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Research

Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe

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Summary

- An analysis was made of seed traits along a north–south gradient spanning 19° of latitude in Europe using *Aesculus hippocastanum*, which originates from Greece and the Balkans and has been introduced throughout Europe.
- Because summer temperatures decrease with increasing latitude in Europe, we tested the hypothesis that the heat sum during seed development influences physical, physiological and biochemical seed traits.
- Seeds from Greece (within the natural range) had a fresh mass five times higher than those from Scotland (most northerly seed lot), and a lower axis moisture content and solute potential. In addition, Greek seeds germinated at cooler temperatures, and were more desiccation tolerant. Principal component analysis (PCA) showed that the observed patterns in seed characteristics were consistent and predictable: a single PCA axis explained 86.6% of the variation in the dataset. This axis correlated strongly with the heat sum accumulated by the seeds during development.
- The results support the notion that seeds from further north were shed less developed as a consequence of cooler temperatures during development, and provide a quantitative explanation for intraspecies variability in recalcitrant seed traits for this and, possibly, other species.

Key words: *Aesculus hippocastanum*, desiccation, heat sum, seed germination, thermal time.

Introduction

The effects of the parental environment during seed development on seed mass, germinability and dormancy are well known for a wide range of species (Fenner, 1991; Wulff, 1995; Gutterman, 2000). However, whilst interseasonal or intersite differences in seed lot response to desiccation have been recorded in some recalcitrant seeded species (Tompsett & Pritchard, 1993; Finch-Savage & Blake, 1994) a relationship to measured environmental conditions has not been explored. Here we assess the effects of air temperature during seed development on seed responses of the recalcitrant seeded species *Aesculus hippocastanum.*

Air temperature during development can affect seed dormancy: dormancy levels are typically inversely related to the heat sum (°C d) accumulated during development, for both herbs (Fenner, 1991) and woody perennials, such as *A. hippocastanum* (Pritchard *et al.*, 1999). However, other seed characteristics may also be influenced by temperature. In a number of species, seed or fruit size is positively related to increasing air temperatures during development (Tompsett & Pritchard, 1993; Stanley *et al.*, 2000). Furthermore, seeds of late-maturing annuals may be shed at an earlier developmental point (i.e. with smaller, less developed embryos), as a consequence of a reduced developmental heat sum (Wagner & Mitterhofer, 1998).

During development, orthodox seeds pass through three distinct phases: embryogenesis, active biosynthesis of reserve material leading to a rapid increase in seed fresh and dry weight, and seed maturation when dry weight accumulation ceases and fresh weight declines markedly (in dry dehiscent fruit types) or remains relatively constant (in fleshy fruit types) (Adams & Rinne, 1980; Ellis *et al.*, 1987a; Welbaum & Bradford, 1989). The transition from the second to the third phase coincides approximately with the acquisition of desiccation tolerance (Kermode & Bewley, 1985; Ellis *et al.*, 1987a; Welbaum & Bradford, 1989). Physiological evidence suggests that seeds of many recalcitrant species do not complete the second phase of development before fruit abscission: seed dry mass increases right up to the time of peak seed fall, for example in *A. hippocastanum* (Tompsett & Pritchard, 1993). During seed development in this species, axis water potential and seed moisture content decline to *c.* −1 to −1.5 MPa and 50%, respectively, by the time of seed shed (Farrant & Walters, 1998; Tompsett & Pritchard, 1998). Taken together, this evidence suggests, at least for *A. hippocastanum*, that seed dry mass, axis water potential and axis water content at the time of seed shed may be a measure of seed developmental status (i.e. how far seeds have progressed in phase II of development).

For recalcitrant species, an effect of developmental status on seed desiccation tolerance is well known. For example, desiccation tolerance has been shown to increase with development, albeit not to the same extent as orthodox species, for seeds of *Acer pseudoplatanus* (Hong & Ellis, 1990; Dickie *et al.*, 1991), *A. hippocastanum* (Tompsett & Pritchard, 1993; Farrant & Walters, 1998), *Camellia sinensis* (Berjak *et al.*, 1993) and *Landolphia kirkii* (Pammenter *et al.*, 1991). Between year variations in recalcitrant seed desiccation tolerance for material collected from the same site have also been recorded (Tompsett & Pritchard, 1993; Finch-Savage & Blake, 1994). However, it is not clear what factors cause these interannual differences.

