T_{sc}) - \mu]/\sigma\} \quad (8)$$

where b is the fraction of non-dormant seeds at the beginning of stratification and G_{max} is the maximum percentage of seeds in the population able to release dormancy.

For estimation of the optimal temperature and water contents for dormancy release of grape seed, the relationship between the percentage of seeds in which dormancy was released and temperature or water content, crossing from the sub- to the supraoptimal range, was modelled as:

$$y=\Phi[(x-\mu1)\sigma1]+\Phi(\mu2-x)/\sigma2]-100\% \quad (9)$$

where μ1 and μ2 are the means, and σ1 and σ2 are the standard deviations of the distributions at sub- or supra-optimal temperature or under sub- or supraoptimal water content, respectively. μ1 and μ2 denote the mean of the lower and upper threshold temperature for germination (Grundy *et al.*, 2000), and here were defined to the mean of the lower and upper threshold temperatures (T_{sl} and T_{su}) or water contents (W_{sl} and W_{su}) for dormancy release. In Equation 9, a normalized final germination was applied, so that the maximum germination was 100%.

Graphpad Prism 5.0 (GraphPad Software) was applied for statistical analysis of the data and parameter estimation, and a global regression with Prism 5.0 was used to perform a regression with constraint parameters (http://graphpad.com/articles/P4Global.pdf). The global regression was also used to test the significant difference between regression lines, which was implemented through comparing the lines between regression with no constraint and regression with constraining all parameters to shared values for all data.

Results

Effect of stratification temperature and time periods on dormancy release

Stratification effect on dormancy release: Dormancy release, quantified by the dynamics of germination, was strongly influenced by the stratification temperature and time periods (Fig. 1). After germination at 30 °C/20 °C in darkness for 30 d, germination of the control (unstratified) seeds was ~23.3%, and the stratification treatment significantly changed the germination of seeds (Fig. 1). When stratified at 0–10 °C and 15 °C/5 °C, the dormancy of seeds was continuously released, which eventually allowed >80% of seeds to germinate (Fig. 1A–D, H). In addition, the stratification not only released the seed dormancy, but also increased germination rates with increasing stratification times at these temperatures (Fig. 1A–D, H), particularly visible at 15 °C/5 °C (Fig. 1H). Although the stratification at 15 °C for 30 d also resulted in dormancy release of 75% of seeds in the population, the stratification effect decreased when the time was prolonged to 60 d (Fig. 1E). When seeds were stratified at 20 °C, the percentage of dormancy release was ~40% during 10 d and this decreased when the stratification time was longer than 15 d (Fig. 1F). Dormancy release could not be observed when seeds were stratified at 25 °C, and the germination decreased with increasing stratification time (Fig. 1G).

As mentioned above, stratification exerted three different effects on dormancy release of grape seeds. At temperatures of 0, 3, 6, 10, and 15 °C/5 °C, stratification continuously released seed dormancy with increasing stratification time, presenting a sigmoidal increase on a percentage germination basis, with the maximum effect at 10 °C (Fig. 2A). When stratified at 15 °C and 20 °C, only dormancy release of some seeds could be achieved after certain durations, i.e. 30 d and 10 d, respectively (Fig. 2B). When stratification was at 25 °C, the dormancy status of seeds, as measured by percentage germination, was maintained or increased with increasing stratification time (Fig. 2C). The effect indicated the simultaneous occurrence of dormancy release and induction, which could be modelled by Equation 2 (Fig. 2).

