Variable desiccation tolerance in *Acer pseudoplatanus* seeds in relation to developmental conditions: a case of phenotypic recalcitrance?

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Abstract. Nine seedlots of the widely planted southern and central European native tree species *Acer pseudoplatanus* L. were collected along a north–south gradient spanning 21° of latitude in Europe. We investigated how the heat sum during seed development influences seed maturity as assessed by physical, physiological and biochemical traits. Using principal component analysis we found predictable and consistent patterns in all traits, which correlated with heat sum. For example, compared with fruits from their native range (Italy and France, heat sum $>3000°$C d), fruits from the coldest location (Scotland; heat sum of $1873°$C d) were shorter (*c.* 30 *v.* 42 mm), germinated over a narrower temperature range (5–20 *v.* 5–35°C) and had smaller embryos (28 *v.* >70 mg) with a higher water content (*c.* 63 *v.* 48%), less negative solute potentials (*c.* -2.4 *v.* -4.1 MPa) and were more desiccation sensitive (critical water potential of -20.2 *v.* -55.4 to -60.7 MPa). The observed level of desiccation-tolerance for the French and Italian seedlots is more consistent with the intermediate category than the previous classification of *A. pseudoplatanus* as recalcitrant. Our results demonstrate that a lower heat sum causes fruits from northern Europe to be dispersed before maximum potential seed quality is achieved.

Keywords: climate change, heat sum, principal component analysis, recalcitrant seed.

Introduction

Orthodox (Type I *sensu* Pritchard 2004) seeds pass through three developmental phases: embryogenesis, reserve deposition and maturation drying, with full desiccation tolerance acquired close to the transition between reserve deposition and maturation drying (Kermode and Bewley 1985; Ellis *et al.* 1987; Welbaum and Bradford 1989). In contrast, recalcitrant (Type III *sensu* Pritchard 2004) seeds fail to enter the maturation drying phase and consequently accumulate dry mass right up until the time of dispersal (Tompsett and Pritchard 1993). Consequently, one possible

explanation for the desiccation sensitivity of recalcitrant seeds is that seed shed occurs before development has progressed fully.

Recent work on recalcitrant seeds of *Aesculus hippocastanum* collected from across Europe has shown that air temperature during seed development significantly influences maturity status at dispersal, as assessed by seed mass, water content, solute potential, germinability and desiccation sensitivity. Under warmer conditions, seed development progressed further before dispersal, and seeds were shed further into the reserve

Abbreviation used: PCA, principal component analysis.

deposition phase (Daws *et al.* 2004*a*). For example, an increase in heat sum of approximately 920°C d resulted in a 10-fold increase in seed dry mass. This was also reflected in a greater relative level of desiccation tolerance. However, it is unclear how applicable these results are to other species with potentially recalcitrant seeds.

Previous work from this group has shown that desiccation tolerance, and other seed factors related to the extent of development at shedding, can exist on a continuous developmental scale and that the position on this scale may not only be genetically, but also environmentally determined (Daws *et al.* 2004*a*). Furthermore, it is likely that in non-native distribution ranges the environmentally determined changes in seed characteristics do not represent beneficial adaptations but rather constraints that are deleterious to fitness. Although the majority of species have seeds that are clearly at either the recalcitrant or orthodox ends of the desiccation tolerance spectrum there may be other species with recalcitrant seeds, whose position on the developmental scale may be significantly affected by climate. For such species, climate change at a rate that is too fast to permit genetic adaptation may have a major influence on the ability of their seeds to germinate and become established (St. Clair and Lynch 2004). Consequently, from both a practical and ecological viewpoint, it is important to know whether the responses of *A. hippocastanum* to climatic conditions are representative of other species with recalcitrant seeds.