A. hippocastanum originates from Greece and the Balkans and has been introduced, in the last *c.* 400 yr, throughout Europe (Howard, 1945). There have been a number of past studies on seed development in recalcitrant species, including *A. hippocastanum* (as already described). However, these have all focused on seed development, at one location, through time. In comparison, this study aimed to test whether climate, at widely differing locations, can affect seed developmental status and consequently desiccation tolerance. To test this proposition, seeds of *A. hippocastanum* were sampled from five distinct sites within Europe with a south–north gradient of *c.* 19° latitude. This gradient included the natural range of this species (Greece) and naturalised populations from further north. Along this transect, it was predicted that seed developmental status, at seed shed, would decrease as a consequence of cooler temperatures. In this study, interpopulation genetic differences could potentially confound any apparent environmental effects on seed quality. However, this is unlikely with *A. hippocastanum* because its comparatively recent introduction throughout Europe (*c.* 400 yr) means that naturalised populations may only have been through 10 or fewer generations and hence have had little opportunity for either genetic drift or directional selection for the seed traits in question. Consequently, any patterns of seed response along this climate gradient are likely to reflect seed responses to environmental conditions rather than genetic change among populations.

Materials and Methods

Seed lot details

Seeds were collected from various European locations (Table 1) in September/October 2002 and sent by air-freight to Wakehurst Place, UK. Seeds were collected from a minimum of five trees per location and dispatched within 3 d of collection. To allow a comparison of seed development times and environmental conditions during development, the timing of flower fall was recorded at each seed location. This

Table 1 Some characteristics of the five seed lots of *Aesculus hippocastanum* studied

		Seed fall/ collection	Degree days	Fresh mass	Moisture content (% f. wt)		Whole seed dry	Axis osmotic potential
Seed lot	End of flowering	date	(°C d)	(g)	Embryonic axis	Whole seed	mass (g)	(MPa)
Aberdeen, Scotland (57°10′ N 2°04′ W)	6/6/02	12/10/02	1666.1	3.5 ± 0.2^{a}	76.6 ± 1.5^{a} (73.5 ± 1.4)	69.0 ± 1.9^{a} (68.4 ± 2.5)	1.2 ± 0.1^{a} (1.16 ± 0.5)	-2.1 ± 0.1
Sowiniec, Poland (52°25′ N 16°53′ E)	15/5/02	15/9/02	2205.4	13.8 ± 0.5^{b}	64.9 ± 0.8^{b}	46.6 ± 0.6^{b}	7.4 ± 0.2^{b}	-2.3 ± 0.0
Wakehurst Place, England (51°03′ N 0°06′ W)	31/5/02	26/10/02	2158.2	15.5 ± 0.5^{c}	69.2 ± 0.8^{c}	49.0 ± 1.0^{c}	7.8 ± 0.2^{b}	-2.4 ± 0.0
Angers, France (47°9′ N 0°32′ W)	9/5/02	21/9/02	2396.5	16.2 ± 0.4^{cd}	60.7 ± 0.8^{d} (58.8 ± 0.8)	42.3 ± 0.5^{d} (45.5 ± 0.7)	9.4 ± 0.3^{c} (9.9 ± 0.64)	-2.7 ± 0.1
Mt Parnes, Greece (38°10′ N 23°45′ E)	25/5/02	17/10/02	2583.7	17.1 ± 0.3^{d}	59.7 ± 0.2^{d}	41.9 ± 0.2^{d}	9.9 ± 0.2^{c}	-3.0 ± 0.2

Figures in brackets refer to results of a small scale preliminary study conducted in 2001 using seeds from Scotland and France. Values are ± 1 SE of the mean. Values within the same column were compared by One-way ANOVA, followed by Fisher's LSD test. Those followed by the same letter are not significantly different ($P > 0.05$).

date was taken as the first day on which all flowers had fallen. Freshly fallen (i.e. naturally dispersed) seeds were collected from the ground and the date of seed collection was taken as the end of development. At each location, a data logger (Tiny Data Logger, Gemini Data Loggers Ltd. Chichester, UK) was used to continuously record air temperature (every 30 min) throughout the entire seed development period (i.e. from the end of flowering to seed shed). The loggers were located in the shade so that they recorded actual air temperature. For the period of seed development, the heat sum (°C d; assuming a base temperature of 0°C) was calculated as follows:

$$\text{Heat sum} = \{\sum[\text{temperature at logging interval} \times \text{logging interval (h)}]\}/24 \qquad \text{Eqn 1}$$

Before shipping, seeds were held at ambient temperatures and following receipt at 15°C, which has previously been shown to have little impact on the dormancy status of seeds from England (Pritchard *et al.*, 1996). Germination and desiccation studies were commenced within 3 d of seed receipt. Upon receipt, the moisture content of the component parts (seed coat, cotyledons and embryonic axis) of the seeds was determined for 25 individual seeds by drying at 103°C for 17 h (ISTA, 1999). In addition, the fresh weight of 150 individual seeds, per batch, was determined.

