The Effect of Chilling and Moisture Status on the Germination, Desiccation Tolerance and Longevity of Aesculus hippocastanum L. Seed

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Effects of 2 °C chilling on the threshold moisture contents and water potentials for various physiological processes were estimated for Aesculus hippocastanum L. seed. Seed harvested at the time of maximum seed fall exhibited a dual response to drying: partial drying from near 50% to 32–40% moisture content progressively increased germination percentage (at 16 °C) up to various peak values; further desiccation was detrimental, confirming that the seeds are 'recalcitrant'. The moisture content for optimum germination was increased by at least 10% as the chilling period was raised from 0 to 9 weeks. A negative linear relationship was found between log<sub>10</sub> mean time to germinate and probit final germination, regardless of pre-treatment, indicating that partial desiccation and chilling are interchangeable in promoting germination of hydrated seed. For nearly fully hydrated seeds, increasing the chilling period from 6 to 26 weeks increased the viability-loss onset point for desiccation injury from near 40% to about 48% moisture content without altering the drying rates of seed tissues. Extending moist chilling in various seed lots from 0 to 26 weeks decreased subsequent longevity at 16 °C. For 26-week-chilled seeds longevity (the period to lose one probit of germination) differed above and below a threshold moisture content of 48%. It remained constant in the moisture-content range 48–38%, but increased progressively as moisture content was raised above 48%. This threshold moisture content coincided with the value above which chilled seed pre-germinated in storage. The results indicate that post-harvest desiccation and chilling alter the water relations of various physiological processes and a schematic summary is presented which relates the results to an axis water sorption isotherm.

Key words: Aesculus hippocastanum L., horse chestnut, chilling, moisture content, water potential, desiccation tolerance, longevity, recalcitrant seed, embryo axis, maturation, germination.

INTRODUCTION

In developmental studies on Aesculus hippocastanum seeds, desiccation to low moisture contents has been observed to induce both a reduction in germination percentage (Tompson and Pritchard, 1993) and an increase in electrolyte leakage from the axis (Farrant et al., 1997). These responses indicate inclusion of this species in the 'recalcitrant' (i.e. desiccation intolerant) seed storage category of Roberts (1973). However, the relationship of desiccation damage to moisture content for seed collected at the time of maximum seed fall has not been detailed and is analysed in the present study in terms of desiccation sensitivity.

A limited amount of seed desiccation can have different effects from the damaging influence of a more complete drying treatment; knowledge of both is important in practical seed handling of recalcitrant tree seeds. For example, Panochit, Wasuwanich and Hellum (1984) showed a clear increase in germination on reducing the moisture content of Shorea roxburghii Miq. seed from approx. 50% to approx. 40%. Furthermore, a similar effect of partial drying on seed of Aesculus hippocastanum (Suszka, 1966) and Litchi chinensis Sonn. (Fu et al., 1994) has been found, but the effect was limited in these reports to an alteration of vigour characteristics. In the present study the quantitative effects of partial desiccation on both final percentage and rate of germination were assessed.

Evidence for the way in which moist storage affects the desiccation tolerance of recalcitrant seeds is equivocal. Changes in desiccation sensitivity of Quercus robur L. appeared to be variable (Finch-Savage, 1992; Finch-Savage, Blake and Clay, 1996), whilst Farrant, Berjak and Pammenter (1986) found that Avicennia marina (Forssk.) Vierh. increased quickly in sensitivity, a result that they associated with progress towards germination during storage. Chilling of fully hydrated Aesculus hippocastanum seed is known to slowly facilitate germination not only at low temperatures but also on subsequent transfer to higher temperatures (Pritchard, Tompsett and Manger, 1996). The opportunity was taken to examine the relationship between post-harvest chilling period and seed desiccation tolerance, and to assess the association of these factors with other physiological processes.

Tropical recalcitrant seeds which must, in most cases, be held at non-chilling temperatures, are limited to storage for up to a year according to current evidence (e.g. Tompsett and Kemp, 1996). Desiccation-intolerant temperate seeds have been kept much longer; for example, cold-stored Quercus robur L. seed (Suszka and Tylkowski, 1980) and moist Aesculus hippocastanum held at 16 °C (Pritchard et al., 1996) can survive well for approx. 3 years. However, only a few studies have been conducted relating water status to recalcitrant-seed longevity (Gosling, 1989; Pritchard et al., 1995) and this aspect is considered further in the present study.

Moisture content is the unit of measurement generally
used for assessing responses of recalcitrant seed to desiccation. However, this method does not facilitate comparisons of physiological processes among species due to the influence of chemical composition on moisture content; by contrast, the use of water potential via the water sorption isotherm may overcome this limitation (e.g. Roberts and Ellis, 1989 for an orthodox-seeded species). This approach has been employed in relation to desiccation and storage physiology for embryos of the recalcitrant-seeded species Quercus rubra L. (Pritchard, 1991), Q. robur (Finch-Savage, 1992; Poulsen and Eriksen, 1992) and Araucaria hunsteinii K. Schum. (Pritchard et al., 1995). The water potential of excised tissues of Aesculus hippocastanum seed at the time of shedding has also been assessed (Tompsett and Pritchard, 1993). In the present study, water potential thresholds for various physiological processes have been determined in relation to post-harvest desiccation and chilling treatments.

