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Research

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

Kathryn J. Steadman[1,2] and Hugh W. Pritchard[1]

[1]Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK; [2]Western Australian Herbicide Resistance Initiative, School of Plant Biology, Faculty of Natural and Agricultural Sciences, University of Western Australia, Crawley, WA 6009, Australia

Author for correspondence:
Kathryn J. Steadman
Tel: +61 86488 2551
Fax: +61 86488 7834
Email: ksteadman@agric.uwa.edu.au

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Summary

• The effect of moist stratification at cool temperatures on *Aesculus hippocastanum* (horse chestnut) seed dormancy release and subsequent thermal time requirement for germination has been investigated.

• Germination performance following over 50 different treatments, each varying in time and temperature of stratification and germination to a total test time of over 3 yr, was used to develop a predictive model for dormancy release and germination.

• Stratification at 2–16°C caused a reduction in base (minimum) temperature for germination (T_b), being fastest at the colder temperatures. Using the sigmoid relationship between rate of reduction in T_b and stratification temperature, seed germination can be predicted in relation to thermal time accumulation above a gradually reducing T_b. Newly shed unstratified seeds, seeds with reduced viability, and seeds on the brink of germination because of T_b being close to stratification temperature, did not conform to the model.

• T_b is not constant during dormancy release in horse chestnut seeds. A reduction in T_b in response to cold stratification may be characteristic of summer annuals, suggesting future applications for this approach in seed ecology studies.

Key words: chilling, dormancy, germination, horse chestnut (*Aesculus hippocastanum*), recalcitrant seed, stratification, thermal time.

Introduction

Horse chestnut (*Aesculus hippocastanum*), a species with seeds that are recalcitrant (desiccation intolerant) in terms of storage behaviour (Tompsett & Pritchard, 1993), is a widely cultivated deciduous tree common in the northern temperate zone. Although unusual for recalcitrant species (Tweddle *et al.*, 2003), freshly fallen seeds exhibit an embryo-based physiological dormancy that is relieved by a period of hydrated storage (stratification) at cold temperatures (Suszka, 1966; Tompsett & Pritchard, 1993, 1998; Pritchard *et al.*, 1996, 1999). However, in the absence of cold stratification some, but not all, seeds in the population are able to germinate at high temperatures (31–36°C; Pritchard *et al.*, 1999). Aspects of these responses are similar to the type 2 nondeep physiological dormancy described for numerous species, particularly summer annuals, such that seeds become able to germinate at lower temperatures as dormancy is lost during cold stratification (Baskin & Baskin, 1998). This response is well described qualitatively in many species, and ensures germination in the spring when successful establishment is most likely to occur.

We previously used horse chestnut to study the effects of a single cold stratification temperature (6°C) on dormancy release. We measured the effects of stratification on the temperature at which germination rate was zero, termed the base temperature (T_b). We established that T_b progressively reduced at a rate of 0.18°C d^{-1} for seedlots from three

consecutive years, although the starting point, T_b at seed fall, differed between harvests (Pritchard *et al.*, 1999). Thus dormancy release in horse chestnut seeds can be described simply in terms of a T_b reduction gradually allowing germination to occur at progressively lower temperatures (Pritchard *et al.*, 1999). In *Solanum physalifolium* T_b apparently reduces during dormancy release at certain alternating temperatures (del Monte & Tarquis, 1997). A reduction in T_b could be inferred from dormancy release data for seeds of *Alnus glutinosa* (McVean, 1955) and *Betula* sp. (Joseph, 1929); the lowest temperature at which germination occurred reduced from 18 to 7°C and from 31 to 15°C, respectively, by 6 wk cold stratification (2–5°C). Batlla & Benech-Arnold (2003) recently described the same response in *Polygonum aviculare* seeds, in which a decrease in the lowest temperature for germination (denoted T_l by the authors) corresponded with dormancy loss, reducing from an estimated initial 18°C at harvest. Dormancy release was faster at cool than at warm stratification temperatures, and the relationship allowed the use of stratification thermal time to predict dormancy status based on the accumulation of thermal stratification units below a ceiling temperature (T_c), similar to that used previously for horse chestnut (Pritchard *et al.*, 1996).

An alternative, theoretical, population-based model describing seed dormancy behaviour has been proposed by Bradford (1996, 2002) in relation to a moving water potential threshold for growth. The model requires estimates of the mean base water potential for germination ($\psi_b(50)$) and its standard deviation, and the hydrotime constant for each seed population. In applying this theory to germination following dormancy release (dry after-ripening) in the winter annuals *Bromus tectorum* and *Elymus elymoides*, an important assumption is that T_b remains constant and is set at 0°C while $\psi_b(50)$ reduces during dormancy release (Bauer *et al.*, 1998; Meyer *et al.*, 2000). However, our previous work on cold stratification-induced dormancy loss in hydrated horse chestnut seeds demonstrated that accounting for the reduction in T_b may suffice for quantifying horse chestnut seed performance (Pritchard *et al.*, 1999).

In the present study, we considerably extend our earlier work to encompass the influence of stratification temperatures between 2 and 21°C on dormancy release in terms of a gradually reducing base temperature for germination. A benefit of directly determining T_b, and allowing T_b to vary, is the subsequent ability to quantify germination performance in terms of thermal time, θ, allowing quantification of the combined effect of temperature-induced dormancy loss and temperature-dependent germination. Thus the relative effects of different temperatures are integrated into a model for the response during both dormancy release and germination phases. Potential reasons for dispersion of the data around the model are discussed, and the model is used to compare the germination response of three other horse chestnut seedlots.

Materials and Methods

Seed collection and storage

Freshly fallen seeds of *Aesculus hippocastanum* L. (horse chestnut) were collected from the ground during the time of maximum seed fall from a row of more than 25 trees at Chailey, East Sussex, UK between 9 and 21 October 1992. All seeds collected had a shiny appearance to the seed coat, indicating that they were healthy and recently shed. Seeds were stored at 16°C in a loosely tied black polythene bag until 22 October 1992. The average moisture content of the material was measured according to the International Seed Testing Association (1999) as 67 and 52% (fresh weight basis) for the embryonic axis and the rest of the seed, respectively ($n = 5$).

