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# Kinetics of dormancy release and the high temperature germination response in *Aesculus hippocastanum* seeds

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## Abstract

**The kinetics of primary dormancy loss were investigated in seeds of horse chestnut (*Aesculus hippocastanum* L.) harvested in four different years. Freshly collected seeds from 1991 held for up to 1 year at temperatures between 2 °C and 42 °C exhibited two peaks in germination (radicle growth), representing a low temperature (2–8 °C) and a high temperature response (31–36 °C). Germination at 36 °C generally occurred within 1 month of sowing, but was never fully expressed in the seedlots investigated. At low temperatures (2–8 °C), germination started after around 4 months. Generally, very low levels of germination were observed at intermediate temperatures (11–26 °C). Stratification at 6 °C prior to germination at warmer temperatures increased the proportion of seeds that germinated, and the rate of germination for all seedlots. Within a harvest, germination percentage (on a probit scale) increased linearly with stratification time and this relationship was independent of germination temperature (16–36 °C). However, inter-seasonal differences in the increases in germination capacity following chilling were observed, varying from 0.044 to 0.07 probits $d^{-1}$ of chilling at 6 °C. Increased sensitivity to chilling was associated with warmer temperatures during the period of seed filling. The estimated base temperature for germination, $T_b$, for newly harvested seeds varied slightly between collection years, but was close to 25 °C. For all seedlots, $T_b$ decreased by 1 °C every 6 d of chilling at 6 °C. This systematic reduction in $T_b$ with chilling ultimately facilitated germination at 6 °C after dormancy release.**

Key words: Horse chestnut, seed dormancy, base temperature for germination, chilling, recalcitrant seed.

## Introduction

Naturally shed seeds of *Aesculus hippocastanum* L. (horse chestnut) are both desiccation-intolerant (recalcitrant) and dormant (Tompsett and Pritchard, 1993), requiring a period at low temperatures (*c.* 2–11 °C) to ensure high levels of germination at 16 °C and 26 °C (Pritchard *et al.*, 1996; Tompsett and Pritchard, 1998). Longer term chilling (up to *c.* 6 months) facilitates both dormancy loss and subsequent germination at low temperatures (Pritchard *et al.*, 1996) although the kinetics of this response has not been quantified so far.

The rate of chilling-induced dormancy loss in horse chestnut seed is temperature-dependent, being faster the lower the temperature in the range 11 °C to 2 °C (Pritchard *et al.*, 1996). A threshold-based thermal time model has been used to describe the change in dormancy status of the seeds with chilling time in relation to improved germination performance at 16 °C (Pritchard *et al.*, 1996). Similar models have been used to describe the seed germination rate characteristics of numerous species over a range of sub-optimal temperatures delimited at the lower end by the extrapolated base temperature for germination, $T_b$ (Washitani and Takenaka, 1984; Covell *et al.*, 1986; Pritchard and Manger, 1990). Variation in $T_b$ between different fractions in a seed population has previously been found to be reasonably small, usually considerably $<5$ °C (Washitani and Takenaka, 1984; Covell *et al.*, 1986; Gummerson, 1986; Ellis *et al.*, 1987*b*; Pritchard and Manger, 1990; Bradford *et al.*, 1993; Phelps and Finch-Savage, 1997). However, $T_b$ has been found to change with dormancy status. In barley (*Hordeum vulgare* L.), $T_b$ increased as moist (15.2% moisture content) after-ripening of the seeds progressed at 40 °C (Ellis *et al.*, 1987*a*). In contrast, $T_b$ was reduced in seeds of *Solanum physalifolium* Rubsy var. *nitidibacatum*

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(Bitter) Edmonds when a dormancy-breaking, alternating temperature regime of 30/15 °C was applied. In *Solanum nigrum* L., seed populations harvested from sites with more freezing days per year yielded higher estimates for $T_b$ (del Monte and Tarquis, 1997). Intra-population variation in $T_b$ has also been observed (Pritchard and Manger, 1990) in recalcitrant seeds of pedunculate oak (*Quercus robur* L.) and sweet chestnut (*Castanea sativa* Mill.). These results point out that $T_b$ could be used as an indirect marker for the temperature limits to dormancy enforcement at the seedlot level. In the present study, the effects of stratification at 6 °C on the low temperature limit to germination ($T_b$) were investigated in dormant recalcitrant seeds of horse chestnut collected in four years. The aim of this study was to identify an endpoint to dormancy loss at 6 °C and thus separate the process of dormancy release from subsequent germination at the same temperature.