MATERIALS AND METHODS

Collection, chilling and desiccation

Four separate batches of mature seed were collected in 3 separate years over a period of 7 years. Batch 1 (harvested 16 Oct. 1984) and Batch 3 (harvested 28 Sep. 1989) comprised freshly fallen seed at approx. 48% moisture content from three large trees at Wakehurst Place, Sussex, UK. Batch 2 (harvested 12 Oct. 1989) and Batch 4 (harvested 9 Oct. 1990) seed were at approx. 50% moisture content and were collected from a large population of small trees (≥ 25) at Chailey in Sussex. Seed was collected either from the branches by gently shaking, or from the ground soon after natural abscission.

Chilling of seed was carried out in folded-over polythene bags at 2 °C for periods of between 3 and 26 weeks. The process preceded desiccation for the treatments of Batches 1, 2 and 4 in Fig. 3, and for the treatments of Batches 3 and 4 in Fig. 6. Individual seeds that pre-germinated during chilling in the treatments of Fig. 3 were not used in the subsequent drying treatment. Chilling was applied after drying for all treatments of Batches 2 and 4 in Fig. 1; moisture content remained within about 1% of the original value during hydrated chilling.

Moisture content assessment and adjustment

Moisture content was assessed gravimetrically employing 103 ± 2 °C for a period of 17 ± 1 h (International Seed Testing Association, 1985) and is expressed on a wet weight basis throughout. Randomly selected groups of three to five individual seeds were used for each sample. The embryonic axis was excised and assessed separately from the remaining tissues for all samples, except in the case of the Batch 1 treatment of Fig. 3.

Seed was dried in a flow of air at approx. 15 °C and near 15% relative humidity. Internal equilibration of moisture among tissues of the seed at the end of all drying was achieved by immediately holding material in inflated 25-µm-gauge polythene bags for 7 d at 16 °C before it was either germinated or stored. To maintain comparability, control treatments that had not been dried were also kept at 16 °C for 7 d. Exceptionally, the 6-, 12- and 26-week-chilled treatments of Fig. 3 were assessed for moisture content and germinated immediately at the end of all desiccation periods.

Germination

Three germination conditions were employed over the 7-year period of the present study. Firstly, 26 °C was employed in combination with a 12-h photoperiod (Tompsett and Pritchard, 1993). Secondly, 16 °C was used in combination with darkness; light was excluded by wrapping the germination boxes in aluminium foil (Pritchard et al., 1996). The former method was employed for the Batch 1 samples in Fig. 3, for the Batch 2 samples in Table 1 and for the Batch 4 samples in Figs 3 and 6B. The latter method was used in all other cases except in Table 3, where, thirdly, seeds were germinated at 6 °C in the dark (Pritchard et al., 1996). The light source was Warm White fluorescent tubes at a photon flux density of approx. 15 µmol m⁻² s⁻¹.

Germination of seed was in 7 × 11 × 17 cm clear plastic boxes on 200 ml of 1% agar-water: there were between 12 and 15 seeds per box. Single boxes were employed for the samples which were chilled for 12 weeks in Fig. 3, and for all samples in Fig. 6A; in all other cases two replicate boxes were used. Germination assessments, which were made at least weekly, were based on protrusion of the axis to > 1 cm. Exceptionally, seeds in the 51 and 53% moisture content storage treatments of Fig. 6B which were already pre-germinated at the time of sampling (amounting on average to 85 and 32%, respectively) were assessed as viable if they produced an epicotyl in the germination test. Soft seed remaining at the end of the germination test was considered non-viable throughout the study.

The comparability of results obtained using the two warmer germination methods was supported using non-dormant material of Batch 4 seed. Results showed no significant differences between the final germination values of 98 and 96%; analysis of the angular transformed data gave a t-test value of 0.46 with 5 d.f. (n = 45–60 per test). Pre-germination is defined as any visible emergence of the axis from within the seed coat before the germination test was performed.

Storage

Three storage experiments were conducted at 16 °C; desiccation and equilibration were carried out after chilling in these experiments using the methods described above. In the first (Table 2, Fig. 6A), seed of Batch 3 was chilled for 12 weeks and then divided into two treatments which were retained at different moisture contents in closed, 16 × 30 × 30 cm plastic freezer boxes. The inside surfaces of the boxes were lined with moist paper towels and ventilation was carried out twice per week.

In the second experiment (Table 2, Fig. 6B), which was conducted on Batch 4 seed, pre-chilling was for 26 weeks. A few seeds, which had pre-germinated during chilling, drying or moisture content equilibration, were excluded before...
appropriate numbers of seeds were allocated to each storage treatment. The material was retained in inflated polythene bags at seven different moisture contents and the bags were ventilated at least weekly. Moisture contents changed little during storage in either of these experiments, s.e. values within these treatments being < 1.3% for Batch 3 seed and < 0.7% for Batch 4 seed (based on 13–30 replicate determinations per treatment).

In a further storage experiment (Table 3), which was based on a continuation of germination tests from the desiccation experiment of Fig. 1B, seed was held at 16 °C in 7 × 11 × 17 cm clear plastic boxes on 200 ml of 1% agar-water (Pritchard et al., 1996). Seed was ventilated periodically over periods of up to 49 weeks. Moisture content was not assessed during 16 °C storage, but a value of 60 ± 3% s.d. (axis moisture content 64 ± 3% s.d.) was determined for comparably stored seed.