Seed stratification and germination

Two experiments are described. In the first, seeds were placed directly into a germination test at 11 temperatures between 2 and 42°C soon after harvest. In the second experiment, seeds were stratified for between 43 and 300 d at constant temperatures, 2, 6, 8, 11, 16 or 21°C, then moved to a germination test at three or four temperatures above the respective stratification temperature.

Seeds were stratified for dormancy release on 200 cm^3 1% agar-water in plastic sandwich boxes (7 × 11 × 17 cm). Two replicate boxes, each containing 15 seeds, were used for each treatment because of restricted incubator space. Boxes were individually wrapped in aluminium foil to achieve darkness. Seeds were also germinated on 1% agar-water in foil-covered boxes; foil was briefly removed from boxes only to check for germination. The criterion for germination was radicle protrusion through the seed coat and extension to >15 mm with normal morphological appearance (Pritchard *et al.*, 1996). Radicle protrusion (< 5 mm) was also recorded. Agar-water was replaced at the first sign of shrinkage to ensure a nonlimiting supply of water. The germination test continued until all viable seeds had germinated. Nonviability of soft, ungerminated seeds was confirmed by cutting through the seed to view necrotic tissues. Stratification and germination tests continued for a combined test time of up to 3.2 yr.

Statistical analysis and model development

The base temperature for germination rate (T_b) was calculated using five percentiles of the population (20, 35, 50, 65 and 80%) in each stratification treatment. Linear regression lines were calculated through the reciprocal of the time for each percentile to germinate ($1/t(g)$) against germination temperature, and T_b was calculated as the value of the intercept on the temperature axis (Pritchard *et al.*, 1999). The treatments in which seeds germinated to < 80% – those involving no stratification, or stratification at 21°C – were

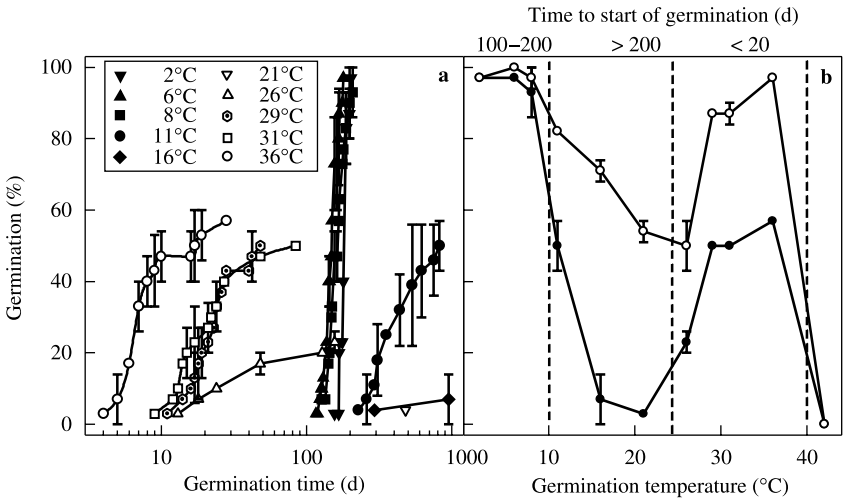

Fig. 1 Germination of *Aesculus hippocastanum* seeds at constant temperatures. (a) Germination progress curves for seeds at constant temperatures in the range 2–42°C. Time is plotted on a log scale to fit all germination curves on one graph. (b) Percentage radicle protrusion (open symbols) and germination (filled symbols) attained with an indication of the difference in time taken for the first seeds to start germinating. Bars, ±1 SEM.

calculated using different percentiles to account for the reduced upper percentile (Pritchard *et al.*, 1999).

Thermal time for germination (θ) is the number of degrees above T_b that the seeds accumulate each day while at temperature T, multiplied by the number of days of that treatment (t), as described by equation 1:

$$\theta = (T - T_b)t \qquad \text{Eqn 1}$$

Thermal time accumulation was calculated following transfer of seeds from stratification to germination temperature (equation 1). Generally, θ did not accumulate during stratification because the temperature was lower than T_b. However, in seeds stratified at 8°C for 120 d and at 11°C for 300 d (the longest stratification time used in each case), T_b reduced during stratification to below the stratification temperature itself. Using the relationship between rate of decline of T_b and temperature, and assuming that the rate of reduction of T_b continued unchanged, θ accumulated during the stratification phase was calculated and added to θ accumulated at the warmer germination temperatures. Seeds that germinated over 80°Cd after the previous seeds germinated were removed from the analysis, up to a maximum of 10% of the population, to eliminate data that may adversely skew the results.

The statistical package GLIM version 4.0 was used for comparison of multiple regression lines using a probit link function. The F distribution was used to check for statistical significance of the increase in scaled deviance caused by constraining multiple regression lines to have the same slope or same intercept (Crawley, 1993).

Results

Germination at single constant temperature

The response of newly shed, dormant horse chestnut seeds to imbibition at a single temperature in the range 2–42°C was highly dependent on temperature (Fig. 1a) and could be segregated into four distinct temperature ranges (Fig. 1b). At low temperatures (2–8°C) maximal germination was observed, reaching over 93% (Fig. 1b). Germination started after 116 d and was completed by 207 d (Fig. 1a). At these low temperatures dormancy release is facilitated, which is then followed by germination. In the medium temperature range (11–21°C) progressively lower levels of germination were obtained as the temperature increased, and germination was first visible (at 11°C) after 200 d (Fig. 1). After 3.2 yr, three and seven seeds were left ungerminated and healthy at 11 and 16°C; on transferral of these seeds to 36°C, three seeds germinated in each case. Germination at high temperatures (26–36°C) reached maximum levels of up to 57% only (Fig. 1). However, in contrast to temperatures below 26°C, germination began within 4–18 d and was generally complete by 50 d (Fig. 1a). The speed and extent of germination improved as the temperature was raised from 26 to 36°C (Fig. 1). In both medium and high temperature ranges some seeds were capable of radicle protrusion but not extension (Fig. 1b), so radicle extension is the preferred measure of germination (Pritchard *et al.*, 1996). Finally, no germination occurred at 42°C.