Differences in the capacity of horse chestnut seeds to germinate at various temperatures are evident. Compared to 16 °C, shorter periods of pre-chilling are required for germination to proceed at 26 °C (cf. Tompsett and Pritchard, 1993, with Pritchard *et al*., 1996). Although horse chestnut is used as an amenity species in northern Europe, it originates from the Balkans (Tutin *et al*., 1968), suggesting that warmer temperatures for germination might be preferred. Whether warm temperatures (>26 °C) could offer improvements in the seed germination test regime for this species is also assessed here.

## Materials and methods

### *Seed collection and storage*

Main collections of *Aesculus hippocastanum* L. (horse chestnut) seed were made in 1988, 1990, 1991, and 1992 from a row of more than 25 trees at Chailey, East Sussex, UK. These harvests were made over about a 2 week period from the second week in October at the time of maximum seed fall. The majority of seeds were freshly fallen, with some lightly shaken from the trees. Seed moisture contents (fresh weight basis) at harvest were 54%, 49%, 53%, and 52% for the seedlots from 1988, 1990, 1991, and 1992, respectively.

Seeds were stored at 16 °C in a loosely tied black polythene bag for 5–6 d and 1–2 d for 1991 and 1992, respectively, before the start of stratification or germination tests. In 1988 and 1990 the seeds were stored at 2 °C in a loosely tied black polythene bag for about 1 week before use.

### *Seed stratification and germination*

Embryos were isolated from the seeds by cutting away the seed coat; this included the excision of the lower part of the radicular pocket in which the embryonic axis resides (Pritchard *et al*., 1996).

Seeds and embryos were stratified and germinated on 200 $cm^3$ 1% agar–water in plastic sandwich boxes ($7 \times 11 \times 17$ cm), with 15 (generally) or 20 seeds per box. Boxes were wrapped in aluminium foil to achieve nominal darkness, and opened only to score germination level. Germination took place in constant temperature incubators set at temperatures between 2 °C and 42 °C. Stratification of seeds was performed at 6 °C. The criteria for germination was axis emergence to >1 cm and a normal morphological appearance of the extending radicle (Pritchard *et al*., 1996). Agar–water was replaced at the first sign of shrinkage. The test continued until all viable seeds had germinated, up to 1 year in some cases; inviability of soft, ungerminated seeds was confirmed by cutting through the seed. Two or three replicates of 20 seeds were used in all tests on 1988 seed. In other experiments, two replicates of 15 seeds were used except for 1990 seeds that were germinated at 16, 21 and 26 °C following stratification for between 7 and 84 d for which three replicates were germinated, and one box of 15 seeds was used for 1990 seeds left at 6 °C to germinate and 1991 seeds that were stratified for 98 d before germination.

In 1991 and 1992, newly harvested seeds were placed at 11 temperatures from 2 °C to 42 °C for germination. Seeds from 1988 and 1990 were sown at 6 °C soon after collection. Additionally, in 1991 the seed coat was removed and the embryos were germinated at 26, 31 and 36 °C.

Seeds were subjected to stratification treatments at 6 °C followed by germination at a warmer temperature (11–36 °C). Germination temperatures of 16 °C and 21 °C were used for 1988 seeds following 56–125 d of chilling. Seeds collected in 1990 were stratified for between 7 d and 84 d, and were then moved to 16, 21, 26, 31, and 36 °C for germination. Seeds collected in 1991 were stratified for between 8 d and 98 d and then germinated at 11–36 °C. In 1992 seeds were stratified for 44, 75 and 101 d followed by germination at 16–36 °C.

### *Statistical analysis*

The statistical package GLIM version 4.0 was used for comparison of multiple regression lines. Analysis for statistical significance of the increase in scaled deviance caused by constraining multiple regression lines to have the same slope used the *F*-distribution with a 95% confidence interval (Crawley, 1993).

## Results and discussion

### *The high temperature response*

Horse chestnut seeds collected in 1991 and 1992 gave 3% germination after incubation at 21 °C for over a year (data not shown). However, as germination temperature was raised from 26 °C to 36 °C, germination of newly harvested seeds progressively increased from *c*. 40% to 80% and from *c*. 15% to 55% for the 1991 and 1992 seeds, respectively, with the germination test complete within 2 months of exposure to the temperature (Fig. 1). Horse chestnut seeds are dormant at the time of maximum seed fall and germination at 16 °C (Pritchard *et al*., 1996) and 26 °C (Tompsett and Pritchard, 1993) benefits from pre-chilling. In contrast, the results in Fig. 1 clearly indicate that freshly harvested horse chestnut seeds have the ability to germinate to ≥50% at the higher temperatures of 31 °C and 36 °C. Thus under certain conditions, a proportion of the population may have no requirement for chilling for germination to progress, and that proportion is variable between years. Such a high temperature response is certainly not a common feature of temperate