The osmotic potential of fully hydrated embryonic axes ($n = 2 \times 5$) from seeds of the different provenances was measured using a WP4 dewpoint potentiameter (Decagon Devices, Pullman, WA, USA) operated in a temperature controlled room (*c.* 21 ± 1°C). Before measurements, excised axes were hydrated by placing them on the surface of 1% agar in water at 21°C for 24 h. Axes were then sealed in 1.8 ml Nalgene cryovials (Nalge Company, Rochester, NY, USA) followed by rapid plunging into liquid nitrogen (Boyer, 1995) for 5 min to ensure complete freezing of the axis tissue. The water potential of the axes was measured once the axes had thawed and reached 21°C. Because when the turgor pressure is zero, that is post freezing, the tissue water potential is equal to the osmotic potential this value was taken as the osmotic potential of the tissue (Boyer, 1995).

Seed germination

Due to space constraints, as a result of the large seed size of this species, and the concurrent investigation of five seed lots, two replicates of 15 (Scotland, Southern England, France, Poland) or 10 (Greece) seeds each were sown on the surface of 1% agar in water in sandwich boxes (6 × 11 × 17 cm). Although this involves comparatively low numbers of seeds per treatment this approach has been successfully applied in other studies on large seeds (Finch-Savage *et al.*, 1992; Tompsett & Pritchard, 1993, 1998). Sandwich boxes were wrapped in light proof bags and incubated at a range of temperatures between 5 and 40°C (5, 10, 15, 20, 25, 30, 35 and 40°C). Germination was defined as radicle emergence by at least 10 mm (Pritchard *et al.*, 1996) and germination was scored every 4–5 d.

Seed desiccation tolerance

Seeds were dried at *c.* 15°C and 15% rh for up to 96 h and sampled at intervals of 8 h (Southern England, France, Poland and Greece) and 6 h (Scotland). At each sample time the moisture content of the embryonic axis was measured for 10–15 seeds and two replicates of 15 (Southern England, France and Poland) or 10 seeds (Scotland and Greece) were germinated at 35°C.

The relationship between embryonic axis gravimetric water content and water potential was determined by drying excised axes at *c.* 15°C and 15% rh for up to 36 h. Axes (2 × 5) were periodically removed and sealed in 50 mm Petri dishes with parafilm and allowed to equilibrate for 18 h at 21°C before water potential determination using a WP4 dewpoint potentiameter. Following water potential determination, axes were dried at 103°C for 17 h for moisture content determination (ISTA, 1999).

Statistical analysis

One-way ANOVA on nontransformed data, followed by Fisher's LSD test, was used to test for differences in seed traits (mass and moisture content) between the five locations.

The seed germination response to temperature has been described using a thermal time approach (Garcia-Huidobra *et al.*, 1982). In this model, seeds accumulate $(T_g - T_{bg})t_g$ units of thermal time when subjected to temperatures T above a base temperature T_b but below an optimum T_o, where t_g is the time since the start of germination. When the thermal time accumulated has reached the critical value (θ_{Tg}) for cumulative fraction g of the population, germination occurs. Thus the thermal time requirement for fraction g of the population can be described as:

$$\theta_{Tg} = (T_g - T_b)t_g \qquad \text{Eqn 2}$$

Within a seed population there is variance in germination times and some authors have reported that at suboptimal temperatures the variation in germination times results from a normal or log-normal distribution of the thermal time required for germination of the whole population (Covell *et al.*, 1986; Ellis *et al.*, 1986, 1987b). Furthermore, the base temperature for germination is generally assumed to be constant (i.e. $T_{bg} = T_b$). The minimum temperature at which germination could occur, for the various seed lots of *A. hippocastanum*, was determined by plotting germination percentage (on a probit scale) against log-thermal time [$\log_{10}(T - T_b)t_g$], where T_b is unknown and estimated by changing the value of T_b until the minimum residual variation is obtained (Ellis *et al.*, 1987b). However, for the Scottish seed lot it was not possible to conduct this analysis because of the low number of germinated seeds. Log-thermal time was used rather than thermal time, because for all seed lots this approach yielded the highest R^2. On a plot of probit(germination) against log(thermal time), the median thermal time required for seed germination of the population corresponds to the thermal time when probit(germination) = 5. The inverse of the slope of the fitted line is the standard deviation of the population responses to thermal time (i.e. the flatter the slope of the fitted line the greater the variation in response to thermal time between individual seeds).

To test whether the water potential isotherms for the seed lots were different, either two separate linear regression lines or a single regression line were fitted to the data for the two extreme seed lots in the population (i.e. Scottish and Greek) using GenStat for Windows, Version 5 (VSN International Ltd, Oxford, UK). Subsequently the significance of the change in residual deviance when using a single or two regression lines was tested using an *F*-test (Sokal & Rohlf, 1995).