Thus the dormancy expressed in this species was conditional on temperature and, in newly shed seeds, was expressed at temperatures < 26°C. When germination was tested at 26–36°C, around half the population did not exhibit dormancy and were capable of completing germination. The optimal single constant temperature that allowed dormancy release followed by germination was 6°C.

Germination following a single upward shift in constant temperature

Stratification resulted in seeds germinating more quickly on moving to a warmer temperature (Fig. 2). As stratification

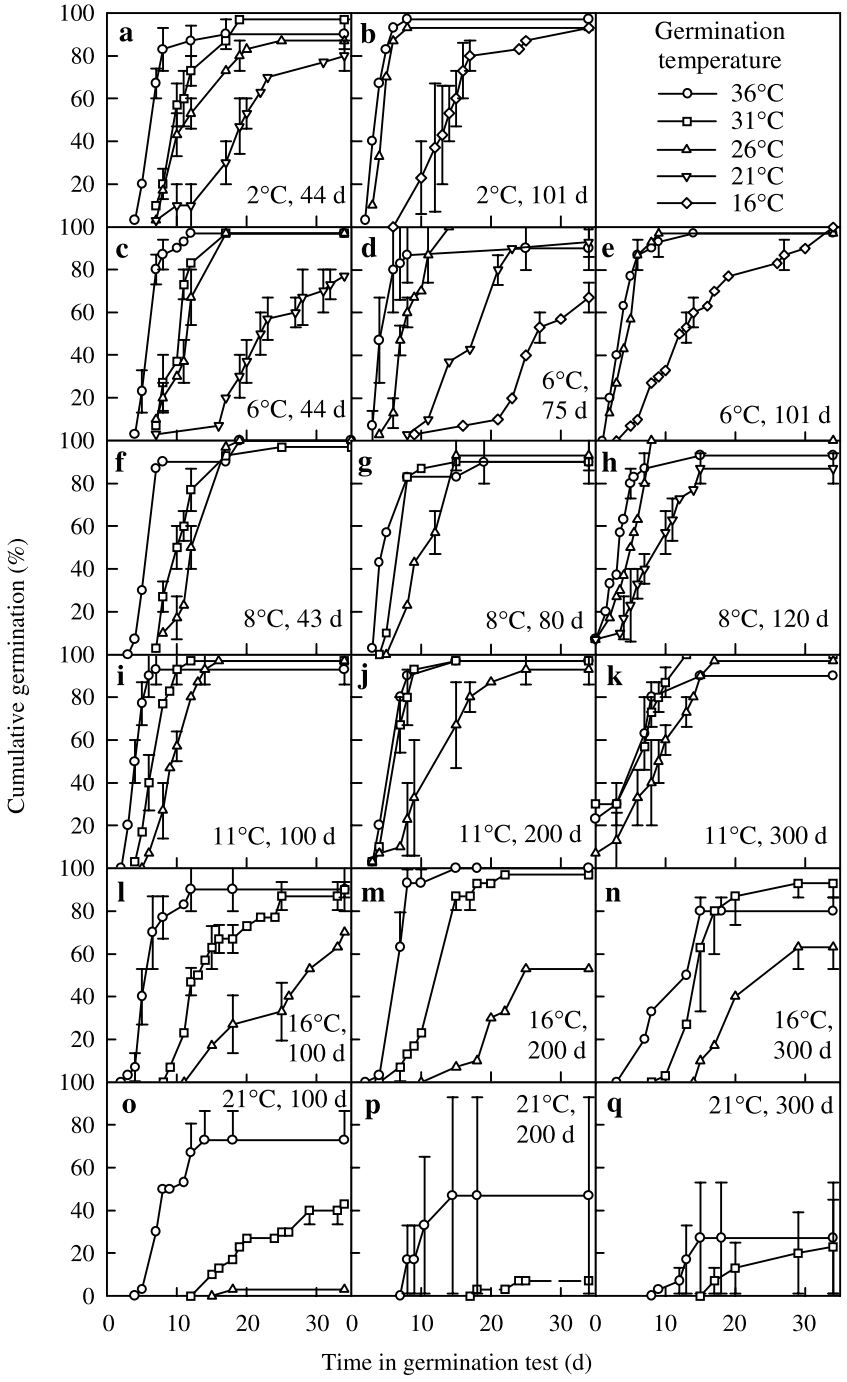

Fig. 2 Cumulative germination of *Aesculus hippocastanum* seeds germinated at 16–36°C following stratification at 2°C (a,b); 6°C (c–e); 8°C (f–h); 11°C (i–k); 16°C (l–n); or 21°C (o–q) for 43–300 d. Bars, ±1 SEM.

time at cold temperatures was extended, the rate of germination in the subsequent germination test increased. For example, at a germination test temperature of 26°C, it took 10 d to reach 50% germination (t_{50}) following stratification at 8°C for 80 d (Fig. 2g), but t_{50} was only 5 d if stratification time was extended to 120 d (Fig. 2h). Furthermore, as stratification continued, cooler temperatures gradually became permissible for germination. In fact, a few seeds germinated just before the end of the stratification treatment when seeds were left at 8°C for 120 d or 11°C for 300 d (Fig. 2h,k). The exception to this trend was in the 21°C stratification treatments (Fig. 2o,p,q), in which the final level of germination reduced and the rate of germination slowed as stratification time increased.