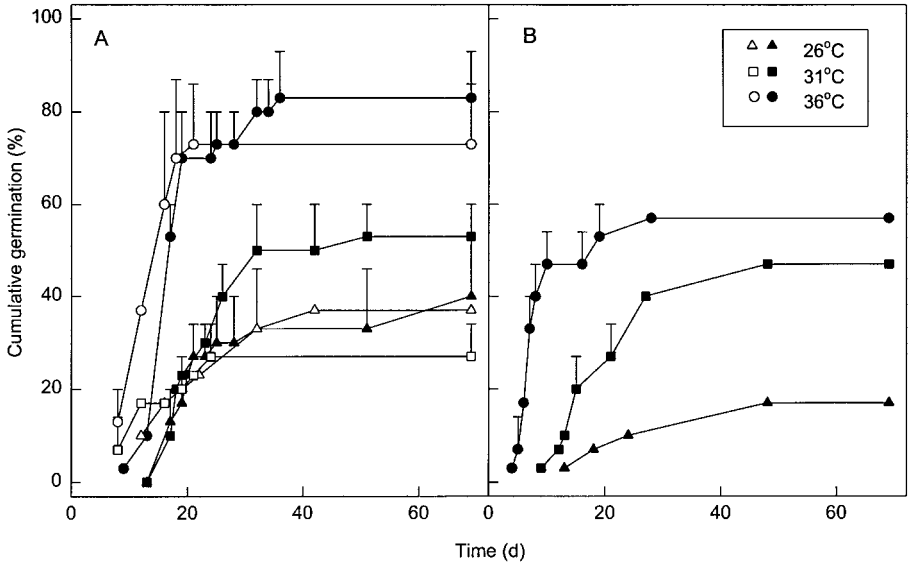


**Fig. 1.** The germination response of *Aesculus hippocastanum* seeds to temperatures between 26 °C and 36 °C. Material from 1991 (A) and 1992 (B) was sown as seeds (closed symbols) and as embryos (open symbols). Bars denote 1 SE of the mean.

tree seed germination (Bewley and Black, 1994; Baskin and Baskin, 1998).

Horse chestnut was originally introduced into Britain from Greece and Albania (Mitchell, 1974). In Greece the main period of seed fall is in October (C Thanos, personal communication) at the start of the winter rains (Meteorological Office, 1973). Interestingly, the absolute and average maximum daily temperatures in October reported for various sites in Greece are around 36 °C and 25 °C, respectively, for example, Tríkkala (Meteorological Office, 1973). As a consequence, in parts of the Balkans some seeds might germinate before winter, whereas this is extremely unlikely in the UK. The origin of the seed stock used to establish the trees from which the seeds used in this study were collected is unknown, thus it is unclear whether the high temperature response is phenotypic and/or genotypic in nature. However, a strong genetic component for a similar high temperature response has been observed in pure lines of *Avena fatua* L. (Naylor and Fedec, 1978).

The inability of the whole population to germinate at high temperatures was not apparently reduced by the presence of the seed coat, as isolated embryos from 1991 generally showed a similar temperature dependency to that of the seeds (Fig. 1A). The lower results for embryos in some cases may have resulted from physical damage during the removal of the radicular pocket from around the embryonic axis. Thus, the suggestion based on the morphology of germination that dormancy in this species is embryo-based (Pritchard *et al.*, 1996) is supported by this quantitative study.

The upper temperature limit for germination of non-stratified seed was observed to lie between 36 °C and 42 °C for the 1991 (Fig. 2) and 1992 (data not shown) seedlots. Whilst germination progressively increased when the temperature was raised from 26–36 °C, intermediate temperatures (11–21 °C) resulted in poor germination