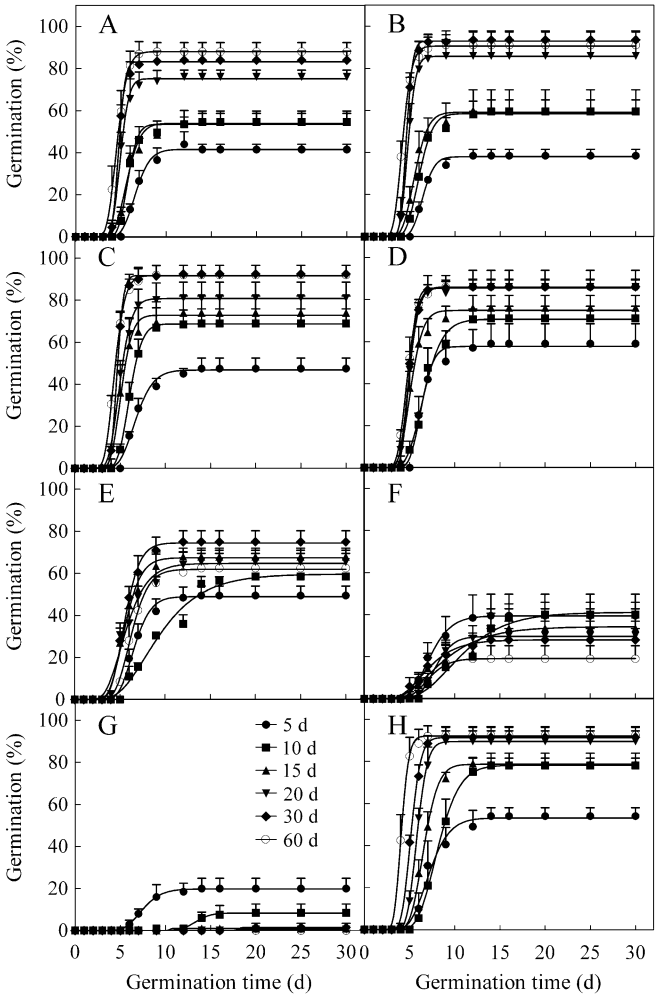

Fig. 1. Time course of germination of grape seeds stratified at different temperatures for different time periods. Seeds were mixed with moist perlite (water content of 2 g H_2O g^{-1} DW), and stratified at 0 (A), 3 (B), 6 (C), 10 (D), 15 (E), 20 (F), 25 (G), and 15 °C/5°C (H) for 5, 10, 15, 20, 30, and 60 d, respectively, and then germinated at 30 °C/20 °C for 30 d. All values are means ±SD, and the solid lines are the logistic regression lines.

Release and induction of seed dormancy and prediction of seed germination: From the regression of the percentage of germinable seeds versus stratification time (Fig. 2), the rate of dormancy release and induction were obtained. The rate of dormancy release linearly increased with increasing temperature from 0 °C to 10°C as:

$$r_{sl}=0.072+0.0066\times T_s \quad (10)$$

and then decreased with increasing temperature from 10 °C to 25 °C (Fig. 3):

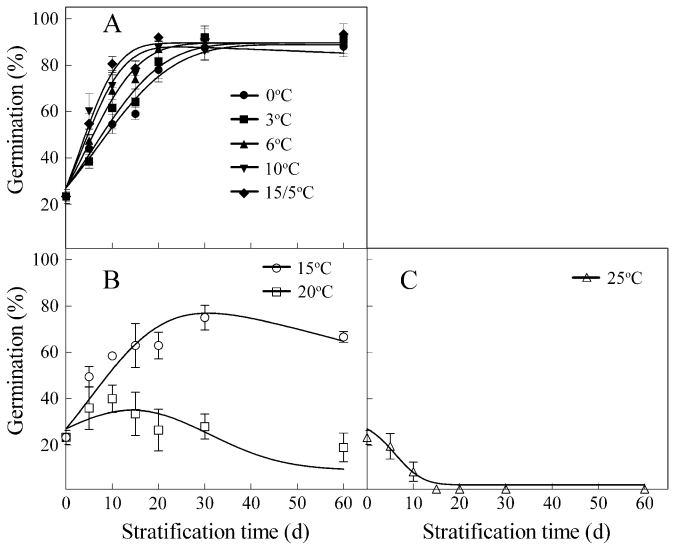

Fig. 2. Different stratification behaviours of seeds stratified at a series of temperatures. The final percentages of germinable seeds stratified at a given temperature were plotted and regressed against the stratification time. The regression was according to Equations 2 and 4. In Equation 2, the parameters *g*1 and *g*2 were constrained to be shared for all data. Data are means ±SD and the solid lines are the regression lines.

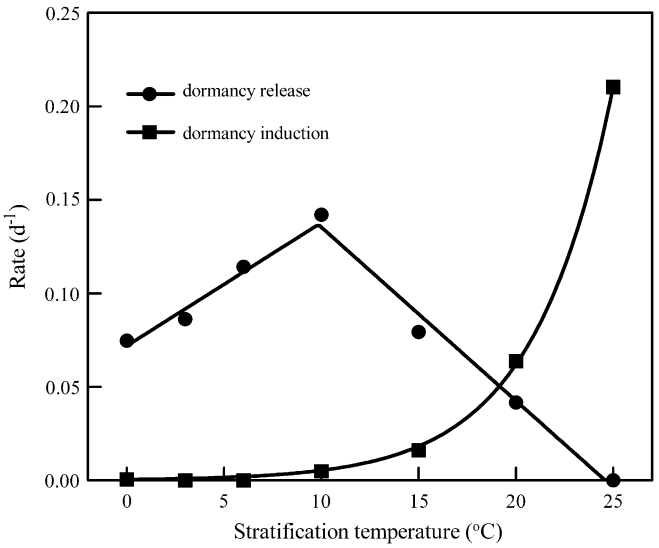

Fig. 3. Change of rate of dormancy release and induction. From the regression between the stratification time and germination of seeds after stratification (Fig. 2), the estimated rates of dormancy release (filled circles) and induction (filled squares) were plotted and regressed versus temperature of stratification. The rate of dormancy release showed a two-phase linear change, while induction exhibited an exponential increase with temperature. The intercepts of the two-phase linear line on the temperature axis were –10.9 °C and 24.5 °C.

$$r_{su} = 0.137 - 0.0093 \times (T_s - 9.8) \quad (11)$$

where r_{sl} and r_{su} are the rate of dormancy release over a range of the corresponding temperatures, respectively.