Acer pseudoplatanus L. was chosen for this study because it has been widely planted in parts of Europe that are outside of its native range of central and Southern Europe (Jones 1945). Seeds from *A. pseudoplatanus* trees in England are reported to be recalcitrant (Hong and Ellis 1990; Dickie *et al.* 1991; Greggains *et al.* 2000) because seeds are killed by drying to comparatively high water contents (∼20%). However, this may not be valid for seeds collected within the native range. We hypothesised that: (a) seeds from within their native range are likely to develop under conditions that are significantly warmer than those in Northern Europe, (b) such seeds would have progressed further in development and (c) these seeds have a greater level of seed desiccation tolerance than those from further north. These hypotheses have not previously been fully tested for *A. pseudoplatanus* although Larcher (1985) found that fruits from Germany can survive storage at −16°C, presumably as a consequence of insufficient water for rapid nucleation and growth of ice crystals, and may therefore tolerate a considerable degree of desiccation stress. There is also some evidence that fruits of *A. pseudoplatanus* can respond to environmental conditions during development. For example, fruits collected from Cambridge (Southern England) had a significantly greater dry mass than those collected in the same year from further north (Lancashire, Northern England) (Warr 1986).

Therefore, the aim of this study was to investigate the effects of climatic conditions during development on seed characteristics, in particular desiccation tolerance, for seedlots of *Acer pseudoplatanus*. This was achieved by collecting seedlots from seven locations throughout Europe, which span 21 degrees of latitude and differ in summer climatic conditions, including two locations within the species' natural range (France and northern Italy). Subsequently we correlate seed physical and physiological characteristics (including the responses to desiccation) with the heat sum experienced by the seeds during development.

Materials and methods

Seedlot details

Fruits, at the point of natural dispersal, were collected from a range of European locations in October 2002 and 2003 (see Table 1). Fruits were collected from a minimum of five individual trees per location (with the exception of Greece) and subsequently bulked. For the Greek seedlot, fruits were collected from a single tree, although this tree was in close proximity to con-specific individuals (less than 100 m). Following collection, fruits were sent within 2 d to Wakehurst Place, UK, by air freight. To allow a comparison of seed development times and environmental conditions during development, the timing of flower fall was recorded at each location. This date was taken as the first day on which all flowers had fallen. Fruits were collected from the trees when natural dispersal had commenced and the collection date was assumed to be the date when seed development ended. 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:

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

Germination studies were conducted for the 2003 seedlots, and were commenced within 3 d of seed receipt. Three replicates of 20 fruits (with the wing removed), from each location, were sown on the surface of 1% agar in water in sandwich boxes (6 × 11 × 17 cm) at 5, 10, 15, 20, 25, 30 and 35°C with an 8-h photoperiod provided by warm fluorescent tubes. Germination was scored every 4–5 d and defined as emergence of the radicle by at least 2 mm.

The water content of the pericarp and embryo (i.e. with the testa removed) was determined for 25 individual fruits by drying at 103°C for 17 h (ISTA 1999). The solute potential of fully hydrated embryos ($n = 3 \times 8$–10) from the different provenances was measured using a WP4 dewpoint potentiometer (Decagon Devices, Pullman, WA) operated in a temperature-controlled room (21 ± 1°C). Different numbers of embryos were used to reflect differences in embryo size between locations. Before measurement, embryos (i.e. with the testa removed) were hydrated by placing them on the surface of 1% agar in water at 21°C for 24 h. Embryos were then sealed in 1.8-mL Nalgene cryovials (Nalge Co., Rochester, NY), plunged into liquid nitrogen (Boyer 1995) and held for 5 min to ensure complete freezing of the embryo. The water potential of the embryos was measured once the embryos had thawed and reached 21°C. Since the tissue water potential after cells have ruptured to reduce turgor pressure to zero is only due to solute potential this value was taken as the embryo solute potential (Boyer 1995).

Table 1.   Some characteristics of the nine seedlots of *Acer pseudoplatanus* studied
Values are ± 1 SE of the mean. Critical water potential values determined experimentally following desiccation experiments