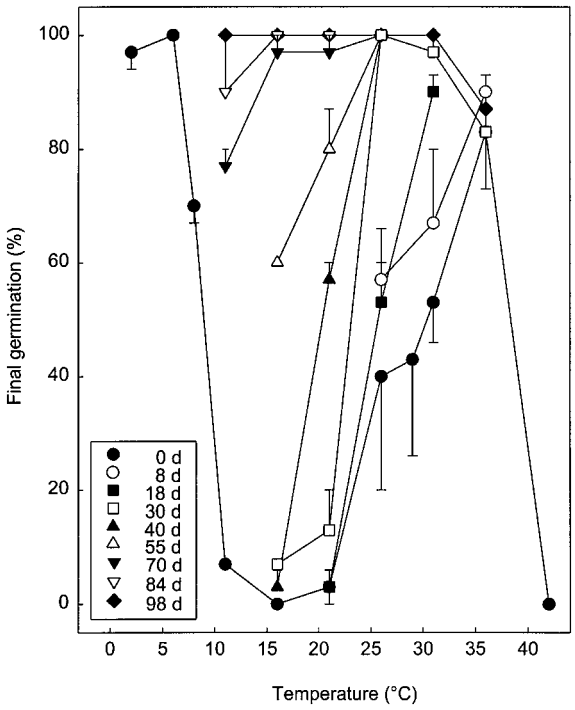


**Fig. 2.** The effects of test temperature on the germination of *Aesculus hippocastanum* seeds after various periods of stratification at 6 °C. Seeds from 1991 were either held at constant temperature between 2 °C and 42 °C (●) or stratified at 6 °C for the periods indicated on the figure and then moved to 11–36 °C for germination. Bars denote 1 SE of the mean.

levels for seeds which did not receive any chilling treatment (Fig. 2). Additionally, the few seeds that were considered to have germinated, did so only after around 7 months at those temperatures. Suszka (1966) noted that horse chestnut seeds remained dormant at 15 °C and 20 °C for at least 175 d. Reduced germination levels have also been reported for seeds at 11 °C and 16 °C, unless the seeds were pre-chilled (Pritchard *et al.*, 1996).

There appears to be a marked change in the germination response of horse chestnut seeds between 16 °C and 26 °C. Below 16 °C, germination improved with decreasing temperature, whilst between 26 °C and 36 °C, the reverse relationship was apparent (Fig. 2). Similarly, some dormant, pure lines of *Avena fatua* seeds exhibit suppression of germination at temperatures in the range 16–24 °C, but germinate well at both lower and higher temperatures (Naylor and Fedec, 1978). Although radicle emergence from non-chilled horse chestnut seed was apparently optimal at 36 °C, subsequent root development was better at 31 °C (data not shown). Therefore, it is recommended that for the rapid production of seedling material from freshly harvested seed a germination test temperature of 31 °C is used.

### *Influence of stratification on final germination*

Final germination at intermediate and high temperatures was improved by stratification at 6 °C prior to germination (Fig. 2). As the length of time of stratification at 6 °C increased, germination gradually improved at all temperatures up to and including 36 °C. In 1991, after only 30 d of chilling, full germination was observed at 26 °C and 31 °C, surpassing that at 36 °C. Seeds chilled for 98 d exhibited high levels of germination at all temperatures $\leq$36 °C, including the intermediate temperatures which were non-conducive for germination when seeds had not been chilled. Thus, one effect of chilling was substantially to widen the range of temperatures over which germination occurred. This type of response has been observed previously in many other species exhibiting primary dormancy (Baskin and Baskin, 1998). However, unlike horse chestnut seeds (Fig. 3), it is more usual for chilling to increase the capacity for germination at warmer rather than lower temperatures (Bewley and Black, 1994).

Linear relations were observed between the length of time of stratification and final germination on a probit scale (Fig. 3). In 1990, 14.4 d of stratification were required to increase germination by 1 probit (e.g. from 50% to 84%), irrespective of germination temperature in the range 16–36 °C (Table 1). Similarly, the 1991 seeds required 14.2 d chilling to increase germination by the same amount. In contrast, 22.6 d of chilling were needed for a 1 probit increase in germination for the 1992 seedlot. Analysis of a limited data set for seeds collected in 1988, in which germination was measured at only 16 °C and