Sorption isotherm determination

The relationship between moisture content and water potential (the sorption isotherm) was assessed at 16 °C for whole axes from Batch 2 seed that had been held for 10 months at 2 °C; after chilling, viability was 98% (n = 60) and axis moisture content was 63%. Water potentials employed ranged from −0.5 to −6.0 MPa and there were five Petri dishes (5 cm diameter) per concentration. Each dish contained 10 ml of autoclaved solution and was sealed with Nescofilm®. Other details were as described elsewhere (Tompsett and Pritchard, 1993).

Statistical analyses

Mean times to germinate were assessed (in d) as the ratio \( \Sigma(Dn)/\Sigma n \), where \( n \) is the number of seeds that germinate on day \( D \) and \( D \) is the number of days from the beginning of the germination test. Values were assessed at the end of the period of the germination test, which extended to over 3 months in some cases and which varied according to when the final germination occurred. Fourteen treatment combinations of partial desiccation and chilling duration and the five undried controls in Fig. 1 were examined. Each was assessed by two replicates giving 38 in total, four of which failed to germinate; analyses were thus based on a total of 34 replicates, 11 in Fig. 2A and 23 in Fig. 2B.

As in our previous recalcitrant-seed studies on storage, probit analysis was performed to determine parameters for germination against time (Tompsett, 1983; Pritchard et al., 1995); longevity is quantified as \( \sigma \) (time in weeks for the loss of one probit of germination), which is calculated as the reciprocal of the regression coefficient. Analysis of variance was performed for the single sampling occasion in the storage experiment of Table 3.

Probit analysis was also performed to relate germination in desiccation experiments to moisture content as in Tompsett (1982). In addition, a value for \( MC_{\text{slow}} \), defined as the moisture content at which 50% of the seed population remain germinable after drying, was calculated for some treatments. This value, being the point on the germination scale where variation is lowest, can be assessed more accurately than the point at the top of the sigmoid shaped curve where viability-loss is first seen on drying.

RESULTS

Dual effect of drying on germination

The effect of desiccation on germination was investigated in detail in experiments on Batch 2, 3 and 4 seed. For these seed lots, which exhibited some dormancy, a dual effect of drying on germination was found; an initial promotive effect observed on partial drying was followed, on further drying, by a detrimental effect (Fig. 1). The two effects, which were expressed following a period of chilling, are considered separately below. Harvest moisture contents of the whole seed (approx. 50%) and the embryonic axis (approx. 65%) were similar in both years of collection (Fig. 1).

The results of partial desiccation treatments exhibit three main features. Firstly, partial desiccation caused an increase in germination percentage with almost all periods of chilling. In the case of the 3 week chilling treatment, for example, maximum germination was increased from < 10% to 25–35% when seed moisture content was reduced prior to chilling from 48–51% to 40–44% (Fig. 1).

Secondly, the lower limit to this promotive effect was observed at higher moisture contents as longer chilling periods were imposed. Thus, on drying down from the harvest moisture content, germination percentage increased to a maximum at moisture contents of 32 and 36%, 40 and 44%, 40 and 44%, and ≥ 48 and 46% (the Batch 2 value preceding that for Batch 4) in the case of seeds subject to 0, 3, 6 and 9 week chilling, respectively (Fig. 1). Corresponding data for the embryo axis moisture contents were 39 and 46%, 52 and 58%, 52 and 58% and 63 and > 63%, respectively for data of the two batches (Fig. 1).

Thirdly, germination percentage was increased as the period of chilling was extended. At the harvest moisture content, considering both years' experiments, germination increased from 15–40% up to near 80% as chilling was protracted from 6 to 9 weeks. Similarly, in the case of seed which had been partially dried to 40–44% moisture content, germination was increased from 15–30% to about 80% when chilling was prolonged from 3 to 6 weeks.

The relationship between final germination percentage and germination rate was assessed, employing results from the initial part of the dual response to desiccation. \( \log_{10} \) mean time to germinate decreased linearly as probit germination percentage increased, irrespective of the combination of partial desiccation and chilling employed (Fig. 2). Fitting single parallel regression lines for the treatments within each year did not significantly alter the scaled deviance compared to free fitting (\( F < 0.93 \)). However, the data for the 2 years of seed collection could not be represented by a single line; scaled deviance obtained for single line fitting (85.5, 34 d.f.) was greater than the values obtained when either parallel separate lines (70.2, 33 d.f.) or unconstrained lines (69.1, 32 d.f.) were fitted. The results for Batch 2 (Fig. 2A) and Batch 4 (Fig. 2B) show that mean time to germination varied from 22 to about 80 d among all treatments as final germination decreased from > 90 to 7%.
The relationship between whole seed moisture content and germination for *A. hippocastanum* seed of (A) Batch 2 and (B) Batch 4. After drying, seed was chilled for 0 (○), 3 (□), 6 (+) and 9 (★) weeks and germinated at 16 °C. Corresponding embryonic axis moisture contents are indicated by a non-linear scale on the upper axis. Bars represent one s.d. of the mean.