Location	End of flowering	Collection date	Heat sum (°C d)	Fruit length (mm)	Seed dry mass (mg)	Embryo water content (% FW)	Embryo solute potential (MPa)	Critical water potential (MPa)
Aberdeen, Scotland 2002 (57°10′N, 2°04′W)	16 May 2002	8 October 2002	1873	28.9 ± 0.8	28.0 ± 2.5	67.1 ± 1.9	−2.4 ± 0.1	−15.7 ± 1.5
Aberdeen, Scotland 2003	24 May 2003	16 October 2003	2023	30.7 ± 1.0	39.7 ± 2.8	63.4 ± 1.5	−2.6 ± 0.1	−20.2 ± 2.0
Bergen, Norway (60°12′N, 5°19′E)	15 May 2003	10 October 2003	2120	33.8 ± 0.5	51.1 ± 2.2	57.8 ± 0.6	−2.8 ± 0.2	−21.0 ± 1.3
Poznan, Poland (52°25′N, 16°53′E)	3 June 2003	11 October 2003	2332	36.8 ± 0.5	69.1 ± 0.5	54.6 ± 1.0	−2.9 ± 0.1	−29.1 ± 1.8
Ardingly, England 2002 (51°03′N, 0°06′W)	25 May 2002	11 October 2002	2440	38.2 ± 0.5	64.0 ± 1.9	57.8 ± 0.7	−2.9 ± 0.1	−26.9 ± 1.3
Ardingly, England 2003	19 May 2003	6 October 2003	2651	38.4 ± 1.3	71.8 ± 0.3	59.7 ± 0.6	−3.4 ± 0.1	−38.4 ± 2.3
Thessaly, Greece (39°15′N, 21°40′E)	23 May 2003	15 October 2003	2678	36.9 ± 0.7	74.3 ± 1.5	53.5 ± 0.9	−3.7 ± 0.1	−42.0 ± 5.8
Angers, France (47°9′N, 0°32′W)	20 May 2003	20 October 2003	3016	41.8 ± 0.9	70.4 ± 4.3	47.9 ± 1.5	−4.2 ± 0.1	−55.4 ± 2.5
Mt Lessini, Italy (45°41′N, 11°13′E)	29 May 2003	13 October 2003	3176	44.0 ± 0.9	83.9 ± 3.7	52.3 ± 1.5	−4.1 ± 0.1	−60.7 ± 4.0

Seed desiccation tolerance

Desiccation studies on the 2003 seedlots were commenced within 3 d of receiving fruits. Whole fruits were dried at approximately 15°C and 15% RH to a range of target water contents between the initial water content and approximately 10%. Periodic re-weighing of the fruits allowed target masses and hence water contents to be achieved. At each target water content, the water content of 25 individual embryos was determined and three replicates of 20 fruits (with the wing removed) sown at 5°C (Hong and Ellis 1990).

The relationship between embryo gravimetric water content and water potential (isotherm) was determined by drying embryos at approximately 15°C and 15% RH for up to 36 h. Embryos ($n = 8$–10) 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 was determined with a WP4 dewpoint potentiometer. Following water potential determination, embryo water content was determined.

Statistical analysis

A preliminary visual inspection of the water potential isotherms for the seven seedlots from 2003 revealed upper and lower extremes for the Scottish / Norwegian and the French / Italian lots respectively. To test for differences in the slope and / or intercept of the regressions, all four possible pair-wise comparisons of the regressions lines of these two extreme responses was undertaken by ANCOVA (Underwood 1997). This was achieved by fitting, in GENSTAT 5 (4th Edition, Lawes Agricultural Trust, IACR Rothamsted), a regression model assuming that each seedlot had a different slope and intercept, i.e. an interaction between the group term (seedlot) and the dependent variable (water content). Subsequently, the significance of the interaction term was assessed by a *t*-test. Since in each case the interaction term was non-significant, it was dropped and the model recomputed (i.e. assuming parallel lines). Subsequently, the significance of the parameter estimates of this model was tested by a *t*-test to assess whether the intercepts of the lines differed.

Using Probit analysis, implemented in GENSTAT 5, we determined the relationship between germination percentage (*y*) and embryo water potential (*x*). Probit analysis enables calculation of the lethal dose for 50% viability loss (in this case the water potential corresponding to 50% viability loss) and its standard error. This value was calculated as it provides a measure of the population response to desiccation (Daws *et al.* 2004*b*).

Principal component analysis (PCA) implemented in PC-Ord (McCune and Mefford 1997) was used to explore the inter-correlation among fruit and embryo characteristics of the seedlots and to determine whether responses could be reduced to one or more explanatory axes. Two separate analyses were conducted, the first with the seven 2003 seedlots, the second with all nine seedlots (2002 and 2003). The first analysis included fruit length, embryo water content, embryo dry mass, embryo solute potential, percentage germination at 35°C and the median water potential for embryo viability loss. While 35°C may appear an unusual temperature for germination in this species, this temperature was used in an attempt to separate the germination responses of the seven seedlots. In addition, the ability to germinate at high temperatures is related to seedlot provenance in other tree species with a similar southern European native range (e.g. *A. hippocastanum*; Daws *et al.* 2004*a*). The second analysis used the same variables, apart from germination at 35°C. Broken-stick eigenvalues were used to determine which axes should be considered for interpretation; if the broken-stick eigenvalue is less than the actual eigenvalue for an axis, then that axis contains more information than expected by chance (Jackson 1993). Subsequently, seedlot scores on the resulting axes of variation were correlated with the heat sum accumulated during seed development to test

whether this independent variable was able to explain the observed patterns of response.