Table 1 Base temperature (T_b) for percentiles of the population and overall population mean for *Aesculus hippocastanum* seeds

Stratification		T_b (°C) for proportions of the seed population										T_b (°C)		
Temperature (°C)	Time (d)	20%	*n*	35%	*n*	50%	*n*	65%	*n*	80%	n	mean	SD	*n*
2	44	13.3	4	13.4	4	13.9	4	15.1	4	17.2	4	14.6	1.6	5
	101	5.2	3	5.6	3	5.3	3	4.5	3	4.0	3	4.9	0.7	5
6	44	14.2	4	15.7	4	15.3	4	15.9	4	17.4	4	15.9	1.2	5
	75	13.0	4	13.2	4	13.5	4	13.2	4	13.3	4	13.2	0.2	5
	101	4.8	4	4.7	4	4.6	4	5.2	4	5.3	4	4.9	0.3	5
8	43	19.1	3	18.6	3	18.5	3	18.8	3	19.2	3	18.8	0.3	5
	80	16.9	3	18.0	3	17.0	3	15.4	3	15.3	3	16.5	1.1	5
	120	6.0	3	7.8	3	5.0	3	5.7	3	5.0	3	5.9	1.2	5
11	100	20.1	3	19.5	3	18.8	3	19.1	3	18.4	3	19.2	0.7	5
	200	14.3	3	15.1	3	16.5	3	17.0	3	18.0	3	16.2	1.5	5
	300	–	–	11.3	3	9.4	3	9.3	3	13.4	3	10.0	1.1	4
16	100	23.4	3	24.2	3	24.1	3	24.2	3	24.3	3	24.4	0.4	5
	200	23.5	3	23.3	3	23.1	3	23.4	3	23.9	3	23.5	0.3	5
	300	20.2	3	20.1	3	19.4	3	19.1	3	21.5	3	20.0	1.0	5
		3%	*n*	10%	*n*	20%	*n*	35%	*n*	50%	*n*	mean	SD	*n*
Untreated		24.0	4	24.6	4	25.6	4	27.0	4	25.0	4	25.3	1.2	5
21	100	23.4	3	25.8	3	28.1	2	29.0	2	–	–	26.6	2.5	4
	200	26.1	3	28.9	2	–	–	–	–	–	–	27.5	2.0	2
	300	24.6	3	24.0	2	26.1	2	–	–	–	–	24.9	1.1	3

T_b was calculated from the regression of the reciprocal of time for 20, 35, 50, 65 and 80% of the seeds to germinate against germination temperature following stratification at 2–16°C for between 43 and 300 d, and for 3, 10, 20, 35 and 50% of the seeds to germinate against germination temperature for freshly fallen untreated seeds or following stratification at 21°C. *n* = number of points on the regression line for calculation of T_b.

Relationship between T_b and stratification temperature

The base temperature for germination rate (T_b) systematically decreased with stratification time at 2–16°C (Table 1). Lower temperatures (2–8°C) were more effective than higher temperatures at reducing T_b (11–21°C). Linear regression lines were fitted through the mean T_b values calculated for the stratification treatments at 2–16°C (treatments resulting in over 80% germination) (Fig. 3). As the seeds all originated from one seedlot, the five lines were constrained to a common origin, and the fit of the data was not significantly different in comparison to allowing the lines to run free ($F = 0.44$; tabulated $F_{4,8} = 3.84$, $P = 0.05$). The common origin of 25.3°C derived in this way was the same as the mean value calculated from $1/t$ lines for the original data for seeds germinated immediately upon collection (Table 1), and T_b did not reduce during 300 d stratification at 21°C (Fig. 3).

The rate of change of mean T_b produced by each of the stratification temperatures of 2–16°C are plotted in Fig. 4. Between 6 and 11°C a linear relationship existed ($r^2 = 0.99$) with a reduction in stratification temperature of 1°C causing an increase in the rate of reduction in T_b by 0.0283°C d^{-1}. However, the relationship between temperature and rate of dormancy release was negatively sigmoidal when considered over the wider range, caused by the effect of stratification at 2°C being similar to 6°C, and that at 16°C being similar to stratification at 11°C (Fig. 4).

The effect of temperature on T_b can be defined by combining equation 2, which describes the change in T_b with time at a single stratification temperature (Fig. 3), with equation 3, which defines the sigmoidal relationship between temperature and rate of change in T_b (Fig. 4):

$$T_b = 25.26 - (st \times R_{T_s}) \quad \text{Eqn 2}$$

$$R_{T_s} = (0.1958/1 + e^{(T_s - 8.9974)/1.3583}) + 0.0135 \quad \text{Eqn 3}$$

(R_{T_s}, rate of change in T_b (°C d^{-1}) at stratification temperature T_s (°C); *st*, length of time of stratification measured in days). The combination of these gives equation 4, which allows an estimation of T_b for this seedlot when stratification temperature (T_s) and stratification time (*st*) are known:

$$T_b = 25.26 - st[(0.1958/1 + e^{(T_s - 8.9974)/1.3583}) + 0.0135] \quad \text{Eqn 4}$$

Statistical modelling of germination in terms of thermal time

The germination progress curves following stratification at 2–21°C have been plotted with percentage germination on a

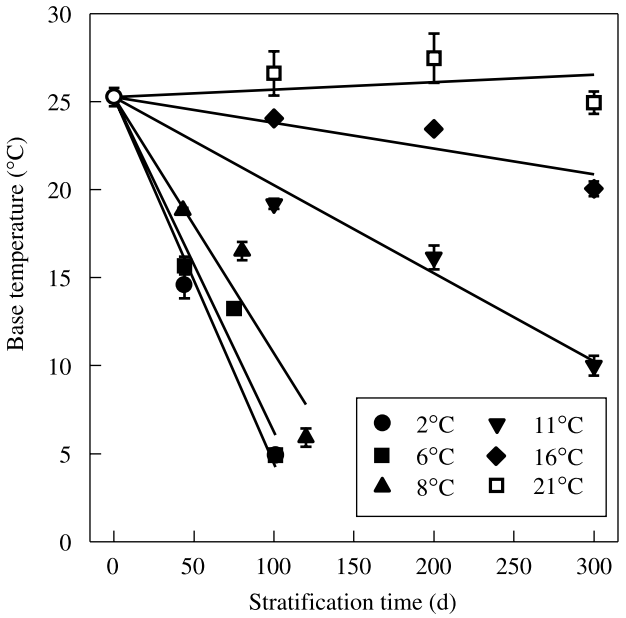

Fig. 3 Relationship between base temperature for germination (T_b) and length of time of stratification at 2–21°C for *Aesculus hippocastanum* seeds. Bars, ±1 SEM. Only the treatments that resulted in over 80% germination (filled symbols) were involved in multiple regression analysis to define the common origin.