The relationship between germination (probability scale) and mean time to germinate (log scale) at 16 °C for *A. hippocastanum*. Seeds were from (A) Batch 2 (intercept 9.56, s.e. 0.79) and (B) Batch 4 (intercept 10.11, s.e. 0.14); the common slope was −6.22, s.e. 0.52. The seed moisture contents (%) at which chilling was applied were: 51 (●), 49 (○), 48 (□), 46 (▲), 44 (●), 42 (△), and 40 (▼). Data are derived from the same experiments as those shown in Fig. 1. The mean germination time for seeds giving a final 50% germination (d) is indicated by dashed lines. Individual replicates with germination > 0% from treatment combinations of partial desiccation and chilling and undried controls in Fig. 1 were employed. Further details are in the section on statistical analyses.
Mean time to 50% germination for pooled data was 34.5 d (Fig. 1A) and 42.3 d (Fig. 1B), indicating that, on average, seeds germinated 23% quicker in 1989 than in 1990.

The second component of the dual effect was expressed when moisture content was reduced below the lower limit at which the partial desiccation effect was observed. In the case of chilled-seed treatments (≥3 weeks), this destructive component is indicated by the loss of viability occurring below about 40-45% seed moisture content (Fig. 1).

A further three experiments were carried out to explore desiccation relationships between germination and moisture content (Fig. 3; Table 1); chilling requirements had been fully satisfied for these seed batches prior to desiccation so that only the second component of the dual effect was observed. The results obtained showed that increasing the period of chilling before drying progressively decreases desiccation tolerance. Thus, the moisture content at which the onset of viability loss was observed on drying increased from near 40% to about 50% moisture content (embryo axis equivalents were 41 and 58%, respectively) as the period of chilling was extended from 6 to 26 weeks. The results can be alternatively expressed by calculation of an MC_{50} value from the parameters shown in Table 1. The parameter MC_{50} is defined as the moisture content at which 50% of the seed population remains germinable after drying. Values of MC_{50} increased from 28 to 40% as the duration of chilling was protracted from 6 to 26 weeks. Corresponding MC_{50} estimates employing moisture contents of the axis (for the two treatments where these data were determined) were 28 and 45% in the case of 12- and 26-week-chilled seed, respectively (parameters used are in Table 1).

The quantification of desiccation tolerance by probit analysis can also be extended to two of the treatments in Fig. 1 for which sufficient data are available. The two treatments which were chilled for 9 weeks subsequent to drying produced almost identical relationships (F = 0.61); the pooled-data estimate for MC_{50} was 38% (Table 1).

### Table 1. Regression coefficients and intercepts for probit analysis of various relationships between germination and moisture content (MC) during desiccation of A. hippocastanum seed

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Seed batch number</th>
<th>Tissues used for moisture content assessment</th>
<th>Figures and symbols to which data refer</th>
<th>Regression coefficient ± s.e. (probit % germination MC %⁻¹)</th>
<th>Intercept of regression line ± s.e. (probit % germination)</th>
<th>Scaled deviance</th>
<th>d.f.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (6 weeks) → D</td>
<td>1</td>
<td>Whole seed</td>
<td>3 (●)</td>
<td>0.205 ± 0.025</td>
<td>−5.766 ± 0.708</td>
<td>8.3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Whole seed</td>
<td>3 (○)</td>
<td>0.235 ± 0.034</td>
<td>−7.368 ± 1.082</td>
<td>5.1</td>
<td>10</td>
</tr>
<tr>
<td>C (12 weeks) → D</td>
<td>2</td>
<td>Embryonic axis</td>
<td>n.a.</td>
<td>0.160 ± 0.041</td>
<td>−4.473 ± 0.654</td>
<td>18.8</td>
<td>8</td>
</tr>
<tr>
<td>C (26 weeks) → D</td>
<td>4</td>
<td>Whole seed</td>
<td>3 (■)</td>
<td>0.183 ± 0.018</td>
<td>−3.739 ± 0.748</td>
<td>7.4</td>
<td>7</td>
</tr>
<tr>
<td>D → C (9 weeks)</td>
<td>2, 4</td>
<td>Whole seed</td>
<td>1 (□)*</td>
<td>0.223 ± 0.020</td>
<td>−8.533 ± 0.749</td>
<td>20.9</td>
<td>10</td>
</tr>
</tbody>
</table>

Data refer to the range of moisture contents over which loss of viability was observed. For two treatments a comparison is made between analyses using whole seed moisture contents and those using the embryonic axis values. The sequence of chilling (C) and desiccation (D) is given with the duration of chilling in parentheses. Seed batches employed are indicated and cross-reference to original data is made where appropriate.

* Single analysis of combined data sets for treatments at ≤48% moisture content.

n.a., not applicable.
reversed during desiccation, the point of coincidence being at approx. 40–50 h (Fig. 4).

The effect of 7 d of equilibration on moisture movement within seeds was assessed; equilibration was applied immediately after drying. Although the exact time course of moisture movement during equilibration is not given, the relationship between moisture content at the start and at the end of the process is presented for the whole seed and for the embryonic axis in Fig. 5. The results are based on pooled data for three experiments. Whole-seed moisture content did not change significantly during the equilibration period, shown by the fact that the slope of the regression line for post-equilibration against pre-equilibration moisture content was near unity (1.14). By contrast, the slope for the embryonic axis line was much shallower (0.72) and was convergent with the line for the whole seed. During equilibration, the moisture content of the axis always increased, returning, where it was lower at the start of equilibration, to a value relatively higher than that of the whole seed.