Results

Germinability, embryo size and water relations

Fruits that developed under warmer conditions were significantly larger (in terms of overall fruit length. For example, Italian fruits were ~15 mm longer than Scottish fruits (heat sum of 3176 *v.* 1873°C d, respectively). Similarly, embryos from warmer conditions had at most three times greater dry mass than those from cooler locations and were shed with lower water contents (Table 1). The solute potential of embryos from warmer conditions was also more negative than those from cooler locations (Table 1) with extreme values of −2.4 and −4.2 MPa for Scottish seeds from 2002 and French seeds from 2003.

Initial germination for all seven seedlots from 2003 was highest at 5°C, reaching 70–98% for all except the Scottish fruits, which exhibited a maximum of only 27% (Fig. 1). As the temperature increased to 20°C germination percentages progressively declined, reaching close to 0% for all except the French and Italian fruits. Between 20 and 35°C germination increased for the French and Italian and to a lesser extent the English and Greek fruits while remaining close to 0% for the Polish, Norwegian and Scottish fruits.

Seed desiccation sensitivity

For all nine seedlots there was a similar, linear relationship between log(embryo water potential) and log(water content) (Table 2). However, for a given water content, there was a tendency for the Scottish and Norwegian lots to have more negative water potentials, while the French and Italian
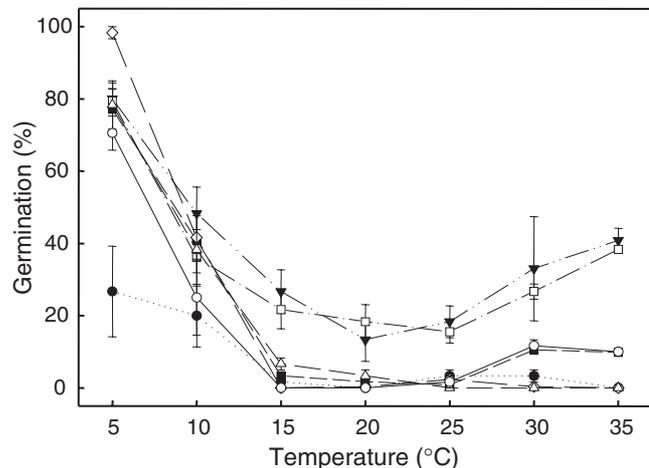

Fig. 1. The effect of constant temperatures on the germination percentage (after 12 weeks) of seven seedlots of *Acer pseudoplatanus* (England 2003, ○; France, ▼; Greece, ■; Italy, □; Norway, ◇; Poland, △; Scotland 2003, ●). Error bars are ± 1 s.e. of the mean.

Table 2. Constants describing the relationship between water potential (MPa) and water content (on a dry mass basis) for embryos of *Acer pseudoplatanus* in the equation $\log_{10}$(water potential) $= A + B[\log_{10}$(water content)]
***$P < 0.001$**

Seedlot	A	B	R^2
Scotland 2002	0.792	−1.324	0.969***
Scotland 2003	0.765	−1.288	0.987***
Norway	0.780	−1.313	0.993***
Poland	0.764	−1.303	0.986***
England 2002	0.760	−1.303	0.987***
England 2003	0.758	−1.306	0.988***
Greece	0.741	−1.333	0.976***
France	0.675	−1.309	0.992***
Italy	0.695	−1.319	0.990***

Fig. 2. (*A, B*) The relationship between germination percentage and embryo water potential for nine seedlots of *Acer pseudoplatanus* (England 2002, ▽; England 2003, ○; France, ▼; Greece, ■; Italy, □; Norway, ◇; Poland, △; Scotland 2002, ▲; Scotland 2003, ●). Error bars are +1 s.e. of the mean. Lines were fitted by Probit analysis. The initial increase in germination for the Scottish seedlots was not included when fitting the Probit model to the data.

embryos tended to have less negative water potentials (Table 2). This was supported by pair-wise comparisons of the extreme seedlots (Scotland / Norway and France / Italy). For all comparisons there was no evidence for non-parallelism of the two pairs of regression lines ($P > 0.05$) and the intercepts of the regression lines were significantly different ($P < 0.001$). The equations from linear regression of the data for each seed lot (Table 2) were subsequently used to convert the embryo water content values from the desiccation screen to water potential.