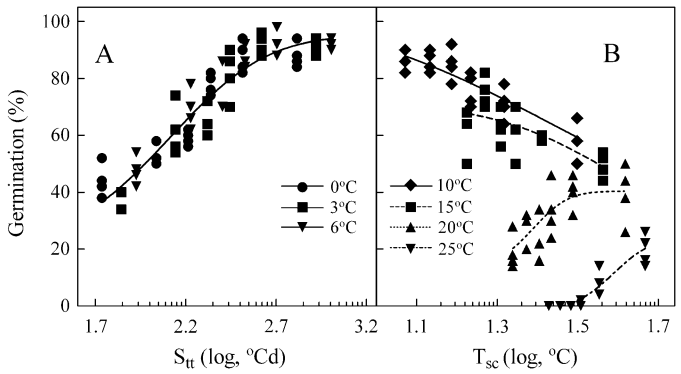

Fig. 4. Change of percentage of germinable seeds with stratification thermal time (S_{tt}) and 'ceiling' temperature (T_{sc}). Calculating from Equations 5 and 6, the S_{tt} (A) and T_{sc} (B) were regressed against the percentage germination after stratification (Equations 7 and 8). The regression lines were not significantly different at suboptimal temperatures from 0 °C to 6 °C (A), but were significantly different at supraoptimal temperatures (B).

The inflexion of the regression lines of the rate of dormancy release defined the optimal temperature for dormancy release (T_{so}), ~9.8 °C (Fig. 3). From Equations 10 and 11, T_{sb} and S_{Tc} at sub- ($T_s \leqslant 9.8$ °C) and supra- ($T_s \geqslant 9.8$ °C) optimal temperature were calculated as –10.9 °Cd and 107.5 °Cd, respectively. At this time, the change in the percentage of germinable seeds with S_{tt} (Fig. 4A) or T_{sc} (Fig. 4B) could be analysed via the thermal time approaches (Equations 5–8). At suboptimal temperatures, the increase in the percentage of germinable seeds with S_{tt} followed one regression line when seed were stratified at 0–6 °C (Fig. 4A; $F_{6,72}$=0.84; Tabulate $F_{6,72}$=2.23, P=0.55), while at supra-optimal temperatures the change in the percentage of germinable seeds with T_{sc} varied greatly (Fig. 4B). On the other hand, if T_{sc} was assumed to be constant but S_{Tc} was assumed to follow a log-normal distribution (Equation 6), the regression lines had a similarly significant difference among the supraoptimal temperatures (data not shown).

The method above did not integrate the factor of dormancy induction into the quantification of dormancy release, while dormancy induction occurred with dormancy release at the same time, and its rate continuously increased with increasing temperatures (Fig. 3):

$$r = 0.00047 \times e^{0.24 \times T} \quad (12)$$

Thus, the factors of dormancy release and induction were combined together to quantify the effect of stratification time and temperature on dormancy release (Equations 2, 10, 11, and 12). From the value of *g*1 and *g*2 obtained from the regression line, –0.52 and –1.26, respectively, the percentage of seeds that can germinate when stratified at suboptimal temperature was estimated ($T_s \leqslant 9.8$ °C):

$$y = 0.9 \times \Phi\left[-0.52 + \left(0.072 + 0.0066 \times T_s\right) \times t_{sg}\right] \times \left[1.1 - \Phi\left(-1.26 + 0.00047 \times e^{0.24T} \times t_{sg}\right)\right] \quad (13)$$

while at supraoptimal temperature ($T_s \geqslant 9.8$ °C):

$$y=0.9\times\Phi\{-0.52+[0.137-0.0093\times(T_s-9.8)]\times t_{sg}\}\times[1.1-\Phi(-1.26+0.00047\times e^{0.24T}\times t_{sg})] \quad (14)$$

Thus the germination of seeds stratified at a given time and temperature, in both the sub- and supraoptimal range, could be predicted. To evaluate the function of the equation in the prediction, the independent data obtained from stratification treatment on another seed population (sample 1) were compared with the percentage of germinable seeds predicted from Equations 13 and 14. A good correlation was obtained between the predicted and observed data (Fig. 5, solid line, R^2=0.85). However, the equation slightly underestimated the percentage of germinable seeds, showing a slope slightly greater than 1 (Fig. 5, dashed line). Underestimation was possibly due to the different times at which the seeds were harvested, which may influence the seed dormancy status.

Modelling optimal temperature for dormancy release: Judging from the rate of dormancy release, it appeared that the optimal temperature for dormancy release (T_{so}) was ~9.4 °C (Fig. 3). Nevertheless, a method to model the percentage of seed germination after stratification against stratification temperature (Equation 9) gave a changed value of T_{so} with stratification time (Fig. 6). When normalized germination was plotted and regressed versus the stratification temperature (Equation 9), germination showed a bell-shaped change with the stratification temperature (Fig. 6A). It was clear that germination of seeds stratified at 0–10 °C increased, and that at 10–25 °C decreased with increasing stratification time (Fig. 6A). With increasing stratification time, the regression lines approached the maximum germination percentage at 0–10 °C, while at 10–25 °C the lines shifted to a lower temperature (Fig. 6A). This change on both sides of the line was more directly reflected in an exponential decline in T_{sl} and T_{su} with stratification time (Fig. 6B and Table 1). The stratification temperature corresponding to maximum germination in the regression lines was the T_{so}, which decreased