Longevity effects and water relations

The effect of storing seed at 16 °C was assessed in three experiments. In the first two experiments the effect of moisture content on storage life was assessed, using seed which had been fully chilled to release dormancy before

Fig 4. Relationship of embryonic axis (○) and whole seed (●) moisture content (MC), plotted on a log₁₀ scale, to drying time for A. hippocastanum. Before drying, material employed was either (A) unchilled (Batch 2) or (B) chilled for 26 weeks (Batch 4). The fitted lines represent the regression of log₁₀ moisture content on time; \( r^2 \) was within the range 0.95–0.98 for all lines. In A, intercepts were 1.705 and 1.852 \( \log_{10} \) MC % and slopes were \(-0.0026\) and \(-0.0067 \log_{10} \) MC % h⁻¹ for whole seeds and embryonic axes, respectively; in B, corresponding intercepts were 1.721 and 1.991, and slopes were \(-0.0024\) and \(-0.006\).

Fig 5. The post-drying relationship of moisture content before equilibration at 16 °C to that at the end of 7 d of equilibration is presented for A. hippocastanum seed tissues. Data are for the whole seed (●) and axis (○) and are derived from Figs 1 and 6B. Linear regressions for whole seed and axis data gave slopes of 1.14 and 0.72, intercepts of 5.82 and 21.63 and \( r^2 \) values of 0.98 and 0.95, respectively.
The effect of moisture content on probit germination percentage after various periods of storage is presented for *A. hippocastanum* seed held at 16°C. In A, 12-week-chilled seed of Batch 3 was used and mean whole seed moisture contents during storage were 49% (E) and 45% (D) with embryonic axis moisture contents of 62 and 52% respectively; germination was at 16°C. In B, 26-week-chilled seed of Batch 4 was used, mean whole seed moisture contents during storage were 53% (E), 51% (D), 48% (+), 45% (*), 44% (_), 41% (^) and 38% (y) with embryonic axis moisture contents of 74, 71, 67, 62, 61, 55 and 52% respectively; germination was at 26°C. Parameters for fitted lines and corresponding axis moisture contents are in Table 2. Arrows indicate extreme values on the probit scale.

Both % pre-germination during storage and moisture content values for the seed and embryonic axis (with numbers of seeds employed) are shown.

* 60 seeds sown.
† Insufficient data below infinity probits available for analysis.
‡ 120–180 seeds sown.

Results for the first storage experiment employing 12-week-chilled seed of Batch 3 are in Fig. 6A and Table 2. The rate of viability loss during storage was 0.102 probits per week at the lower moisture content of 45% (axis 51%). At the higher moisture content of 49% (axis 62%), seed drying. The results showed a linear decline of probit percentage germination with time (Fig. 6). In addition, immediate desiccation damage was observed on drying below approx. 48% moisture content prior to storage (Fig. 6).

### Table 2. Regression coefficients and intercepts for the relationship between probit % germination and period of storage (weeks) at 16°C for *A. hippocastanum* data presented in Fig. 6

<table>
<thead>
<tr>
<th>Figures and symbols to which data refer</th>
<th>Mean moisture content (± s.d.)</th>
<th>Percent pre-germination during storage</th>
<th>Intercepts (probits ± s.e.)</th>
<th>Regression coefficient (probit loss per week ± s.e.)</th>
<th>Scaled deviance</th>
<th>d.f.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6A (●)</td>
<td>49 ± 2</td>
<td>62 ± 3</td>
<td>13</td>
<td>22*</td>
<td>—†</td>
<td>—†</td>
</tr>
<tr>
<td>6A (○)</td>
<td>45 ± 5</td>
<td>51 ± 6</td>
<td>14</td>
<td>0*</td>
<td>0.339 ± 0.262</td>
<td>—†</td>
</tr>
<tr>
<td>6B (●)</td>
<td>53 ± 3</td>
<td>79 ± 4</td>
<td>23</td>
<td>88†</td>
<td>2.185 ± 0.195</td>
<td>41.6</td>
</tr>
<tr>
<td>6B (○)</td>
<td>51 ± 2</td>
<td>70 ± 7</td>
<td>25</td>
<td>47†</td>
<td>0.905 ± 0.139</td>
<td>—†</td>
</tr>
<tr>
<td>6B (□)</td>
<td>48 ± 2</td>
<td>66 ± 4</td>
<td>25</td>
<td>0†</td>
<td>0.701 ± 0.148</td>
<td>—†</td>
</tr>
<tr>
<td>6B (▲)</td>
<td>46 ± 2</td>
<td>61 ± 3</td>
<td>24</td>
<td>0†</td>
<td>0.324 ± 0.167</td>
<td>—†</td>
</tr>
<tr>
<td>6B (▼)</td>
<td>41 ± 2</td>
<td>53 ± 6</td>
<td>30</td>
<td>0†</td>
<td>1.033 ± 0.217</td>
<td>—†</td>
</tr>
</tbody>
</table>

* 60 seeds sown.
† Insufficient data below infinity probits available for analysis.
‡ 120–180 seeds sown.
The effect of imbibed storage at 16 °C on subsequent germination at 6 °C of A. hippocastanum seed