Desiccation of all seed lots resulted in a steady decline in viability, with all seeds killed by water potentials as low as −80 MPa (Fig. 2). However, the water potential that resulted in 50% viability loss differed between the seed lots (Table 1) with the extreme seedlots (Scotland and Italy) differing in critical water potential by more than 40 MPa. In addition, there was a pronounced increase in germination following mild desiccation from approximately −3 to −4 MPa for both Scottish seedlots.

Principal component analysis of seed traits

Principal component axis I (eigenvalue = 5.2, broken-stick eigenvalue = 2.5) explained 87.1% of the variation of the six seed traits for the 2003 seedlots, while PCA axis II was non-significant (eigenvalue = 0.5, broken-stick eigenvalue = 1.5). In addition, the loadings for the traits along axis I were similar, ranging from |0.38| to |0.43|. The seedlots were arranged along axis I as follows with seedlot scores: Scotland (−3.2), Norway (−2.1), Poland (−0.8), England (−0.1), Greece (0.5), France (2.7) and Italy (2.9). Furthermore, heat sum during seed development (see Table 1) was significantly correlated with seed lot scores along this axis (Pearson product-moment correlation; $r = 0.99$, df = 5, $P < 0.001$). Including data for the 2002 seedlots, PCA axis I (eigenvalue = 4.5, broken-stick eigenvalue = 2.3) explained 90.0% of the variation in seed traits, with similar trait loadings ranging from |0.44| to |0.45| along axis I. PCA
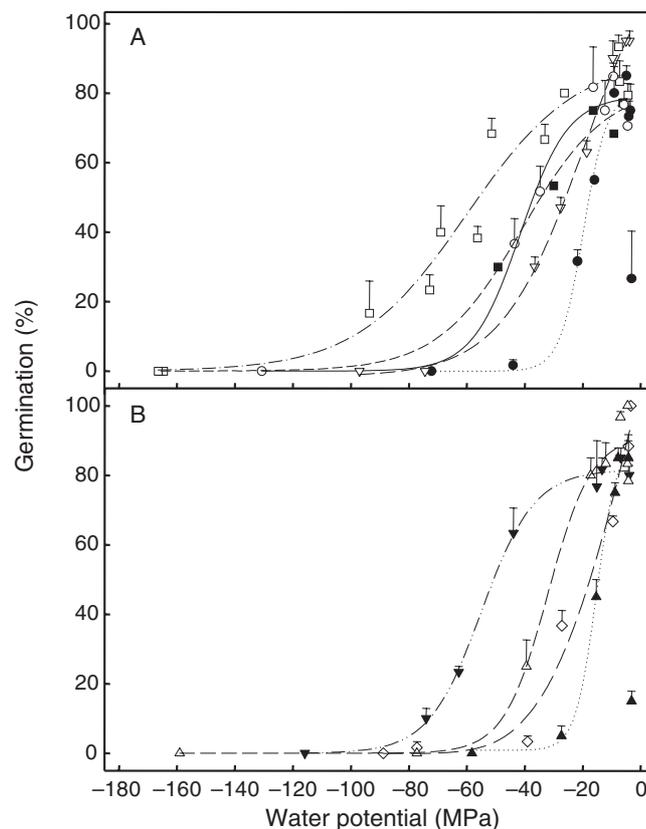
axis II was non-significant (eigenvalue = 0.2, broken-stick eigenvalue = 1.3). The seedlots were arranged along axis I with seedlots scores of −3.4, −2.4, −1.2, 0.0, −0.3, 0.5, 1.2, 2.7 and 3.0 for Scotland 2002, Scotland 2003, Norway, Poland, England 2002, England 2003, Greece, France and Italy, respectively. These scores were significantly correlated with heat sum (Pearson product-moment correlation; $r = 0.98$, df = 7, $P < 0.001$).