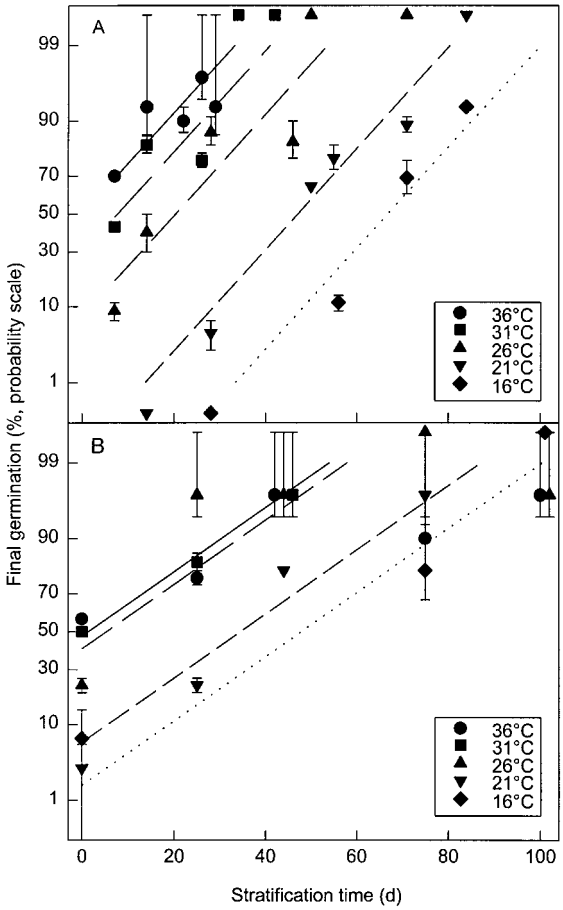


**Fig. 3.** The effects of stratification time at 6 °C on germination of *Aesculus hippocastanum* seeds from 1990 (A) and 1992 (B) sown at various test temperatures. Parameters of the fitted lines are given in Table 1. Data points for each temperature occurring after peak germination were not included in the analysis. Bars denote ±SE of the mean.

21 °C (data not shown), revealed that 26.1 d of chilling were required to increase final germination by 1 probit. It was not possible to constrain the data for the dependency of germination on chilling period for the 4 years to a single slope. Thus the kinetics of dormancy release exhibited a clear inter-seasonal variability, with germination capacity improving faster in seed lots from 1990 and 1991 than in 1988 and 1992.

These results corroborate earlier findings which showed dormancy loss rate (DLR), based on the change in germination performance post-chilling, was faster in seeds from 1989 compared to those from 1988 (Pritchard *et al.*, 1996). Such differences in response were ascribed to the 1989 seeds having a higher ceiling temperature for dormancy release ($T_c$ (*D*)) and thus a greater accumulation of chilling units per day of stratification than 1988 seed. It was also suggested that warmer temperatures during the maturation of the seed contributed to the higher $T_c$ (*D*). Consequently, an analysis was made of the variation in temperatures between the four harvest years using data

**Table 1.** *Intercepts and regression coefficients (mean ± SE) for the relationship between germination and stratification time at 6 °C for three seed lots of* Aesculus hippocastanum

Within years, the regression coefficients for the germination temperature response could be justifiably constrained to the same value (*F*-statistics were non-significant; $P = 0.05$). Note that 50% germination equals a probit value of 0.

| Germination temperature (°C) | Intercept (probits) | | |
|---|---|---|---|
| | 1990 | 1991 | 1992 |
| 16 | −4.65 ± 0.19 | −3.68 ± 0.16 | −2.11 ± 0.19 |
| 21 | −3.28 ± 0.15 | −2.89 ± 0.13 | −1.53 ± 0.13 |
| 26 | −1.41 ± 0.11 | −0.58 ± 0.09 | −0.23 ± 0.13 |
| 31 | −0.53 ± 0.11 | −0.21 ± 0.09 | −0.06 ± 0.13 |
| 36 | 0.01 ± 0.09 | 0.64 ± 0.07 | −0.06 ± 0.10 |
| Regression coefficient (probits $d^{-1}$) | 0.0696 ± 0.0028 | 0.0704 ± 0.0027 | 0.0442 ± 0.0022 |
| $r^2$ | 0.91 | 0.92 | 0.91 |
| *F*-statistic | 0.61 | 1.16 | 3.53 |

recorded from a site (Wakehurst Place) close to the seed collection point. Cumulative temperatures above 0 °C were calculated for the main seed filling phase from *c.* 70 d after anthesis (DAA) to *c.* 140 DAA (Tompsett and Pritchard, 1993), i.e. from August until the second week in October, using recorded daily temperature minima. These values were plotted against the chilling time required to increase germination by 1 probit, as shown in Fig. 4. As seasonal temperatures became warmer, rising from 800 to 900 thermal units over the main seed filling phase (which is equivalent to about 1 °C higher minimum temperature per day), stratification times for germination improvement nearly halved to 14 d. The data presented in Fig. 3 thus support the hypothesis that seed developing in relative warm years (e.g. 1990 and 1991) have higher $T_c$ ($D$) values for net dormancy loss than those maturing in cooler years (e.g. 1988 and 1992). In this way, for any given temperature in the chilling range (*c.* 2–11 °C) the warmer season seeds will accumulate more chilling units (thermal time) for dormancy release than cooler season seeds. This means that final germination capacity will be apparently more sensitive to chilling in warm season material (Fig. 3).