<table>
<thead>
<tr>
<th>Duration of chilling pre-treatments (weeks)</th>
<th>Period of imbibed storage (weeks)</th>
<th>Seeds rotten during 16 °C storage (mean %)</th>
<th>Final germination at 6 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>49</td>
<td>3</td>
<td>145</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>7</td>
<td>120</td>
</tr>
<tr>
<td>6</td>
<td>43</td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td>9</td>
<td>40</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

The material used was provided by continuing the desiccation experiment of Fig. 1B with seeds that remained dormant during the 16 °C germination assessment. In the present experiment rotten seeds were removed during storage and the values given were calculated on the basis of the 30 seeds per sample in the original experiment. Germination at 6 °C is based on the indicated number of sound seeds.

Fig 7. The relationship of moisture content to water potential at 16 °C is presented for embryonic axes of A. hippocastanum seed (●). Bars represent ± one s.d. of the moisture content mean. Also, a schematic representation is given for the water potential and moisture content boundaries at which various physiological processes occurred in the present study. The four following processes were included: development during partial drying (□); desiccation injury (◆) (point of onset of viability loss); germination competence (□) (for pre-chilled seed); and longevity changes (■) (for pre-chilled seed). Double-headed arrows draw attention to observed variability in the onset limits for desiccation injury. Sources for the data are given in the text. Points indicated on the diagram demonstrate its use. Thus, drying in the range from Point A (water potential at harvest) to Point B facilitated seed development, whilst drying below this range caused desiccation injury in all treatments (Fig. 1). Increasing the period of chilling raised the point of onset of desiccation injury within the range from Point B to Point C (Figs 1B and 6B). At water potential values above Point A, changes in longevity were observed and germination could proceed for pre-chilled seed. Pre-harvest maturation is thought to occur in the range from 0 to −1 MPa, in line with declining moisture contents (Tompsett and Pritchard, 1993); however, this process is not shown as there are no sorbtion isotherms available for immature seed axes.
Overall, 96% of the transferred seeds germinated (equivalent germination decreased significantly as the period of chilling was extended. The results are presented in Table 3. Post-storage germination percentage combined within each pre-chilling period and the results are shown. The former fit was adopted, indicating that the rate of loss of viability during storage increased about fourfold as moisture content was reduced within this range (Fig. 6B, Table 2). Secondly, for treatments within the lower range, from 38 to 48% moisture contents (axis 52 to 67%), the rate of reduction in germination ability during storage was unaltered \( F = 3.43 \) for parallel against free fitting. The common slope for rate of loss was 0.38 probits per week (Fig. 6B, Table 2) and intercepts on the germination axis for these treatments, indicating initial germination at the start of storage, decreased from 82 to 15% (Table 2).

Some germination was observed for seeds in the three highest moisture content treatments of these long-term storage experiments. Immediately prior to the start of storage in the Batch 4 experiment (i.e. after transfer from 2 °C, during a period of 9-d retention at near 16 °C), a mean of 73% of the seeds germinated in the highest moisture content treatment of 53%. During the subsequent period of 16 °C storage further germination occurred; in the latter case, mean germination increased by a further 15%, giving a total of 88% (Table 2). By comparison, lower total germination (22 and 47%) was observed for seeds in the 49 and 51% moisture content treatments of the Batch 3 and Batch 4 experiments, respectively (Table 2). No germination was observed at any stage in pre-chilled seeds held with moisture contents \( \leq 48\% \), which is equivalent to axis moisture contents of \( \leq 62\% \) (Table 2).

A third storage experiment was conducted by continuing the germination tests that were carried out at 16 °C for the partial desiccation treatments of Fig. 1B. The end of the original germination tests was at 40–49 weeks. At this point 46 seeds that were rotten, representing a mean 3–15% of the seeds originally sown per treatment, were discarded. The remaining ungerminated seeds were transferred to 6 °C; this procedure enabled dormancy to be broken and germination to be assessed. Analysis of variance showed there was no significant effect \( F = 0.031; \text{ d.f. } 1,16 \) of moisture content on final germination (data not shown), but there was a significant main effect of chilling \( F = 13.19; \text{ d.f. } 1,16 \). Consequently, moisture content treatment data were combined within each pre-chilling period and the results are presented in Table 3. Post-storage germination percentage decreased significantly as the period of chilling was extended. Overall, 96% of the transferred seeds germinated (equivalent to 83% including the rotten seeds which had been removed) and 90% produced epicotyls (data not shown).

### Axis water potential

In order to elucidate further the role of water in physiological processes, the relationship between moisture content (in the range from 29 to 73%) and water potential (in the range \(-60 \) to \(-0.5 \text{ MPa} \)) was examined for the embryonic axis of stored seed (Fig. 7). A close similarity was observed between the resulting sorption isotherm and the one presented earlier for axes of fresh seed (Tompsett and Pritchard, 1993). Water potential ranges in which various physiological processes occurred are also given in Fig. 7; these relationships are further discussed below.