Discussion

Germination, embryo size and water relations

All six of the seed / fruit traits we investigated contributed substantially to axis I of the PCA and in addition, the location of the seedlots along axis I correlated strongly with heat sum during development. Consequently, the observed trends in fruit and embryo traits were highly consistent and predictable and could be explained on the basis of climatic conditions. Thus, fruit length, embryo mass, germinability and desiccation tolerance increased

while embryo water content and solute potential decreased with increasing heat sum. This is consistent with previous studies (Kermode and Bewley 1985; Ellis *et al*. 1987; Welbaum and Bradford 1989) that have reported seed water content to decrease and dry mass and desiccation tolerance to increase during the second phase of seed development. Furthermore, for *Aesculus hippocastanum* and *Quercus robur*, seed water potential and solute potential decrease during development (Finch-Savage *et al*. 1992; Farrant and Walters 1998). This suggests that for the seedlots of *A. pseudoplatanus*, seed development progressed further for seeds from warmer locations, i.e. reserve deposition had progressed further. This is reinforced by the seedlot from the coldest location (Scotland) exhibiting a positive effect of partial desiccation on germination; partial desiccation may facilitate a continuation of the maturation processes that occur on the plant (Tompsett and Pritchard 1998). Similar patterns of seed responses to climate have also been observed for the recalcitrant species *A. hippocastanum* where developmental status was also correlated with developmental heat sum (Daws *et al*. 2004*a*).

Fruits from all seven locations exhibited maximum germination at 5°C. Hong and Ellis (1990) and Webb and Wareing (1972) have also reported that 5°C is a suitable germination temperature for this species. Germination of the French and Italian fruits also occurred at 30 and 35°C, while for seedlots from cooler locations, germination declined with increasing temperature. Webb and Wareing (1972) found that germination of Welsh fruits steadily decreased at temperatures greater than 5°C with germination reaching 0% at 20 and 25°C. The increase in germination we observed, at high temperatures, for the French and Italian fruits appears to be the first report of this phenomenon for *A. pseudoplatanus* although a similar response has been observed for recalcitrant seeds of *A. hippocastanum* (Pritchard *et al*. 1999). This improvement in germinability across the temperature range with increasing developmental heat sum is consistent with the observation that increasing air temperatures during development typically increase germinability in a wide range of species (Fenner 1991; Gutterman 2000).

Plant species may exhibit germination responses that suggest adaptation to local environmental conditions (e.g. Cruden 1974; Thompson 1975; Daws *et al*. 2002). Thus, the responses we observed may reflect adaptation and genotypic variation rather than phenotypic responses to environmental conditions. However, this is unlikely for three reasons. First, the observed patterns with respect to fruit size, embryo mass and water content were not only observed between locations, but also between years for collections from England and Scotland made from the same trees in two consecutive years that differed in summer conditions (Table 1). Second, in the United Kingdom, sycamore is an introduced species and has only been widespread since

the 19th century (Jones 1945). Since this species is a tree it is unlikely that sufficient generations have passed for considerable genetic adaptation to local environmental conditions to occur. Third, the observed trends (smaller, more desiccation-sensitive seeds) outside the native range of this species (i.e. United Kingdom and Norway) are unlikely to be of adaptive benefit.

Embryo desiccation tolerance

On a water content basis, the levels of desiccation tolerance for embryos from England, Poland and Greece are comparable with published data for English seedlots. Embryos from England, Greece and Poland had a water content (fresh mass basis) range for 50% viability loss of 23–29% [values calculated from the critical water potentials (see results) using the equations in Table 2] *v.* 19–29% for English seeds in three separate studies (Hong and Ellis 1990; Dickie *et al*. 1991; Greggains *et al*. 2000). Seedlots from cooler locations (Norway and Scotland) were more desiccation sensitive (33–39%) while those from warmer locations (France and Italy) were more desiccation tolerant (water content for 50% viability loss of approximately 15% for both seedlots).