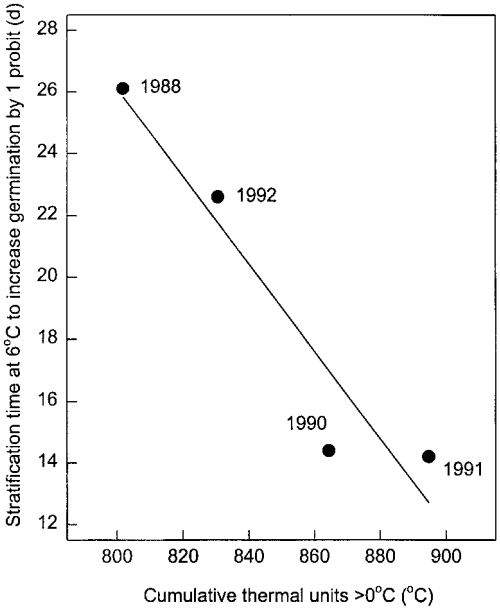


**Fig. 4.** The relationship is shown between the cumulative thermal units above zero that occurred during the 10 weeks prior to maximum seed fall and the time of stratification at 6 °C required to increase germination by 1 probit for *Aesculus hippocastanum* seeds. Material was collected between 1988 and 1992. The regression line through the data is $y = 138.9 - 0.14x$; $r^2 = 0.91$.

### *Influence of stratification on rate of germination*

Stratification at 6 °C not only improved the final level of germination (Figs 2, 3), but the rate of germination also increased. For example, seeds harvested in 1992 took around 6.5, 5 and 4 d to reach 50% germination at 36 °C following 44, 75 and 100 d of chilling (Fig. 5). The linear regression lines were calculated through the reciprocal of the time for a particular percentile to germinate ($1/t(G)$) at each of usually four germination temperatures. For simplicity, three percentiles of the population were chosen: 20, 50 and 80%. From these lines, $T_b$ was calculated for each stratification treatment. As stratification period at 6 °C increased, $T_b$ reduced. For example, for 1992 seed, $T_b$ moved from 16 °C to 8 °C as the chilling period increased from 44 d to 101 d (Fig. 5). The results suggest a systematic reduction in $T_b$ with stratification time, an hypothesis that was tested further by comparison of $T_b$ movement during stratification in seeds from 1990 and 1991.

The value of $T_b$ for each treatment was calculated in the same way as described above, using germination to three percentiles at a minimum of three germination temperatures. Generally 20, 50 and 80% were the chosen percentiles, though in a small number of cases for which germination did not reach 80%, alternative percentiles were used that were representative of the proportion of seeds that did germinate. For example, unstratified seed

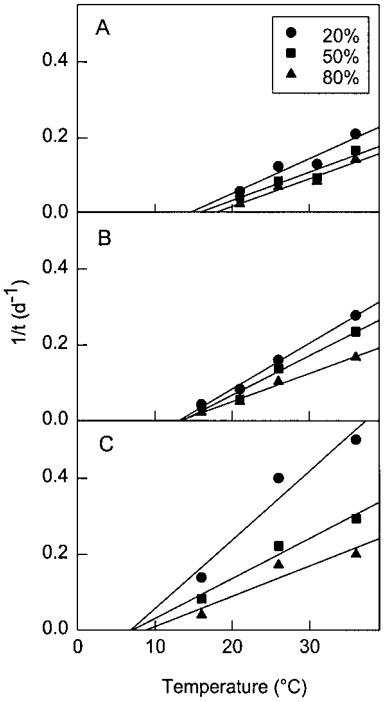


**Fig. 5.** The relationship between reciprocal of time for germination and temperature is shown for *Aesculus hippocastanum* seeds from 1992 stratified at 6 °C for 44 (A), 75 (B) and 101 d (C). Data for 20, 50 and 80% germination are indicated on the figure. Mean ± SE base temperatures for germination rate ($T_b$) are 15.6 ± 0.9 °C (A), 13.3 ± 0.2 °C (B) and 7.6 ± 0.8 °C (C).