The relationship between germination percentage (y) and axis water potential for viability loss (x) was explored, for each seed lot, using Probit analysis implemented in GenStat 5. This approach assumes that each individual seed is a statistically independent unit and hence a replicate. Probit analysis is frequently used for analysing mortality curves and enables calculation of the lethal dose (LD_{50}; in this case the median water potential for axis viability loss) and its standard error. For the Scottish and English seed lots where mild desiccation resulted in an improvement in germination percentage, the initial points before the upturn in germination were excluded from the fit.

Principal component analysis (PCA) was used to explore the intercorrelation among seed lot characteristics to determine whether responses could be reduced to one or more axes of response. Whole seed moisture content, whole seed dry mass, axis solute potential, base temperature for germination and the median water potential for axis viability loss were included in the analysis. Only one variable each, for seed moisture content and dry mass, was included (i.e. fresh mass and axis moisture content were excluded) to prevent the analysis being biased towards these variables. Subsequently, the seed lot scores on the resulting axes of variation were correlated with the heat sum during seed development to test whether this independent variable was able to explain the observed patterns of response.

Results

Seed size and moisture content

Seeds that developed under warmer conditions were significantly larger (in terms of both fresh and dry mass: One-way ANOVA, $P < 0.05$) and had significantly lower (One-way ANOVA, $P < 0.05$) moisture contents at shedding than seeds that developed under cooler climatic conditions (Table 1). Seeds from warmer conditions also had embryonic axes with more negative osmotic potentials than those from cooler locations (One-way ANOVA, $P < 0.05$; Table 1).

Seed germination and dormancy

Figure 1 indicates that for all the seed lots, germination at constant temperatures was maximal at 30–35°C; germination was reduced at temperatures higher or lower than this optimum. In addition, for the Greek seeds, and to a lesser extent the French seeds, at least part of the seed population was able to germinate at cooler temperatures than the other seed lots. Thus, some seeds of the Greek seed lot germinated at 15°C: no seeds of the Polish, English or Scottish populations germinated at 20°C.

The minimum temperature for germination (T_b) of the seed lots increased in the following order: 19.0, 21.9, 23.1, 23.9°C for the Greek, French, Polish and English seeds, respectively (Table 2). However, it should be noted that a single value of T_b is not appropriate for all seeds within each population, that is there was a narrow range of values of T_b within each population (Fig. 2), which the mathematics of the thermal time equation are unable to account for. While it was not possible to calculate T_b for the Scottish seeds (see the Materials and Methods section), T_b is likely to be between 20 and 25°C because some seeds germinated at 25°C: none germinated at 20°C (Fig. 2). Seeds of the Greek population also had a greater spread in the thermal time requirements for germination than the other seed lots (standard deviation of the thermal time for germination of log 0.687°C d compared with log 0.387, 0.419, and 0.489°C d for the English, Polish and French seeds, respectively) (Fig. 2).

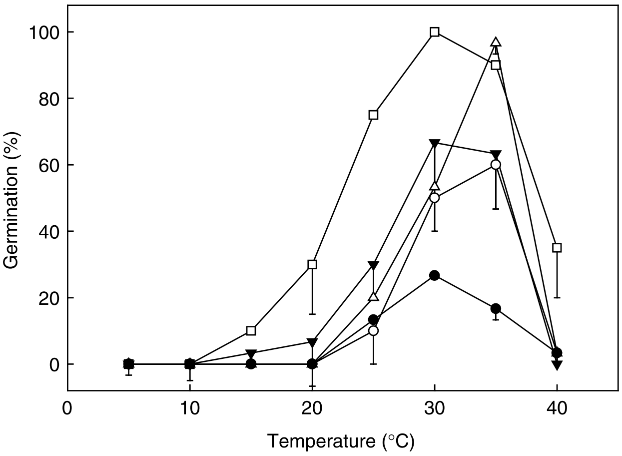

Fig. 1 The effect of constant temperatures on the germination percentage (after 12 wk) of seeds of five seed lots of *Aesculus hippocastanum* (Scotland, closed circle; England, open circle; France, closed triangle; Poland, open triangle; Greece, square). Error bars are shown as – 1 SE of the mean unless smaller than the symbol.

Table 2 The base temperature for germination, and constants describing the relationship between probit(germination) and log(thermal time) (°C d) for seeds of *Aesculus hippocastanum* in the equation probit(germination) = A + B[$\log_{10}$(thermal time)]

Seed lot	Base temperature (°C)	A	B	R^2	*P*-value	Average October air temperature (°C) Max.	Min.
Scotland	Not done	–	–	–	–	17.1	0.1
Poland	23.1	−0.441	2.387	0.655	< 0.05	20.8	−1.3
England	23.9	−0.855	2.587	0.833	< 0.05	19.5	−1.8
France	21.9	0.104	2.044	0.623	< 0.05	23.8	−2.2
Greece	19.0	2.034	1.456	0.676	< 0.05	29.7	10.5

The average maximum and minimum air temperatures in October (the main month of seed shed) are included for the nearest meteorological station to the collection site (from Meteorological Office, 1983).