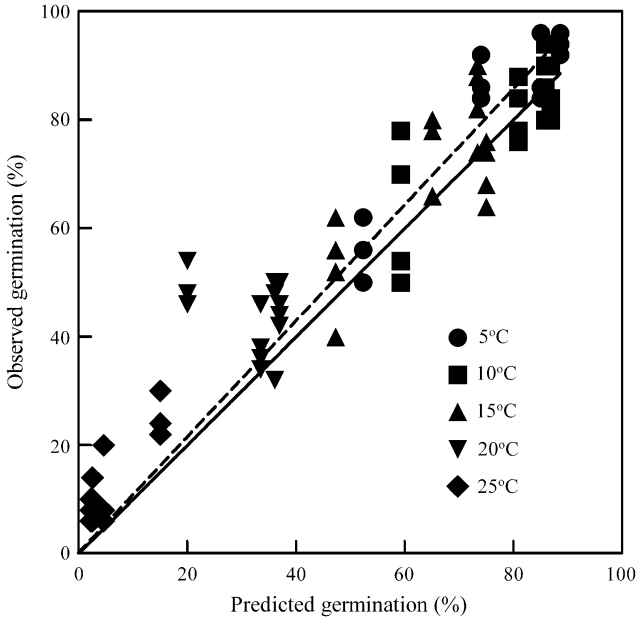

Fig 5. The relationship between observed germination percentages of seeds stratified at different temperatures for a series of periods versus predicted germination percentages (using Equations 13 and 14). Seeds were stratified at 5, 10, 15, 20, and 25 °C for 7, 14, 21, and 40 d respectively, and then germinated at 30 °C/20 °C for 30 d. The solid line represents regression with $y=x$ (R^2=0.85, n=80) and the dashed line shows the regression with $y=1.07\times x$ (R^2=0.87, n=80).

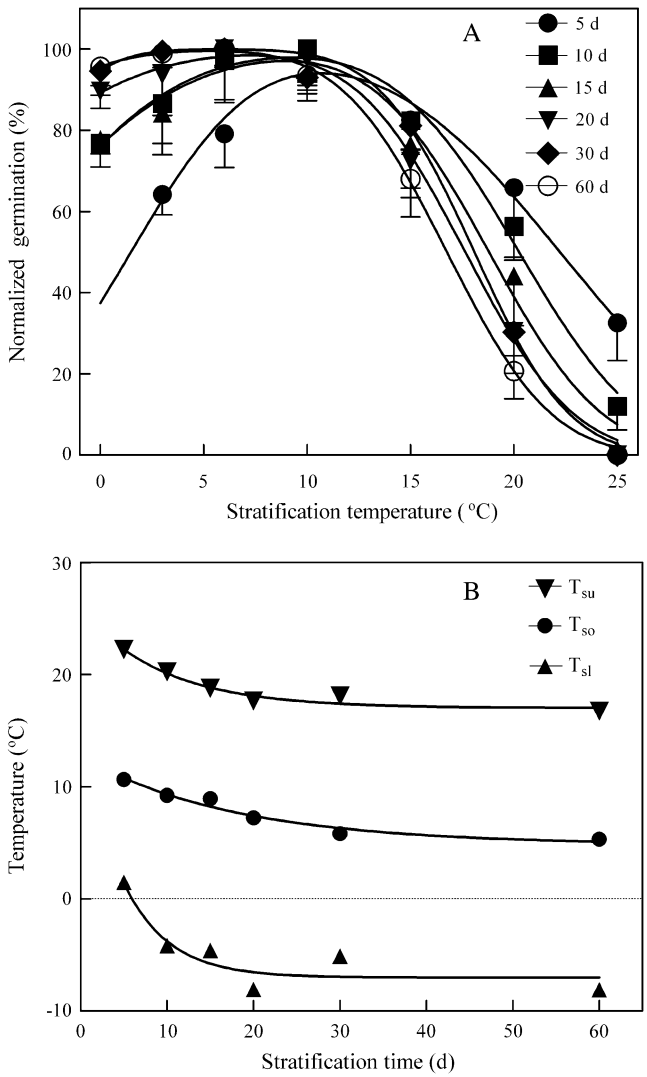

Fig. 6. The change in T_{sc}, T_{sl}, and T_{su} for dormancy release with stratification time. Germination of seeds stratified at different temperatures was normalized to the highest level of germination in one period, and then regressed according to Equation 9 (A, solid lines). Germination of seeds stratified at 0 °C for 5 d was excluded from the regression. The optimal temperature for stratification (T_{so}) was calculated from the regression line, and exponentially changed with stratification time as lower and upper threshold temperatures (T_{sl} and T_{su}, B). The bars show ±SD. The R^2 and parameters of A are shown in Table 1.

Table 1. R^2, parameter values, and its 95% confidence interval (CI) of the regression in Fig. 6A
Sd, stratification time.