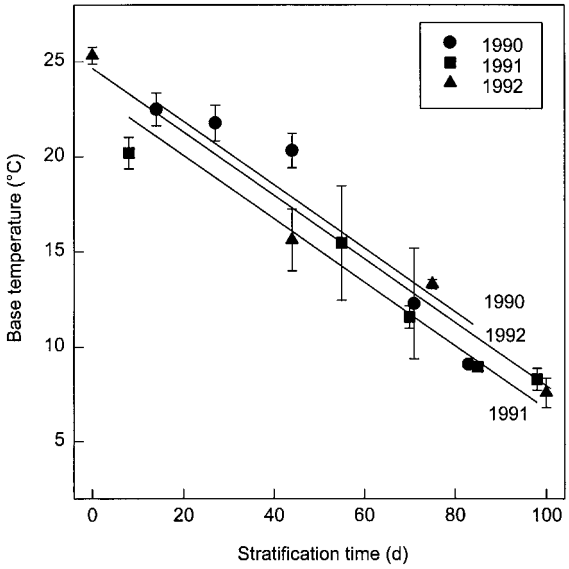


**Fig. 6.** The relationship between base temperature for germination rate ($T_b$) and stratification time at 6 °C is shown for *Aesculus hippocastanum* seeds collected in 1990 to 1992. Bars denote ± SE of the mean. Fitted lines have a common slope of (mean ± SE) 0.167 ± 0.013 °C d$^{-1}$ and intercepts of 25.2 ± 0.9 °C (1990), 23.4 ± 1.0 °C (1991) and 24.6 ± 1.1 °C (1992).

from 1992 only reached 23, 50, 50, and 57% germination at temperatures of 26 °C, 29 °C, 31 °C, and 36 °C, respectively. Thus, it was only possible to evaluate the regression lines for a limited range of population fractions. In this case, the time for the population to germinate to 3, 10, 20, and 35% was calculated from the raw germination data.

It is commonly assumed that $T_b$ is a single value and the error of its estimation is not usually reported (Covell *et al.*, 1986; Gummerson, 1986; Bradford *et al.*, 1993; Ellis *et al.* 1987*a*, *b*). However, $T_b$ varied between fractions of the horse chestnut seed population (Fig. 5). The standard error around the mean was between 0.2 and 3.0 °C amongst the 14 estimated values for $T_b$ displayed in Fig. 6, though there was no evidence in the present data of a sequential change in $T_b$ across the sub-populations. These observations are in agreement with Phelps and Finch-Savage, who found that $T_b$ varied amongst sub-populations of tomato and cabbage seeds (Phelps and Finch-Savage, 1997). For simplicity, the small variation in $T_b$ observed for horse chestnut was not included in further analysis of the data.

The pattern in the shift in $T_b$ with stratification at 6 °C is presented in Fig. 6. A similar response was observed between years such that $T_b$ decreased by 1 °C every 6 d of chilling up to around 100 d of treatment. Constraining the lines to be parallel was not significantly different to a free fitting ($F = 1.78$). However, the initial $T_b$ in the seeds harvested in different years was estimated to vary slightly, from 25.2 °C in 1990, to 23.4 °C in 1991, and 24.6 °C in 1992. These estimates are close to the lower limit identified for the high temperature germination response of around 26 °C (Fig. 2). Although some germination at 26 °C was observed in non-chilled seed from 1991 (Fig. 2), seedlots from three other years (1983, 1988 and 1989) exhibited no germination at this temperature unless the seeds were pre-chilled (Tompsett and Pritchard, 1993). This apparent contradiction in responses could have many causes. Non-conformity of the germination response to a predictive germination rate model were observed in sweet chestnut seeds held within 2 °C above $T_b$ (Pritchard and Manger, 1990). In the case of horse chestnut, however, failure of freshly harvested seed to germinate at just above $T_b$ could relate to the ageing kinetics of the seeds in hydrated storage, which become progressively faster as temperature is raised from 11 °C to 21 °C (Steadman, 1997). The ageing effect is presumably exacerbated at even higher temperatures, meaning that in some years the seeds could have aged before slow germination could be completed at 26 °C.

Dormancy in horse chestnut seed at low and intermediate temperatures can be understood in terms of $T_b$. The systematic reduction in $T_b$ with stratification enables the seeds to germinate at progressively lower temperatures, and explains the widening in the temperature response in Fig. 2. Dormancy release at 6 °C can be

described in a single equation [1] in terms of reduction of $T_b$ from the starting value of newly harvested seed at maximum seed fall for that seed lot ($T_{b\,SF}$) by stratification for $t_s$ days, thus

$$T_b = (-0.176t_s) + T_{bSF} \quad (1)$$

At the time of natural seed fall, $T_b$ is approximately 25 °C, so seeds can germinate at non-lethal temperatures above this value, resulting in the high temperature response (Fig. 2). As chilling at 6 °C continues, $T_b$ decreases, and so gradually lower temperatures become permissive for germination of the seed population. Seeds placed at 6 °C for germination (i.e. not moved after stratification) will not germinate until $T_b$ is below 6 °C. From the quantified response of seeds in 1990, 1991 and 1992, it is predicted that between 104 and 115 d of stratification at 6 °C, depending on year of seed development, are necessary for $T_b$ to reach 6 °C. Germination progress curves for the low temperature response for each of the three years indicate that first emergence is observed in germination tests at 6 °C soon after this period (Fig. 7).