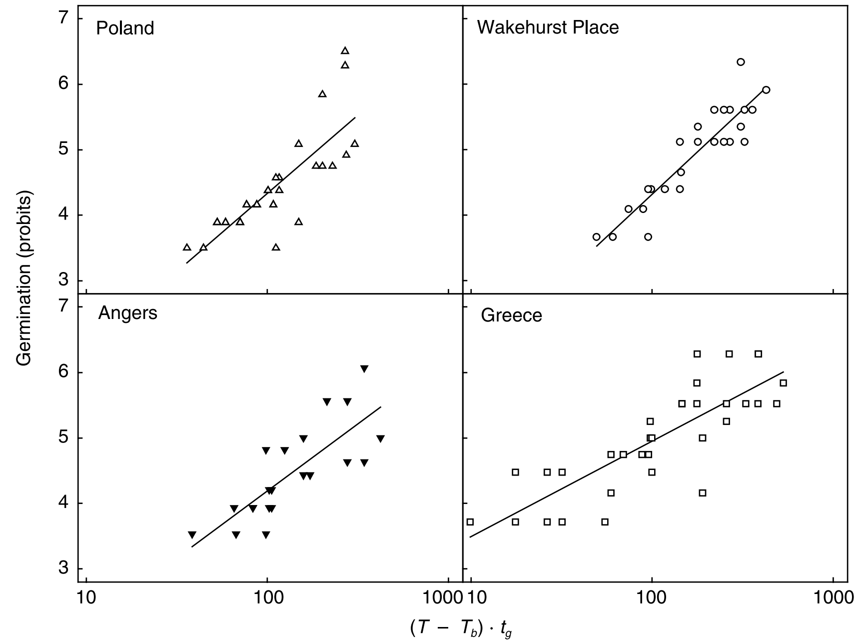

Fig. 2 Thermal time requirements (log scale) for germination of four seed lots (excluding Scotland) of *Aesculus hippocastanum* assuming base temperatures for germination of 19.0, 21.9, 23.1 and 23.9°C for Greek, French, Polish and English seed lots, respectively.

Seed desiccation sensitivity

For all seed lots there was a similar, linear relationship between log-axis water potential and log-water content (Fig. 3). The fitted lines for the Scottish and Greek seed lots were significantly different ($F_{1,24} = 5.92$, $P < 0.05$): for a given axis water potential, the Scottish seeds had a higher axis water content than the Greek seeds. The equations from linear regression of the data for each seed lot (Table 3) were subsequently used to convert the axis moisture content values in the desiccation screen to water potential.

Desiccation of all five seed lots resulted in seed death, with all seeds killed by axis water potentials as low as −30 MPa (Fig. 4). However, the median axis water potential at which mortality occurred differed between seed lots. Median values (± 1 SE) were −5.1 ± 0.65, −9.2 ± 0.53, −10.5 ± 0.41, −12.8 ± 2.1 and −16.2 ± 0.83 MPa, for Scottish, Southern English, Polish, French and Greek seed lots, respectively. In addition, partial desiccation to *c.* − 3.5 MPa resulted in an increase in germination for the Scottish and English seed lots.

Table 3 Constants describing the relationship between water potential (MPa) and water content (percentage water on a fresh weight basis) for axes of *Aesculus hippocastanum* in the equation $\log_{10}$(water potential) = A + B[$\log_{10}$(water content)]

Seed lot	A	B	R^2	*P*-value
Scotland	0.765	−1.346	0.983	< 0.001
Poland	0.714	−1.238	0.956	< 0.001
England	0.729	−1.410	0.957	< 0.001
France	0.722	−1.361	0.991	< 0.001
Greece	0.685	−1.300	0.970	< 0.001