Sd (d)	R^2	Parameter	Estimate	95% CI		Sd (d)	R^2	Parameter	Estimate	95% CI	
				Lower limit	Upper limit					Lower limit	Upper limit
5	0.81	μ1	1.47	−0.69	3.63	20	0.97	μ1	−8.08	−20.47	4.31
		σ1	4.63	1.26	8.00			σ1	6.54	−2.43	15.51
		μ2	22.23	20.89	23.56			μ2	17.65	17.05	18.25
		σ2	6.41	4.05	8.78			σ2	4.12	3.28	4.96
10	0.93	μ1	−4.19	−8.30	−0.09	30	0.98	μ1	−5.11	−23.94	13.73
		σ1	5.93	1.63	10.23			σ1	3.18	−8.37	14.73
		μ2	20.25	19.47	21.02			μ2	18.09	17.66	18.51
		σ2	4.65	3.51	5.79			σ2	3.61	3.03	4.18
15	0.93	μ1	−4.60	−9.81	0.61	60	0.99	μ1	−8.11	−33.38	17.17
		σ1	6.44	1.04	11.86			σ1	4.74	−9.56	19.04
		μ2	18.79	17.94	19.65			μ2	16.76	16.34	17.18
		σ2	4.32	3.09	5.55			σ2	3.97	3.37	4.55

like T_{sl} and T_{su} (Fig. 6B). The T_{so} changed from 10.6 °C at 5 d of stratification to ~5.3 °C at 60 d.

Effect of water content on dormancy release of seed

After equilibration at different RHs, seeds were stratified at different water contents and temperatures for 90 d. The results showed that the water content at which the seeds were stratified significantly influenced subsequent seed germination, and the effect was closely related to the stratification temperature (Fig. 7). When stratification was at 5 °C, the percentage germination of seeds increased with increasing water content of seeds, reaching a plateau level when the water content was higher than ~0.40 g g^{-1} (Fig. 7A). When stratification was at 20 °C or 30 °C, the percentage germination first increased with increasing water content of seeds, and then decreased (Fig. 7B, C). The viability test of seeds stratified at different RHs for 90 d indicated that the ageing process did not occur (data not shown). Thus, lower water contents clearly promoted the effect of stratification on dormancy release, especially of warm stratification. For release of dormancy of seeds, the means of the lower (W_{sl}) and higher (W_{su}) threshold water contents were 0.23 g g^{-1} and 0.30 g g^{-1} at 20 °C, and 0.14 g g^{-1} and 0.27 g g^{-1} at 30°C (Table 2), while the optimal water content (W_{so}) was 0.26 g g^{-1} at 20 °C and 0.23 g g^{-1} at 30 °C. The optimum water content for dormancy release changed with the temperature of stratification, and moved to a lower potential at higher temperature (Fig. 7D).

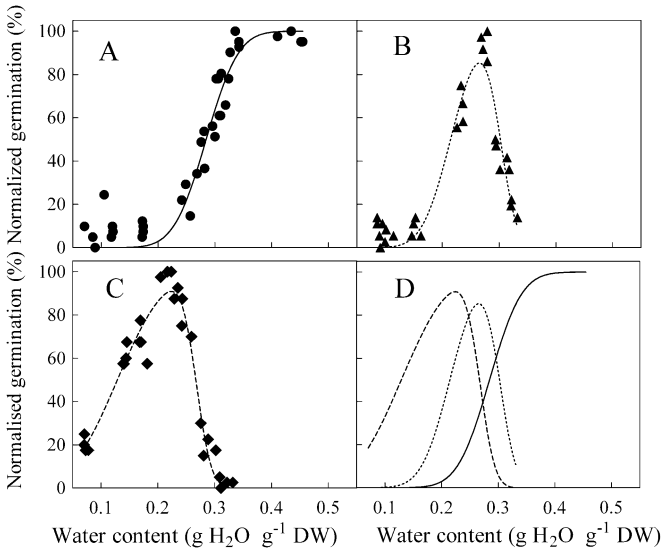

Fig. 7. Effect of water content on dormancy release of grape seeds stratified at different temperatures. After equilibration at different relative humidities, seeds were stratified at 5 (A), 20 (B), and 30 °C (C) for 90 d, and then germinated at 30 °C/20 °C for 30 d. After subtracting the minimum germination, seed germination was finally normalized to the greatest germination and regressed to the water content. The normalized germination of seeds stratified at 5 °C related to water content with Equation 1, and at 20 °C and 30 °C with Equation 9. The changes in normalized germination at different water contents and temperatures are shown in D (solid line, 5 °C; dotted line, 20 °C; dashed line, 30 °C). All values are means ±SD. The R^2 and parameters are shown in Table 2.

Discussion

The percentage germination of newly harvested 'Beichun' grape seeds was ~23.3%, showing that these seeds have dormant characteristics like those of other grape species or varieties (Singh, 1961; Manivel and Weaver, 1974; Ellis *et al.*, 1983; Spiegel-Roy *et al.*, 1987). Dormancy release of 'Beichun' grape seed is temperature dependent during stratification, i.e. the dormancy of seeds is effectively released by stratification of 0–10 °C and 15 °/5 °C, partially released by 15 °C and 20°C, and inhibited or induced by 25 °C (Figs 1, 2). Totterdell and Roberts (1979) argued that stratification not only resulted in dormancy release, but also could induce dormancy, and that these two independent

Table 2. R^2, parameter value, and its 95% confidence interval (CI) of the regression in Fig. 7
ST, stratification temperature.