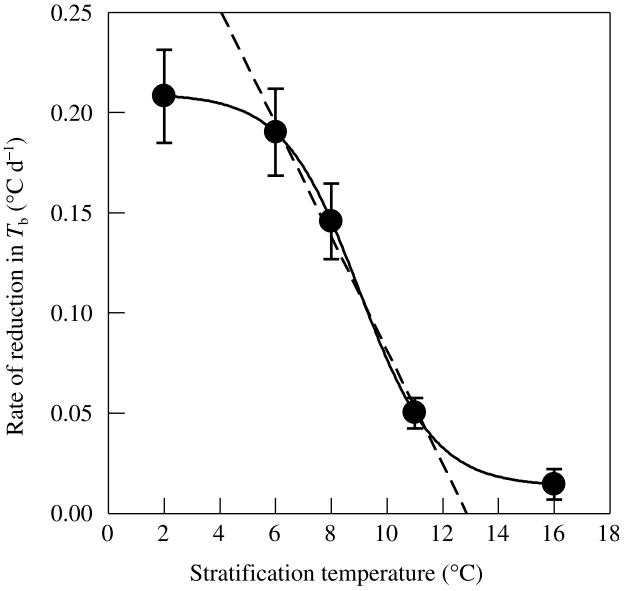

Fig. 4 Relationship between rate of reduction in base temperature (T_b) with temperature used for stratification of *Aesculus hippocastanum* seeds. Bars, ±1 SEM. The least-squares regression through 6, 8 and 11°C (dotted line) has the equation: $y = 0.3648 - 0.0283x$ ($r^2 = 0.991$). The four-parameter sigmoidal curve through 2–21°C (solid line) has the equation: $y = (0.1958/1 + e(x^{-8.9974/1.3583}) + 0.0135$ ($r^2 = 0.998$).

probit scale (linearized probability) against θ (Fig. 5). On a probit scale, cumulative normally distributed data produce a straight line (Finney, 1977). Comparison of log scale with a linear scale (data not shown) revealed that the distribution of θ within the horse chestnut seed population is better described as log-normal, as for the recalcitrant seeds of two other temperate trees, *Castanea sativa* and *Quercus robur* (Pritchard & Manger, 1990).

Probit germination of seeds from many of the 59 different treatments were similar with respect to θ, requiring the accumulation of an average of 100°Cd to germinate, irrespective of stratification time or subsequent germination temperature. All regression lines for germination of seeds following stratification at 2°C could be constrained to be parallel (Fig. 5b,c; Table 2). This was also the case for seeds stratified at 16°C (Fig. 5m–o). However, seeds stratified at 6, 8 or 11°C did not have consistent responses to thermal time. In each case the longest stratification treatment, 6°C for 101 d (Fig. 5f); 8°C for 120 d (Fig. 5i); and 11°C for 300 d (Fig. 5l) was significantly different, and the remaining regressions for each stratification temperature were parallel (Table 2). The remaining data across all five stratification temperatures (2, 6, 8, 11 and 16°C) were approximately parallel ($F_{4,218} = 3.65$; tabulated $F_{4,200} = 3.84$, $P = 0.005$, $r^2 = 0.60$; Fig. 5).

The three excluded treatments (6°C, 101 d; 8°C, 120 d; 11°C, 300 d) responded to θ similarly ($F_{4,54} = 3.36$; tabulated $F_{4,60} = 3.65$, $P = 0.01$) with a slower probit increase in germination per log θ than shorter stratification treatments. T_b had reduced to below the stratification temperature in these treatments (Table 1), allowing a small amount of thermal time to be accumulated before the seeds were transferred to the germination temperature, and a few seeds in the 8°C for 120 d and 11°C for 300 d treatments germinated before the end of the allotted stratification period. The wider distribution of θ requirement for germination among the population (low slope) occurred only in treatments where T_b was close to the stratification temperature, and may be evidence of the seeds being on the brink of germination.

Seeds tested for germination immediately at seed fall (no stratification) and those stratified at 21°C were the only treatments that did not reach 80% germination. Not only was germination capacity reduced, but they were associated with a systematic difference in T_b within sequential proportions of the population, with early germinating seeds having a lower apparent T_b than later germinating seeds (Table 1). Additionally, θ accumulation required for germination was highly variable (Fig. 5a,p–r). Seeds stratified at 21°C exhibited reduced germination capacity as stratification time continued (Fig. 5p–r). At this temperature seed ageing was hastened, with tissue necrosis and fungal infection being visible in those remaining ungerminated.

The treatments in which seeds were stratified at 2–16°C for over 6 wk, were not on the brink of germination, and were not exhibiting reduced viability are plotted together in Fig. 6. While germination rate in response to thermal time