### DISCUSSION

#### Dual effect of drying followed by chilling

The promotive effect of artificial drying from about 50% down to 32–40% moisture content on germination (Fig. 1) may represent a continuation of the earlier maturation processes which occur naturally on the trees as the seeds become drier (Tompsett and Pritchard, 1993). A similar post-harvest process has been observed in other species. In *Zizania palustris* L. embryos it occurs within the same range of moisture contents (Aldridge and Probert, 1992); and in *Acer pseudoplatanus* L. seeds it is found over a rather higher range of about 55–80% moisture contents (Hong and Ellis, 1990). The present observations support earlier suggestions that seed of *Aesculus hippocastanum* (Tompsett and Pritchard, 1993) and *Quercus robur* (Finch-Savage and Blake, 1994) may not have completed developmental growth at the time of seed fall.

Partial desiccation is not, however, essential for the completion of such developmental processes in *A. hippocastanum* seed, since naturally shed, undried material germinates at 16 °C after chilling for 9 weeks. Similarly, partial desiccation was not essential for these processes in the case of seeds which had been harvested from the tree between 90 days after anthesis (DAA) and the time of maximum seed fall (Tompsett and Pritchard, 1993). Likewise, post-harvest chilling is not an absolute requirement, since germination occurred in a proportion of the seeds on drying even in the case of unchilled material (Fig. 1). Data for both germination rate and final germination (Figs 1 and 2) indicate that chilling and partial desiccation are interchangeable processes and can be viewed as modulators of developmentally-associated changes in seed quality.

The relationship between final germination and rate of germination (vigour) was generally of the same type as previously reported for various ‘orthodox’-seeded crop species (Ellis and Roberts, 1980). However, in the present case faster germination rates were detected for the 1989 seed lot than for the lot collected in 1990 (Fig. 2); such inter-seasonal differences may be temperature-related, as has been suggested for *Arum maculatum* L. seed (Pritchard, Wood and Manger, 1993).

The lower moisture content limit to desiccation maturation, indicated by peak germination on drying, increased with lengthening periods of chilling up to 9 weeks (Fig. 1). Progress towards germination during treatment would have

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been limited in this experiment due to the unfavourable temperature (2 °C) and the moisture content conditions applied. Desiccation damage is likely to have accumulated in the seeds during moist cool storage, explaining the shift in optimum germinability observed. Longer-term desiccation damage is further discussed below in relation to storage at 16 °C.

Desiccation below the range 32–40% moisture content resulted in loss of viability (Fig. 1). Peak germination on drying failed to reach 100% in any chilling treatment; a similar failure to reach 100% germination following desiccation maturation was observed in earlier studies on other species, although the effect was not well detailed (Hong and Ellis, 1990; Aldridge and Probert, 1992). Overall, these findings suggest that, at moisture contents near that for optimum germination (Fig. 1), opposing effects may operate; the negative effect of desiccation damage in one part of the seed lot may combine with the positive effects of desiccation maturation in another part of the seed lot. If this is so, some seeds will already be non-viable when seed in the treatment reaches the apparent optimum moisture content for maturation, thus explaining why germination remains below 100%.

**Effect of chilling followed by drying**

Whilst increasing the periods of moist chilling prior to drying reduced dormancy, it also increased the threshold moisture content for viability loss by 10% (Fig. 3, Table 1). The latter result is most probably explained by a slow progress towards germination over time. In support of this contention, some seeds in the population chilled for 26 weeks pre-sprouted at 2 °C. A comparable association has been described for *Hordeum vulgare* L. and *Vigna radiata* L. (Hong and Ellis, 1992), which are orthodox; in these species, desiccation sensitivity increased with the period of time that these seeds were held under conditions of hydration favouring germination. Similar results have been found for desiccation-sensitive material employing the following analytical methods: ultrastructure changes and electrolyte leakage in the case of *Camellia sinensis* L. (O. Kuntze) (Berjak, Vertucci, and Pammenter, 1993); and ultrastructure and germination indices for *Avicennia marina* (Farrant et al., 1986). By contrast, for immature *Zizania palustris* material, Vertucci et al. (1995) concluded that cool storage of subemerged embryos for up to 9 months did not reduce desiccation tolerance, despite loss of dormancy.

Although desiccation tolerance for *Aesculus hippocastanum* was reduced as the cold storage period increased, the distribution of seed deaths over moisture content on drying, shown by the similarity in the sigmoid relationships between germination and moisture content in all cases (Fig. 3), remained unchanged. A sigmoid relationship of this type was originally described for *Araucaria heterophylli* seeds (Tomsett, 1982) and was subsequently found to extend to dipterocarps (Tomsett, 1986), *Quercus* spp. (Pritchard, 1991; Finch-Savage, 1992) and to *Z. palustris* (Kovach and Bradford, 1992). Such normal distributions may be explained by the pattern of desiccation sensitivity of individual seeds, by the range of moisture contents within individual seeds, or by a combination of both factors. Whichever explanation applies, however, the closeness of the slopes for probit germination against whole-seed moisture content (Table 1) suggests that the pattern of desiccation damage exhibited by the seeds in each treatment is the same. The lowering of the intercept values obtained, however, implies that the processes take place at higher moisture contents as chilling progresses. From this analysis we conclude that, for different chilling treatments, different threshold levels of relevant factors are required, on chilling, to obtain the same response at the individual-seed level for the parameter under consideration. This approach is essentially the same as that adopted to interpret the pattern of population response in relation to seed dormancy relief by Bradford (1996) and Pritchard et al. (1996).