ST (°C)	R^2	Parameter	Estimate	95% CI		ST (°C)	R^2	Parameter	Estimate	95% CI	
				Lower limit	Upper limit					Lower limit	Upper limit
5	0.94	μ	0.285	0.279	0.291						
		σ	0.045	0.034	0.055						
20	0.91	μ1	0.215	0.203	0.226	30	0.95	μ1	0.129	0.120	0.138
		σ1	0.039	0.022	0.057			σ1	0.065	0.051	0.080
		μ2	0.303	0.298	0.308			μ2	0.268	0.263	0.273
		σ2	0.023	0.015	0.030			σ2	0.021	0.015	0.027

processes may occur at the same time. In *Rumex*, dormancy was released as long as the temperature was <15 °C, but simultaneously could be induced at any temperature. Similarly, the model shows that the dormancy of 'Beichun' grape seed can be released at temperatures reaching ~24.5 °C (the average 'ceiling' temperature), but can also be induced at any temperature (Fig. 3).

The effect of temperature on the rate of dormancy release is closely related to species. In some species, such as *Orobanche* spp. (Kebreab and Murdoch, 1999) and *L. rigidum* (Steadman, 2004), the rate continuously increases with increasing temperature, and also in some species, i.e. *Rumex* (Totterdell and Roberts, 1979), the change in rate is independent of temperature, while in other species, e.g. *Aesculus hippocastanu* (Pritchard *et al.*, 1996), the rate decreases with increasing temperature. However, for 'Beichun' grape seeds, the rate increases initially and then decreases with increasing temperature (Fig. 3). Also, the rate of dormancy induction of 'Beichun' grape seeds also increases with increasing temperature. These results were similar to those for *Rumex* seeds (Totterdell and Roberts, 1979).

The occurrence of dormancy induction in grape seed may weaken the effect of the thermal time approach in quantitatively describing the change of germinable seeds in relation to time and temperature, especially at supraoptimal temperature. At suboptimal temperature, due to the slow rate of dormancy induction, e.g. the rate at 0–6 °C (Fig. 3), dormancy induction has little influence on the percentage of seed that can be released from dormancy. Thus, the same regression exists with the percentage of germinable seeds against S_{tt} (Fig. 4A). When the rate becomes more rapid at supraoptimal temperatures, such as the rate at 10–25 °C (Fig. 3), it may decrease the percentage of seeds that can be released from dormancy and the more rapid the rate, the greater the decrease. Thus, the regression with the percentage of germinable seeds against T_{sc} depends on the temperature (Fig. 4B). However, some more effective methods such as Equations 13 and 14 can be applied to quantify the germinable seeds at a given time and temperature, in both the sub- and supraoptimal range. The methods can eliminate the influence of dormancy induction on dormancy release.

Generally, summer species will release dormancy at colder temperatures in winter (Batlla and Benech-Arnold, 2007), but the seeds of summer perennial species of grape can release dormancy at both colder and warmer temperatures, which requires the regulation of the water content. At 5 °C, dormancy is released well in 'moist' conditions, and the releasing effect increases with increasing water content (Fig. 7A), while at warmer temperatures of 20 °C and 30 °C, 'dry' conditions are more effective to release dormancy, and the effect is normally distributed around the optimum water content (Fig. 7B, C). Bair *et al.* (2006) hypothesized that after-ripening occurs at an optimum range of Ψ, and at lower or higher ranges the effect of dormancy release decreases with Ψ. However, in the present work, the optimum water content for dormancy release of grape seeds also showed a temperature-dependent change, e.g. the optimum water content was 0.26 g g^{-1} and 0.23 g g^{-1} at temperatures of 20 °C and 30°C, respectively. Thus the conditions for dormancy release of grape seeds seem to be very wide ranging, and dormancy can be released in either 'moist' or 'dry' conditions. An alteration of membrane fluidity may be a mechanism to explain the different water content requirements for stratification at various temperatures. Membrane fluidity is thought to control dormancy release, because it influences signal transduction pathways (Murata and Los, 1997; Hallett and Bewley, 2002). For dormancy release, membranes may need to transform to a critical phase that can receive and transfer a dormancy release signal, which is influenced by temperature and water content. At higher temperatures or water contents, membranes have higher fluidity (Alonso *et al.*, 1996; Murata and Los, 1997; Bryant *et al.*, 2001). Thus, when the membrane fluidity reaches a critical phase that is optimal for dormancy release with a 'moist' level at cold temperature, the water content for stratification must be lower than the 'moist' level to reach the critical phase at warm temperatures, and the higher the temperature, the lower the water content requirement.

When considering the occurrence of dormancy induction during stratification (Fig. 2) and the variation of the optimal stratification temperature (Fig.5) and water content (Fig. 6), it is very difficult to determine the appropriate conditions for dormancy release in grape seed. Thus, various protocols for grape seed dormancy release were suggested in previous studies (Singh, 1961; Manivel and Weaver, 1974; Ellis *et al.*, 1983; Spiegel-Roy *et al.*, 1987).