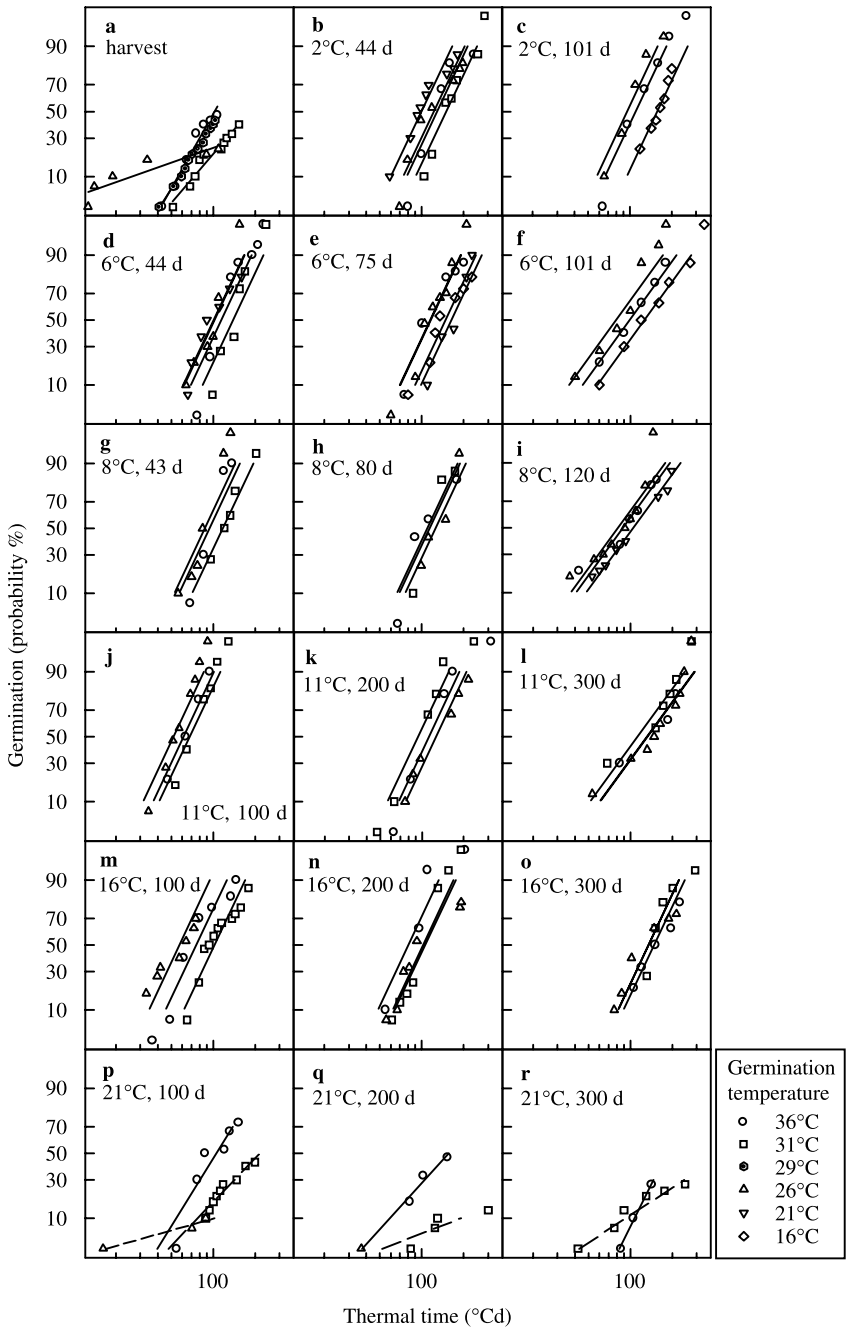

Fig. 5 Cumulative germination (probability scale) with thermal time (θ,°Cd) on a log scale for *Aesculus hippocastanum* seeds germinated at 16–36°C at seed fall (a); or following stratification at 2°C (b,c); 6°C (d–f); 8°C (g–i); 11°C (j–l); 16°C (m–); or 21°C (p–r) for 43–300 d. Regression lines are constrained to be parallel where statistically acceptable (Table 2).

accumulation was the same in these seeds, constraint of the individual parallel regression lines to be a single line (equation 5) was associated with a significant increase in scaled deviance ($P < 0.001$):

$$\text{probit}(g) = 3.8306 (\log \theta) - 7.7346 \qquad \text{Eqn 5}$$

(g, germination (%) in response to θ, thermal time (°Cd) accumulation). This was because of the spread in the data, with θ at which 50% germination was observed (θ_{50}) having extremes of *c.* 50 and 150°Cd. The possibility that the θ requirement for germination varies with dormancy status of the population was excluded. While there appears to be some suggestion of an increase in θ_{50} with stratification time in some of the data (Fig. 5), this was not consistent, and correlation between θ_{50} and dormancy status (T_b) was low ($r = -0.252$). Imprecise measurement of temperature may be the cause of some of the spread. For example, it is possible that instead of requiring more θ for germination to occur at 16°C, for which regressions lay to the right of most of the other lines (Fig. 5c,e,f), the seeds may have been experiencing a slightly lower temperature within the large constant-temperature

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Table 2 *F* statistic and level of significance for comparison of multiple regression lines of probit germination of *Aesculus hippocastanum* seeds against log θ following stratification at 2, 6, 8, 11 or 16°C

Stratification			Parallel lines			Single line		
Temperature (°C)	Time (d)	n[a]	$F(df_1,df_2)$[b]	sig[c]	r^2	$F(df_1,df_2)$	sig	r^2
Comparisons for all seeds stratified at 2–16°C								
2	44, 101	7	$F_{6,40} = 2.11$	NS	0.97	$F_{12,40} = 31.4$	***	0.61
6	44, 75, 101	11	$F_{10,47} = 5.08$	***	0.90	$F_{20,47} = 12.1$	***	0.71
8	43, 80, 120	9	$F_{8,31} = 8.33$	***	0.83	$F_{16,31} = 7.67$	***	0.73
11	100, 200, 300	9	$F_{8,35} = 10.7$	***	0.88	$F_{16,35} = 31.7$	***	0.47
16	100, 200, 300	9	$F_{8,41} = 2.90$	*	0.86	$F_{16,41} = 16.6$	***	0.56
Comparisons excluding treatments with $T_b < T_s$								
6	44, 75	8	$F_{7,34} = 2.21$	NS	0.92	$F_{14,34} = 9.54$	***	0.73
8	43, 80	6	$F_{5,14} = 1.74$	NS	0.92	$F_{10,14} = 6.05$	**	0.74
11	100, 200	6	$F_{5,23} = 3.74$	*	0.96	$F_{10,23} = 71.7$	***	0.49

[a]n = number of regression lines being compared. [b]F statistic with numerator and denominator degrees of freedom df_1 and df_2. [c]Level of significance at which constraint accepted defined by the F distribution: NS, $P > 0.05$; *, $P > 0.01$; **, $P > 0.001$; ***, $P < 0.001$, constraint rejected because of significant increase in scaled deviance. Regression lines were constrained to be parallel or single lines, with and without inclusion of treatments in which base temperature had reduced to below stratification temperature ($T_b < T_s$).