Bradford proposed that chilling-induced dormancy release may be effectuated by a reduction in the hydrotime constant for germination and by a shift in the base water potential for germination to more negative values (Bradford, 1996). This study did not determine the hydrothermal time sensitivity of horse chestnut seeds. However, the results in Fig. 6 indicate that, for this material, it is possible to characterize the dormancy-breaking response in terms of reducing $T_b$ alone. Thus, seed germination at low temperatures is achieved by the sequential removal of dormancy, facilitated by a lowering of $T_b$, until the stratification temperature becomes permissive for germinative growth *per se*.

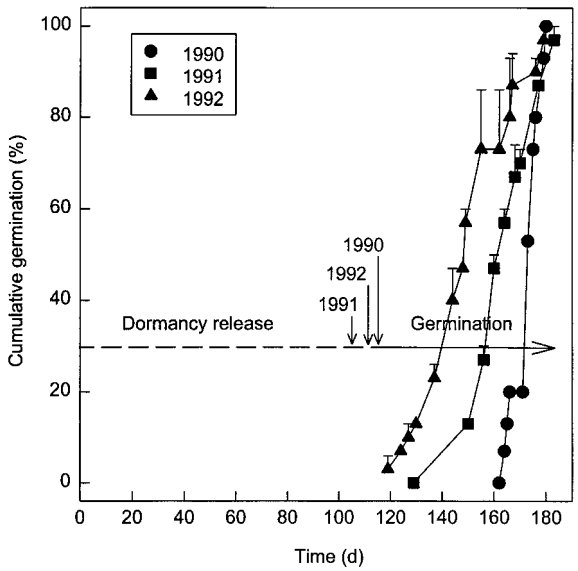


**Fig. 7.** Cumulative germination progress curves for *Aesculus hippocastanum* seeds collected in 1990–1992 and held at constant 6 °C. The base temperature is estimated to have been reduced to 6 °C after 104–115 d (indicated by vertical arrows), after which germination progresses. Bars denote ±SE of the mean.

In crop seed it is often difficult to separate the culmination of dormancy release from the initiation of visible germination (Cohn, 1996). However, Fig. 7 shows that it is possible to separate these two physiological processes quantifiably in horse chestnut. It appears that the process of dormancy release is complete by *c.* 104–115 d and is then separated from visible germination by a lag phase of ≥7 d (Fig. 7). The results support the argument that dormancy release should be clearly distinguished from the germination process itself (Vleeshouwers *et al.*, 1995; Hilhorst, 1997).

## Conclusions

Freshly harvested populations of horse chestnut seed are capable of germinating to a certain extent at high temperatures (31 °C and 36 °C) and to full capacity at low temperatures (2–6 °C). Variable, but generally very low germination at intermediate temperatures (16–26 °C) apparently defines the temperature limits for conditional dormancy. Chilling at 6 °C increases the capacity (total and rate) of the seeds to germinate at all temperatures from 11–36 °C. However, the sensitivity of the seed populations to chilling varies considerably between years, being greater in years when temperatures during seed filling are warmer by as little as 1 °C on the daily minimum temperature. This implies that the thermal history of the seeds on the parent tree influences their subsequent response to dormancy breaking treatment.

Based on studies on three seed populations, the estimated base temperature for germination, $T_b$, in freshly harvested seeds is close to 25 °C. $T_b$ reduces by 0.17 °C per day of chilling at 6 °C so that $T_b$ reaches the chilling temperature after *c.* 110 d. Following this dormancy release phase, visible germination at 6 °C occurs after a further lag of >7 d. These studies indicate that conditional dormancy exists in horse chestnut seeds at low temperatures, but that it is systematically alleviated by chilling.

In this report dormancy release in horse chestnut at one temperature has been quantified and it has been shown how it relates to cardinal temperatures for physiological processes. A further report will consider the effects of other dormancy-breaking temperatures and integrate the relative effects of different temperatures into a consolidated, predictive model for the response of these seeds during both cold stratification and the germination phase.

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