Nevertheless, modelling can overcome the difficulty and clearly explain the conditions for dormancy release in various situations. Although dormancy release and induction occur simultaneously during stratification, the threshold model is still helpful for the characterization of the effect of stratification on seed dormancy when modified as in Equations 2 and 9. The model can describe the effect of stratification not only at a certain temperature (Fig. 2), but also at or with a series of temperatures (Fig. 6) or water contents (Fig. 7), in both the sub- and supraoptimal range. While dormancy can be released over a wide range of temperatures, the effect is modulated by the available water content. This means that the dormancy release of grape seeds is not constrained to a given set of conditions, but will change according to environmental conditions. Seed dormancy is a characteristic which adapts to environmental conditions (Bewley, 1997; Baskin and Baskin, 1998; Batlla and Benech-Arnold, 2007), and its removal is closely related to the subsequent growth conditions, such as temperature, light, and soil moisture (Benech-Arnold *et al.*, 2000). As dormancy is alleviated, germination is allowed to proceed at colder temperatures (Batlla and Benech-Arnold, 2003; Steadman, 2004). Thus, the seed will continue to germinate at colder temperatures, which is not advantageous for seedling growth. However, in grape seeds, induction of secondary dormancy at colder temperatures (Figs 2B, 3) prevents the germination at disadvantageous temperatures. In addition, stratification at a lower temperature can release seed dormancy more effectively over a longer period (Fig. 6B), which ensures that some of the seeds that have been induced to secondary dormancy at the disadvantageous temperature are fully released from dormancy again when the temperature declines during winter. The cold stratification requires 'moist' soil conditions, and if the water content is not 'moist' enough in winter, seed dormancy may not be released. However, grape seeds can also release dormancy even with a limited water content in other seasons, when temperatures are sufficiently warm.

Since seeds can evolve a dormancy mechanism to avoid germination proceeding in an adverse environment, they should accordingly evolve adaptive ways to release dormancy for the occurrence of germination under appropriate conditions. In grape seeds the adaptive way is to release dormancy across wider ranges of temperature and water content. This ensures that seeds can germinate as long as the conditions are appropriate, which reflects a strategy to establish a large number of seedlings under appropriate natural conditions (Benech-Arnold *et al.*, 2000). The stratification treatments were only conducted at a fixed temperature or water content, but dormancy release in the wild generally occurs with variable temperatures or in conditions alternating between 'moist' and 'dry'. If dormancy release can occur over a wider range of temperature and water content as the model showed, seeds will probably lose dormancy in the more complicated wild conditions. Whether the model can be applied to predict dormancy release of grape seed in the wild requires a more detailed investigation.

Acknowledgements

This work was supported by the Chinese National Natural Science Foundation Program (30470183) and the Botanical Garden and Systematic Biology Project (KSCX2-YW-Z-058) of the Chinese Academy of Sciences. The authors thank the Germplasm nursery of grapes of the Institute of Botany, the Chinese Academy of Science for providing the grape seeds, and Professor Kent J Bradford, Dr Kathryn J Steadman, and Dr Zhanwu Dai for their helpful suggestions on data analysis and discussion.

References

Alonso A, Meirelles NC, Yushmanov VE, Tabak M. 1996. Water increases the fluidity of intercellular membranes of stratum corneum: correlation with water permeability, elastic and electrical resistance properties. *Journal of Investigative Dermatology* **106,** 1058–1063.

Alvarado V, Bradford KJ. 2005. Hydrothermal time analysis of seed dormancy in true (botanical) potato seeds. *Seed Science Research* **15,** 77–88.

Bair NB, Allen PS, Meyer SE. 2006. A hydrothermal after-ripening time model of seed dormancy loss in *Bromus tectorm*. *Seed Science Research* **16,** 17–28.

Baskin CC, Baskin JM. 1998. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. San Diego, CA: Academic Press.

Baskin JM, Baskin CC. 2004. A classification system for seed dormancy. *Seed Science Research* **14,** 1–16.

Batlla D, Benech-Arnold RL. 2003. A quantitative analysis of dormancy loss dynamics in *Polygonum aviculare* L. seeds: development of a thermal time model based on changes in seed population thermal parameters. *Seed Science Research* **13,** 55–68.

Batlla D, Benech-Arnold RL. 2004. A predictive model for dormancy loss in *Polygonum aviculare* L. seeds based on changes in population hydrotime parameters. *Seed Science Research* **14,** 277–286.

Batlla D, Benech-Arnold RL. 2007. Predicting changes in dormancy level in weed seed soil banks: implications for weed management. *Crop Protection* **26,** 189–197.

Bauer M, Meyer S, Allen P. 1998. A simulation model to predict seed dormancy loss in the field for *Bromus tectorum* L. *Journal of Experimental Botany* **49,** 1235–1244.

Benech-Arnold RL, Sanchez RA, Forcella F, Kruk BC, Ghersa CM. 2000. Environmental control of dormancy in weed seed banks in soil. *Field Crops Research* **67,** 105–122.

Bewley JD. 1997. Seed germination and dormancy. *The Plant Cell* **9,** 1055–1066.

Bradford KJ. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science* **50,** 248–260.

Bryant G, Koster KL, Wolfe J. 2001. Membrane behaviour in seeds and other systems at low water content: the various effects of solutes. *Seed Science Research* **11,** 17–25.