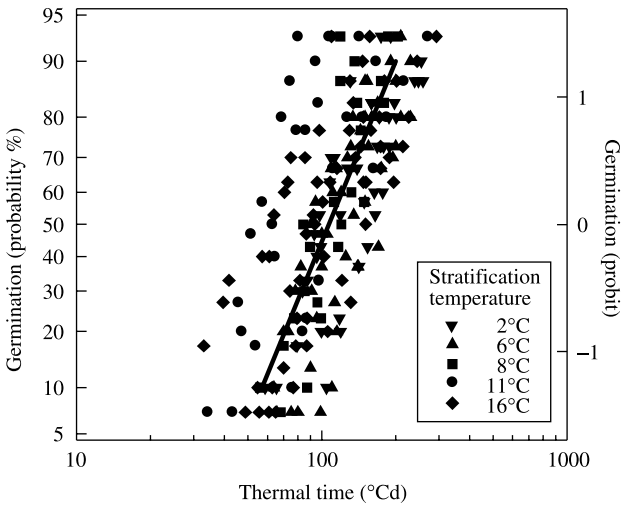

Fig. 6 Cumulative germination (probability scale) with thermal time (θ,°Cd) on a log scale for *Aesculus hippocastanum* seeds that are mature, healthy and not on the brink of germination. Seeds were germinated at temperatures between 16 and 36°C following stratification for between 43 and 300 d at 2–16°C; the regression line has the equation: probit(g) = 3.8306 log θ – 7.7346 (r^2 = 0.56).

room. A reduction by only 1°C would make a noticeable difference: θ at which 50% of the seeds germinated (θ_{50}) would have been reduced from 158 to 144°Cd for seeds stratified at 2°C for 101 d; from 148 to 117°Cd for those stratified at 6°C for 75 d; and from 127 to 114°Cd for those previously at 6°C for 101 d. Unfortunately the temperature in the exact vicinity of the seeds was not logged daily, so it is not possible to confirm these suggestions.

Comparison between horse chestnut populations

A model (equation 6) was produced by combining the equation describing reduction in T_b with stratification (equation 4) and the probit germination response to θ (equation 5). This model described germination following stratification treatments varying in temperature (2–16°C) and time of stratification, as long as the combination involved over 6 wk to account for any minor changes in behaviour in the most immature seeds in the population, seeds were not on the brink of germination, and seedlot viability was not compromised:

$$\text{probit}(g) = 3.8306 \log\left(t\left\{T - \left[25.26 - st\left(\frac{0.1958}{1 + e^{(T_s - 8.9974)/1.3583}} + 0.0135\right)\right]\right\}\right) - 7.7346 \quad \text{Eqn 6}$$

(germination at a temperature, T (°C); for t d; to a certain level, g (%); occurred after stratification for a length of time, st (d); at the stratification temperature, T_s (°C)). Thus, from equation 6 it can be calculated that a group of horse chestnut seeds from this batch stratified for, say, 65 d at 9°C would be expected to achieve 50% germination after 53 d at 20°C, whereas the same level of germination would be achieved after only 18 d at 20°C if stratification was performed over the same period at 7°C.

Germination of three different horse chestnut populations was compared with the present seedlot by predicting θ for germination using equation 6 and an assumed initial T_b at

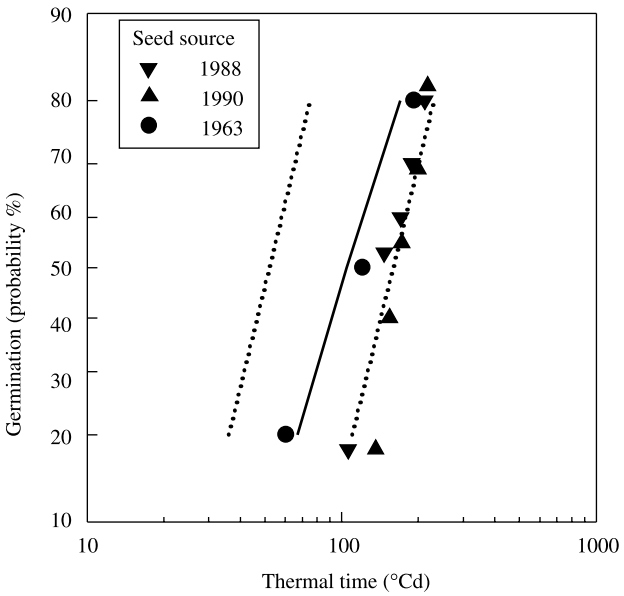

Fig. 7 Comparison of germination of *Aesculus hippocastanum* with thermal time (θ,°Cd) across seedlots. T_{bSF} was not directly determined for these seedlots and was nominally set at 25.3°C. The response of 1992 seeds (solid line, Figure 6) is compared with seeds collected in 1988 and 1990 from the same group of trees in the UK, and with seeds collected in 1963 in Poland (Suszka, 1966). To aid comparison, the extreme observations of the 1992 germination response (Figure 6) are marked by broken lines. Seeds collected in 1988 were stratified at 6°C for 56 d and germinated at 21°C (Pritchard *et al*., 1999). In 1990 seeds were stratified at 6°C for 46 d and germinated at 26°C (Pritchard *et al*., 1999). Seeds collected in 1963 were stratified for 140 d at 10°C and germinated at 20°C (Suszka, 1966).

harvest of 25.3°C (Fig. 7). Data from Pritchard *et al.* (1999) for the germination of seeds collected in 1988 and 1990 from the same row of trees as the present seedlot were compared. Seeds were stratified at 6°C for 56 d in 1988 and for 46 d in 1990, then moved to warmer temperatures for germination. The cardinal temperatures for dormancy loss may vary slightly between seedlots and/or seasons (Pritchard *et al*., 1996, 1999), and a higher T_{bSF} in 1988 and 1990 than in 1992 could be responsible for the difference between years (Fig. 7). Horse chestnut seeds collected in 1963 in Poland (Suszka, 1966) and stratified at 10°C for 140 d behaved remarkably similarly to the seeds in this study (Fig. 7).