Sun and Liang (2001) report water potentials that correspond to the onset of desiccation stress for seeds of a range of species and demonstrate that intermediate (Type II *sensu* Pritchard 2004) species such as *Azadiracta indica*, *Carica papaya* and *Coffea arabica* are killed by potentials in the range −65.6 to −73.6 MPa, which corresponds to the loss of type II 'bound' water (Pritchard 2004). In contrast, species in the recalcitrant category are killed by desiccation to water potentials in the range −1.5 to −23.5 MPa corresponding to the loss of type III 'free' water (Pritchard 2004). Although the critical water potentials in Sun and Liang (2001) were determined by a different methodology from that used in this current study, it is clear that the Norwegian and Scottish seedlots are at the more desiccation-tolerant end of the recalcitrant spectrum (see Table 1). Conversely, the responses of the French and Italian embryos are inconsistent with the recalcitrant category and fall within the type II category. Seed storage behaviour for this species would be further clarified by characterising seedlot responses to subzero temperatures (Ellis *et al*. 1990, 1991*a*; Pritchard 2004).

Our results for both inter-annual and inter-site comparisons suggest that desiccation sensitivity and other traits can be observed as a continuum of responses within a species in relation to maturation status (Daws *et al*. 2004*a*; Pammenter *et al*. 1991), with seed quality constrained in cooler locations. Our previous study (Daws *et al*. 2004*a*) found that, for *A. hippocastanum*, this continuum was confined to the recalcitrant category. However the magnitude of the climatic effect on seeds of *A. pseudoplatanus* is such that they can move from one seed storage category to the next.

The predictability of our results means that it may be possible to infer and model seed responses to a changing climate. It also seems clear for trees grown beyond their natural range, particularly those that produce slowly developing seeds that are potentially desiccation sensitive, that the point of maximum seed quality does not always correspond to the time of natural dispersal. Consequently, identifying the maximum level of desiccation tolerance for a particular species is problematic. For example, it is uncertain whether *A. pseudoplatanus* fruits that develop under conditions warmer than those recorded here would be even more desiccation tolerant. Therefore, when characterising seed responses, including desiccation tolerance, it is important to record provenance and use material collected within that species' native range. Another implication of these results is that the use of pairs of congeneric species' to investigate variable responses to desiccation (e.g. *Acer platanoides* and *A. pseudoplatanus*) is most likely to be valid when both species are collected from their natural ranges.

Our results demonstrate that environmental conditions during seed development systematically affect numerous seed features including the level of seed desiccation tolerance. There is uncertainty over the desiccation tolerance of seeds of several crop species grown outside their natural range, including tea (*Camellia sinensis*), neem (*Azadiracta indica*) and coffee (*Coffea arabica*). One explanation for this may be the use of seed material collected from a range of climatic conditions. For example, tea has been classified as recalcitrant, since flash-dried embryonic axes of seeds collected in South Africa (Berjak *et al*. 1993) experienced desiccation damage below 29% water content. However, seeds from China germinated after drying to a water content of 9% followed by 48 h submersion in liquid nitrogen (Hu *et al*. 1993). Similarly, Amma and Watanabe (1985) reported that seeds from Japan survived storage at 1°C in sealed polythene bags for up to 6 years with viability only dropping from 86% to 73%. This storage duration would be exceptional for recalcitrant material, for which 3 years is considered very good (e.g. *A. hippocastanum*, Pritchard *et al.* 1996 and *Q. robur*, Suszka and Tylkowski 1980).

Neem is native to the Indian sub-continent, and has been widely planted in Africa with different seedlots having been classified as orthodox, intermediate or recalcitrant (Poulsen 1996; Sacandé 2000). Similarly, provenance has been reported to have an effect on desiccation tolerance of coffee seeds. For example, Ellis *et al*. (1991*b*) found differences in the median water content for viability loss for seed lots of the same cultivar collected in different countries.

In conclusion, embryos of *A. pseudoplatanus* can exhibit considerable seed desiccation tolerance suggesting that recalcitrance in this species is phenotypically expressed under sub-optimal growing conditions. Thus, our results highlight that it is important to consider provenance when studying seed traits such as desiccation tolerance and germination. In addition, considerable success has been reported with the storage of type II seeds such as coffee and neem at sub-zero temperatures (Sacandé *et al*. 1998; Dussert *et al*. 2001; Hong and Ellis 2002). Consequently, studies of the responses of sycamore seeds to sub-zero temperatures are worth pursuing, particularly for seedlots from within the species' native range.

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