Chantre GR, Batlla D, Sabbatini MR, Orioli G. 2009. Germination parameterization and development of an after-ripening thermal-time

model for primary dormancy release of *Lithospermum arvense* seeds. *Annals of Botany* 10.1093/aob/mcp070.

Christensen M, Meyer SE, Allen PS. 1996. A hydrothermal time model of seed after-ripening *Bromus tectorum* L. *Seed Science Research* **6,** 1–9.

Covell S, Ellis RH, Roberts EH, Summerfield RJ. 1986. The influence of temperature on seed germination rate in grain legumes. I. A comparison of chickpea, lentil, soybean, and cowpea at constant temperatures. *Journal of Experimental Botany* **37,** 705–715.

Ellis RH, Covell S, Roberts EH, Summerfield RJ. 1986. The influence of temperature on seed germination rate in grain legumes. II. Intraspecific variation in chickpea at constant temperatures. *Journal of Experimental Botany* **37,** 1503–1515.

Ellis RH, Hong TD, Roberts EH. 1983. A note on the development of a practical procedure for promoting the germination of dormant seed of grape (*Vitis spp*.). *Vitis* **22,** 211–219.

Ellis RH, Simon G, Covell S. 1987. The influence of temperature on seed germination rate in grain legumes. III. A comparison of five faba bean genotypes at constant temperatures using a new screening method. *Journal of Experimental Botany* **38,** 1033–1043.

FAO. 2007. Food and Agriculture Organization. http://faostat.fao.org.

Faure O, Dewitte W, Nougarede A, Van Onckelen H. 1998. Precociously germinating somatic embryos of *Vitis vinifera* have lower ABA and IAA levels than their germinating zygotic counterparts. *Physiologia Plantarum* **102,** 591–595.

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. *New Phytologist* **171,** 501–523.

Flemion F. 1937. After-ripening at 5°C favors germination of grape seeds. *Contributions from the Boyce Thompson Institute* **9,** 7–15.

Gan YY, Li SH, Song SQ, Wang WQ, Cheng HY. 2008. Seed dormancy and release of grapes from different provenances. *Biodiversity Science* **16,** 570–577.

Garcia-Huidobro J, Monteith JL, Squire GR. 1982. Time, temperature and germination of pearl millet (*Pennisetum typhoides*, S & H). I. Constant temperature. *Journal of Experimental Botany* **33,** 288–296.

Grundy AC, Phelps K, Reader RJ, Burston S. 2000. Modelling the germination of *Stellaria media* using the concept of hydrothermal time. *New Phytologist* **148,** 433–444.

Hallett BP, Bewley JD. 2002. Membranes and seed dormancy: beyond the anaesthetic hypothesis. *Seed Science Research* **12,** 69–82.

Hardegree SP. 2006. Predicting germination response to temperature. I. Cardinal-temperature models and subpopulation-specific regression. *Annals of Botany* **97,** 1115–1125.

International Seed Testing Association. 1999. International rules for seed testing. *Seed Science and Technology* **27** supplement, 47–50.

Kebreab E, Murdoch AJ. 1999. A quantitative model for loss of primary dormancy and induction of secondary dormancy in imbibed seeds of *Orobanche spp*. *Journal of Experimental Botany* **50,** 211–219.

Manivel L, Weaver RJ. 1974. Effect of growth regulators and heat on germination of tokay grape seeds. *Vitis* **12,** 286–290.

Murata N, Los DA. 1997. Membrane fluidity and temperature perception. *Plant Physiology* **115,** 875–879.

Pritchard HW, Tompsett PB, Manger KR. 1996. Development of a thermal time model for the quantification of dormancy loss in *Aesculus hippocastanum* seeds. *Seed Science Research* **6,** 127–135.

Singh SN. 1961. Germination of grape (*Vitis vinifera* L.) hybrid seeds by chilling. *Current Science* **30,** 62.

Spiegel-Roy P, Shulman Y, Baron I, Ashbel E. 1987. Effect of cyanamide in overcoming grape seed dormancy. *HortScience* **22,** 208–210.

Steadman KJ. 2004. Dormancy release during hydrated storage in *Lolium rigidum* seeds is dependent on temperature, light quality, and hydration status. *Journal of Experimental Botany* **55,** 929–937.

Steadman KJ, Bignell GP, Ellery AJ. 2003*a*. Field assessment of thermal after-ripening time for dormancy release prediction in *Lolium rigidum* seeds. *Weed Research* **43,** 458–465.

Steadman KJ, Crawford AD, Gallagher RS. 2003*b*. Dormancy release in *Lolium rigidum* seeds is a function of thermal after-ripening time and seed water content. *Functional Plant Biology* **30,** 345–352.

Steadman KJ, Pritchard HW. 2004. Germination of *Aesculus hippocastanum* seeds following cold-induced dormancy loss can be described in relation to a temperature-dependent reduction in base temperature (t_b) and thermal time. *New Phytologist* **161,** 415–425.

Totterdell S, Roberts EH. 1979. Effects of low temperatures on the loss of innate dormancy and the development of induced dormancy in seeds of *Rumex obtusifolius* L. and *Rumex crispus* L. *Plant, Cell and Environment* **2,** 131–137.