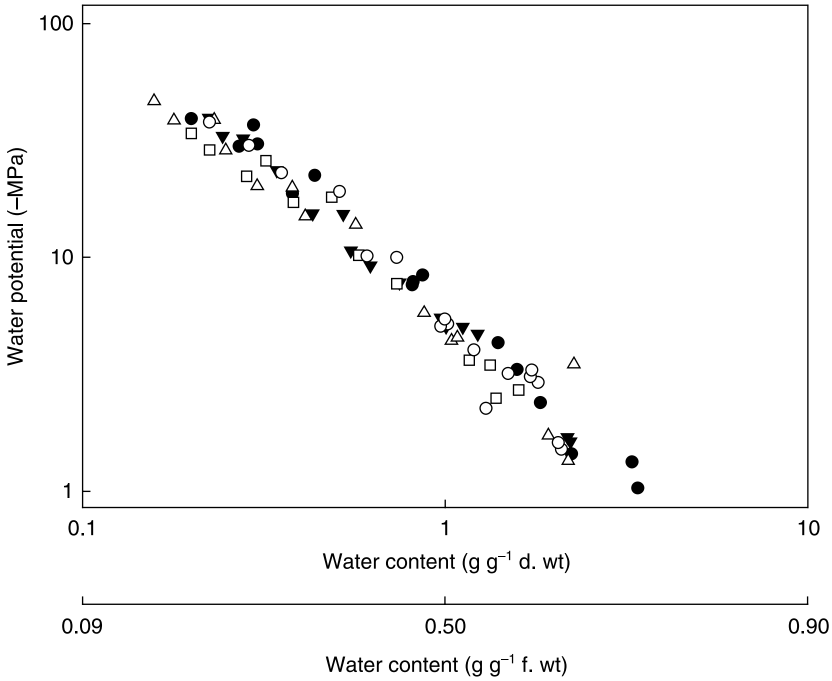

Fig. 3 Water potential isotherms illustrating the relationship between water potential of embryonic axes of five seed lots of *Aesculus hippocastanum* (Scotland, closed circle; England, open circle; France, closed triangle; Poland, open triangle; Greece, square).

Principal component analysis of seed traits

PCA axis I had an eigenvalue of 4.330 and explained 86.6% of the variance in the five seed characteristics. The seed lots were arranged along this axis as follows with seed lots' axis I scores: Scotland (3.847), England (−0.098), Poland (−0.141), France (−1.413) and Greece (−2.196). In addition, seed lot scores on axis I were significantly correlated with heat sum during development (Pearson product-moment correlation; $R = 0.967$, d.f. = 3, $P < 0.01$).

Discussion

In *A. hippocastanum* populations across Europe, there was a relationship between the heat sum during development and seed size, water relations, dormancy, germination rate and desiccation sensitivity. This suggests that environmental conditions can affect a range of seed traits in recalcitrant species, and potentially provides an explanation for the well recorded seed lot variability in desiccation sensitivity for many recalcitrant species.

Seed size and water relations

There was a positive association between developmental heat sum and seed mass, and a negative association with seed moisture content and axis osmotic potential. The growth of recalcitrant seeds is 'indeterminate', that is development continues right up until seed shed and seeds do not undergo a period of rapid desiccation at the end of development (Finch-Savage *et al.*, 1992; Tompsett & Pritchard, 1993; Finch-Savage & Blake, 1994). This suggests that seeds of *A. hippocastanum* from warmer regions were more developed at the time of natural seed shed, presumably because temperature affects the rate of physiological processes such as assimilate import into developing plant organs (Farrar, 1988; Wardlaw, 1990). The effect of temperature on limiting the effective developmental period is reinforced by the large number (*c.* 70%) of nonviable seeds in the Scottish seed lot. Such seeds were typically empty or had underdeveloped cotyledon tissue, which failed to fill the whole volume enclosed by the testa. Similarly, in the annual alpine species *Gentianella germanica* seeds developed under cooler conditions had less mature embryos than those that developed under warmer conditions (Wagner & Mitterhofer, 1998).

The more negative osmotic potential of seeds from warmer conditions suggests these seeds had accumulated more soluble storage compounds. For recalcitrant seeds of both *A. hippocastanum* and *Quercus robur*, axis water potential decreases during seed development (Finch-Savage *et al.*, 1992; Farrant & Walters, 1998). Thus, the lower axis osmotic potential of seeds from Greece also implies seed development progressed further than for the other seed lots.

The observed patterns of response may result from looking at seeds in only 1 yr. However, in our smaller scale preliminary study conducted in 2001, with seeds from Aberdeen, Scotland and Angers, France, similar trends of seed mass and moisture content were observed (Table 1).