Discussion

The base temperature for germination (T_b) is defined as the temperature at which the rate of germination is zero (Garcia-Huidobro *et al*., 1982). Making a correct assessment of T_b is imperative, because this value is the basis for the calculation of thermal time (θ) for germination, with thermal time accumulating more quickly when the difference between the temperature experienced by a seed and T_b is widest (Garcia-Huidobro *et al*., 1982; Covell *et al*., 1986). Based on information derived from nondormant seedlots, T_b is believed to be a single value and to remain constant among seeds in a population irrespective of physiological status. For example, differences in seed quality produced by priming or ageing did not alter T_b in *Allium cepa* seeds (Ellis & Butcher, 1988), and ageing in horse chestnut seeds stratified at 21°C had no impact on T_b. However, we have observed a decrease in mean T_b associated with dormancy release at 6°C (Pritchard *et al*., 1999), and in this study at temperatures between 2 (fast) and 16°C (very slow). Batlla & Benech-Arnold (2003) introduced an additional term, T_l, to describe the lower limit of the temperature range permissible for germination during dormancy release in seeds of the summer annual *P. aviculare*. In the same way as we have described for T_b in horse chestnut, T_l was an index of dormancy status, reducing as dormancy was released by cold stratification. However, in modelling *P. aviculare* germination the assumption was made that T_b was zero and constant, irrespective of dormancy status, and θ for germination was calculated above this base. The fit of our model is drastically reduced if this approach is taken for horse chestnut seeds; calculating θ using a T_b of zero results in θ_{50} ranging from 100 to 800°Cd. Thus, based on our work with horse chestnut seeds, we believe that T_b varies with dormancy status. In modelling horse chestnut germination it is necessary to account for this variation by allowing T_b not only to reflect dormancy status, but also to influence directly the calculation of θ.

The influence of temperature between 2 and 16°C on the reduction of T_b has been quantified, resulting in an equation that describes the change in T_b as dormancy is lost during stratification. Over the range of temperatures usually used for artificial stratification of seeds (5–10°C), the relationship between stratification temperature and dormancy loss rate is linear, which would allow the use of thermal stratification time to model dormancy release. This technique has been applied previously with this species (Pritchard *et al*., 1996), and similarly in *P. aviculare* (Batlla & Benech-Arnold, 2003). However, over the wider range of temperatures used in the present study there was a sigmoid relationship between the effect of temperature on the rate of dormancy loss in horse chestnut (Fig. 3). The lack of linearity negates the use of thermal stratification time as a means to model the dormancy release component, and in the present case the sigmoid equation has been directly incorporated into the final germination model. For ecological studies, in which the temperature experienced can vary widely, this approach would be appropriate for accurate prediction of horse chestnut dormancy release and germination.

The mechanism by which a change in base temperature for germination progression occurs is not clear. However, in developing sunflower seeds a correlation has been observed between the induction of dormancy and a decrease in oleoyl phosphatidyl choline desaturase (ODS) activity, which is

associated with the enhancement of 18 : 2 fatty acids (Hilhorst, 1998). Moreover, sunflower achenes are able to desaturate oleate significantly only at low temperatures (García-Díaz *et al.*, 2002). In addition, chilling-induced dormancy relief in apple buds is associated with enhanced activity of ODS as measured by an increase in linoleic acid content of the cell membranes (Erez, 2000). Thus cold-activated increases in polyunsaturated fatty acids may result in the progressive depression of membrane melting points, thereby permitting regulated metabolism at the lower temperature. In the case of cold-stratified horse chestnut seeds, this could explain their eventual germination at low temperatures.

At harvest only approximately half the population were capable of immediate germination at temperatures > 26°C. Germination performance at high temperatures (31 and 36°C) was improved following stratification even after the first sampling time, 43 d for the coldest temperature and 100 d for the 16°C treatment. This improvement in germinability during stratification at 16°C was unexpected because dormancy release (T_b reduction) was exceptionally slow (< 2°C by 3 months), suggesting that the observed physiological changes were not associated with dormancy release *per se*. It was previously noted that horse chestnut seeds required a combined development and cold stratification time of at least 150 d to achieve 80% germination at 26°C (Tompsett & Pritchard, 1993). Thus it is likely that some horse chestnut seeds are less mature than others at shedding and continue development during the early stages of stratification.

The poor high-temperature germination performance of 21°C stratified seeds, in which there was no apparent dormancy loss, was probably caused by reduced viability rather than maintenance of seed immaturity. Viability loss is slow for seeds in hydrated storage at 16°C, with more than one-third of seeds remaining germinable after 3 yr (Pritchard *et al.*, 1996). On the other hand, freshly harvested, unstratified seeds are expected to be able to germinate eventually when imbibed at 26°C, a temperature marginally higher than T_b at harvest (25.3°C); however only a small proportion of UK populations do so (Tompsett & Pritchard, 1993; Pritchard *et al.*, 1999). Thus, contrary to the performance of some dormant phenotypes of *Avena fatua* (Naylor & Fedec, 1978), horse chestnut seeds appear incapable of maintaining viability for long at around 21–26°C, probably dying in the germination test.

Dormancy release and germination of horse chestnut seeds has been predicted by estimating the θ accumulation above a systematic, temperature-dependent reduction in T_b. Germination of two seedlots collected in different years from the same trees, and one population collected nearly 30 yr previously in Poland, was similar when calculated in terms of θ using parameters measured for the present seedlot. However, sections of the population did not conform to the model, and further investigation is required. These were newly shed, unstratified seeds that exhibited reduced germinability (probably because of immaturity); seed with reduced viability; and seeds on the brink of germination caused by T_b being close to stratification temperature. Additionally, there was significant spread in θ_{50} between treatments in which no consistent pattern was established at this stage. Some of the variation may be linked to imprecise temperature data in which small differences can become large over the extended period involved here. Accounting for the distribution in T_b among the population (Table 1), rather than using mean T_b, may also remove some of the dispersion.

The seeds of many temperate trees and spring-germinating annuals and perennials respond to stratification at cool temperatures (Baskin & Baskin, 1998). It remains to be assessed whether this approach, in which germination is modelled in terms of thermal time accumulation above a gradually reducing T_b, can adequately describe seed dormancy release patterns in other species. The relevance of the model to horse chestnut seedlots from southern European locations, closer to the natural origin of the species, also needs consideration.

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