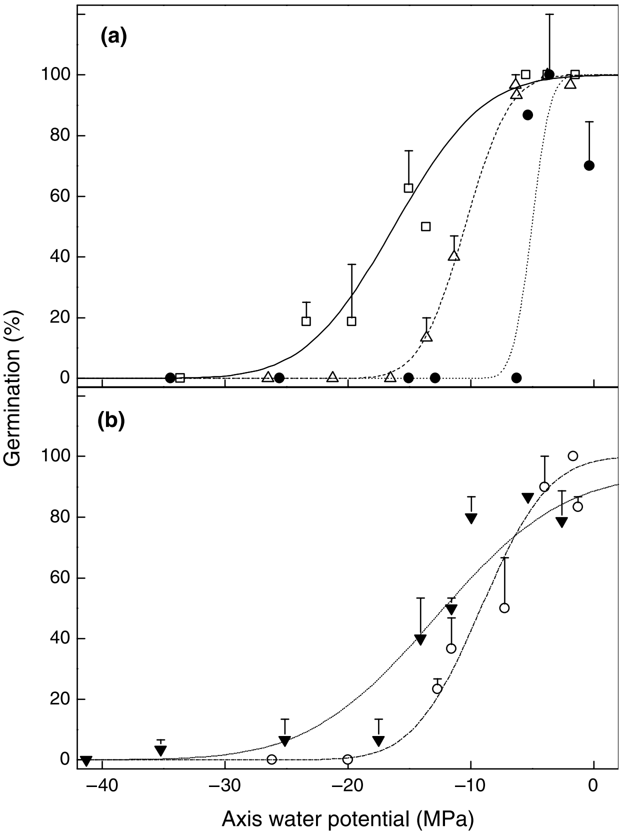

Fig. 4 (a,b) The relationship between germination percentage and embryonic axis water potential for seed lots of *Aesculus hippocastanum* ((a) Scotland, closed circle; Poland, open triangle; Greece, square, and (b): England, open circle; France, closed triangle), dried at 15°C and 15% rh as whole seeds. Error bars are + 1 SE of the mean. Lines were fitted using the parameters derived from Probit analysis. The initial data points, before the upturn in germination for the Scottish and English seeds, were excluded from the fit. Median water potentials for axis viability loss were determined from the equations of the fitted lines. For clarity, maximum germination percentage for the Scottish seed lot is scaled to 100%.

Seed dormancy

Seeds from more southerly locations were able to germinate at cooler temperatures, that is had a lower base temperature for germination (T_b) than those that came from further north. Tompsett & Pritchard (1993) reported that seeds of *A. hippocastanum* from southern England are shed dormant. Dormancy is broken by cold stratification and is reflected in a lowering of T_b as dormancy is lost, facilitating germination at progressively cooler temperatures (Pritchard *et al.*, 1999; Steadman & Pritchard, 2004). In addition, Tompsett & Pritchard (1998) found that partial desiccation from *c.* 50% to 32–40% moisture content also promoted germination. The effects of drying and cold stratification on *A. hippocastanum* seed germination appeared interchangeable and were interpreted as facilitating a continuation of the maturation processes that occur naturally on the parent tree (Tompsett & Pritchard, 1998). In this study, the effect of partial drying on germination was only observed for the Scottish and English seed lots (Fig. 4) which may reflect their being shed less developed than the remaining seed lots. In addition, the lower T_b for the more southerly material supports the view that developmental processes, before shedding, were able to continue further. This relationship between climate and dormancy has been reported for a wide range of orthodox species: warmer climatic conditions typically result in lower dormancy levels (see Fenner, 1991 and references therein).

Seeds of *A. hippocastanum* are unlikely to germinate in the autumn in the UK: temperatures at the time of seed shed rarely reach *c.* 24°C (the minimum temperature at which germination can occur). Consequently, germination occurs in the spring when the temperature window for germination has been widened by chilling (Pritchard *et al.*, 1999). However, for the Greek seeds, T_b was estimated to be *c.* 19°C and some seeds in the population were able to germinate below this calculated value (Fig. 1). The average maximum monthly temperature, in October, close to the site in Greece is 29.7°C (Meteorological Office, 1983, Table 2). Thus, the lower T_b and the lower thermal time requirement for germination, at least for part of the population, suggests that germination of the Greek seed lot (and to a lesser extent the French seed lot) may occur in autumn coincident with the autumn rains. A possible advantage of this strategy would be that seedlings are able to reach as large a size as possible before the onset of summer, thereby reducing drought induced seedling mortality. This possibility is supported by the observation that seeds of the related species *Aesculus californica*, which occurs in Mediterranean-type vegetation in California, USA, germinate in autumn after the start of the rains (Suszka, 1966). However, further studies are required to test whether this occurs for *A. hippocastanum* in Greece and to investigate the ecological implications of these possible differences in the timing of germination.

Desiccation sensitivity

Seed desiccation sensitivity, as measured by the median water potential for viability loss, increased with increasing air temperatures during development (Fig. 4): the median water potential for viability loss ranged from −5.6 to −15.6 MPa. Five discrete levels of critical water potential for seed viability loss have been suggested [−1.8, −5, −12, −50 and −180 MPa (Walters, 1998); −1.5, −4, −12, −23 and −75 MPa (Sun & Liang, 2001)]. The range of water potentials identified in this current study do not neatly fit into these categories. This may partly result from the use of different methodologies in the two studies: in many cases Sun & Liang (2001) and the studies cited in Walters (1998) determined the effect of desiccation on excised embryos, whereas we desiccated whole seeds. Nonetheless, this current study suggests that critical water potentials vary continuously in relation to developmental age and post harvest handling rather than falling within discreet categories. This is supported both by the finding that chilling progressively increases the critical water potential for the onset of desiccation stress in seeds of *A. hippocastanum* (Tompsett & Pritchard, 1998) and the work of Dussert *et al.* (2000) who reported that the critical water activity for viability loss of nine *Coffea* species varied from 0.293 to 0.920.

We believe that this is the first report of a link between climatic conditions across a continent, during development, and the relative level of seed desiccation sensitivity within a single species. An increase in desiccation tolerance during development has been demonstrated previously, for a wide range of recalcitrant species (as already described). Thus, our results, coupled with this known effect, suggest that the increasing desiccation tolerance of seeds that developed under warmer conditions results from development progressing further before seed shed. Finch-Savage & Blake (1994) reported interannual variation in seed characteristics of *Q. robur*, such as mass, moisture content and desiccation sensitivity. In years where seeds were shed with a lower moisture content and with a higher level of desiccation tolerance, this may have reflected faster development before precocious shedding. Thus, in species such as *A. hippocastanum* and *Q. robur* with recalcitrant seeds and a long development period (*c.* 150 d), termination of the growing season may result in the truncation of seed development at different stages in different years as a result of interseasonal variations in climate. Thus, the current findings with *A. hippocastanum* suggest that the greater desiccation sensitivity of seeds from further north is a consequence of a relative reduction in the development period. The Greek seed lot tested here had a higher level of desiccation tolerance than previous seed lots of *A. hippocastanum* investigated (Tompsett & Pritchard, 1993, 1998; Farrant & Walters, 1998), presumably as a consequence of the seeds in these studies having developed in climates cooler than the natural range (i.e. Southern England and Fort Collins, USA compared with Greece). Therefore, in studies to determine a species' desiccation tolerance it is essential to bear in mind the species' native range. If this is not the case, the observed level of desiccation tolerance may reflect seed development under suboptimal conditions rather than the maximum potential for that species. Nonetheless, while it is comparatively easy to observe desiccation tolerance lower than the maximum possible, it is difficult to assess what a species' maximum desiccation tolerance may be. For example, it is not known whether seeds of *A. hippocastanum* that develop under warmer conditions than those experienced by the Greek seed lot would have an even greater level of desiccation tolerance.

In support of the premise that environmental conditions during seed development affect the level of seed desiccation tolerance, there is uncertainty over the desiccation tolerance of several species. Seeds of tea (*Camellia sinensis*) from South Africa have been classified as recalcitrant (Berjak *et al.*, 1993) and seed lots from Japan and China as desiccation tolerant (Amma & Watanabe, 1985; Hu *et al.*, 1993). Neem (*Azadiracta indica*) is native to the Indian subcontinent and has been widely planted in Africa and there is debate over its level of desiccation tolerance: seed lots have been classified as orthodox, intermediate and recalcitrant (Poulsen, 1996; Sacandé, 2000). Similarly, provenance has been reported to have an effect on desiccation tolerance of arabica coffee (*Coffea arabica*) seeds (Ellis *et al.*, 1991). Wild rice (*Zizania palustris*) seeds have been classified as either desiccation intolerant (Probert & Longley, 1989; Vertucci *et al.*, 1995; Horne & Kahn, 2000) or desiccation tolerant (Kovach & Bradford, 1992), although the level of tolerance appears to be a function of the duration of the seed development period (Vertucci *et al.*, 1995). We propose therefore, that the reported differences between seed lots of these species may have resulted from seed lots developing under differing environmental conditions. This possibility of 'phenotypic recalcitrance' requires further investigation, particularly for species that have been widely introduced into a number of climate zones.

Ecological implications

The fact that a single PCA axis explained 86.6% of the variation among the seed lots suggests that the patterns of response for physical characteristics, germination, water relations and desiccation tolerance were consistent. For example, a seed lot able to tolerate desiccation to comparatively low water potentials will germinate at cooler temperatures (i.e. lower T_b), have a lower axis osmotic potential, be larger (greater dry mass) and be shed at a lower moisture content. Furthermore, the significant correlation of the seed lot scores on this axis with heat sum during development suggests that this pattern may reflect differences in developmental air temperature among the *Aesculus* populations.

Our results suggest that the natural dispersal of premature seed from trees introduced into areas outside the plants' native distribution range contributes to lower seed quality (germination performance, water relations and desiccation tolerance). Dispersal may be caused by a range of seasonal influences and their interactions (e.g. temperature, water availability and day length) on the parent tree. However, even under suboptimal conditions, trees nonetheless produce and shed viable seed, albeit of lower quality. They may therefore 'cope' with, rather than exhibit adaptation to, suboptimal conditions. It would be useful to investigate whether these observed effects of climate apply to seeds of other temperate trees and explore their implications for germination and seedling establishment in the field.

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