**Movement of water during and after desiccation**

On drying intact seeds the percentage rate of moisture content reduction for the embryonic axis was twice as fast as that for the whole seed. Consequently, the relationship of axis moisture content to that of the whole seed was reversed at about 40–50 h (Fig. 4). In contrast, for the comparable fruits of *Q. robur* and *Q. nigra* L. the axis moisture content either remained above or became equal to that of the bulk of the other tissues on drying (Finch-Savage, 1992; Connor, Bonner and Vozzo, 1996). The effect in *Aesculus hippocastanum* may be due to the location of the axis within the radicular pocket (Corner, 1976; Pritchard et al., 1996). There, the axis is relatively less enclosed by the cotyledons than is the case for *Quercus*.

Internal moisture changes within the seed were considerable during 7 d of post-drying equilibration. The axis moisture content consistently increased (Fig. 5). Matyssek, Maruyama and Boyer (1991) have shown that a growth-induced change in water potential of about −0.2 MPa can bring about movement of internal water to the growth site. This mechanism might operate in pre-chilled *A. hippocastanum* seed down to about 48% moisture content, at which level germinative growth was still evident (Table 2). At lower moisture contents some passive movement of water from the cotyledons to the axis seems likely. This process may serve to partially protect the axis from desiccation damage after seed abscission. Such findings, combined with other evidence of moisture movement between tissues during storage (Pritchard et al., 1995), re-emphasize the need for care in interpreting seed moisture data in relation to physiological processes.

**Longevity**

The combined data show that moisture content and the period of pre-chilling strongly influence longevity at 16 °C (Fig. 6, Tables 2 and 3). These two factors are discussed below in sequence.

The pattern of longevity for 26-week pre-chilled seed differs above and below a 48% moisture content limit. This
limit is coincident with the critical moisture content value for immediate desiccation damage (Fig. 6). Longevity declined with decreasing moisture content above the limit, whilst below it longevity remained constant as moisture content was reduced. In agreement, longevity decreased as moisture content was reduced from 45% down to the critical moisture content for rapid viability loss at approx. 30% for the recalcitrant-seeded species Araucaria hunsteinii (Pritchard et al., 1995). Additionally, Gosling (1989) presented similar data for Quercus robur fruit stored at 2 °C above and below the critical moisture content for rapid viability loss (at approx. 40%). The results obtained suggest that little physiological benefit to storage life can be expected for mature recalcitrant seed following partial drying. The practical advantage of drying such seed is in the prevention of pre-sprouting.

A sequence may be proposed to account for these effects. In the recalcitrant-seeded species Araucaria hunsteinii (Tomsett, 1983) longevity of hydrated seed decreased as lower oxygen concentrations were imposed. It was argued that this effect may be due to reduced aerobic respiration, a slower rate of repair, and, consequently, quicker accumulation of damage. Furthermore, Ibrahim, Roberts and Murdoch (1983) reached similar conclusions for lettuce seed, which is orthodox, based on the finding that respiration increased as water potential was raised. Thus, in pre-chilled A. hippocastanum seed above 48% moisture content it is possible that efficiency of repair may increase with increasing moisture content, explaining the enhanced-longevity trend reported (Fig. 6). On the other hand, increased oxygen uptake was observed as seeds of Citrus aurantium L., which are orthodox, lost viability in moist storage (Edwards and Mumford, 1985); these complex relationships need further study.

Pre-chilled seed of A. hippocastanum stored at below the threshold moisture content exhibited the same rate of viability loss for all treatments. One explanation is that repair is now impossible and so damage accumulates over time at a relatively constant rate. Any continued reduction in metabolism occurring below 48% moisture content (i.e. about −1 MPa) could exert no further detrimental effect if a lower threshold for effective repair had already been passed. It appears that immediate desiccation-induced viability loss is combined with a slower component of desiccation damage that is somewhat independent of moisture content (Fig. 6). The results obtained suggest that desiccation damage is more complex than had previously been thought (Pritchard, 1991), particularly in respect to its time component.

Comparison of the results for hydrated seed storage data in Fig. 6 and Table 3 with those of Pritchard et al. (1996) indicate that increasing the pre-chilling period at 2 °C from 0 to 26 weeks decreased longevity (σ) at 16 °C from 51 to 6–12 weeks. Although storage of recalcitrant seeds at temperatures which retard germination generally enhances longevity, long-term chilling of A. hippocastanum seed eventually ensures that germination occurs (Pritchard et al., 1996). This suggests an inverse relationship might exist between progress towards germination and longevity (deterioration rate) as has been previously observed for the primed orthodox seeds of Lactuca sativa L. (Tarquis and Bradford, 1992).

Axis water potential in relation to physiology

Axis moisture content is related to water potential in Fig. 7. At the time of maximum seed fall, axis water potential was near −1 MPa (65% moisture content), as found previously (Tomsett and Pritchard, 1993). Despite this high value on shedding, germination is normally prevented by dormancy, a state that can be relieved both by desiccation and by chilling (Fig. 1). In the case of desiccation, the lower limit for water potential below which desiccation maturation processes were not observed was in the region of −3 MPa (Figs 1 and 7). This lower limit coincides with the upper limit for the onset of desiccation-induced viability loss (Fig. 7), illustrating that water potential limits for physiological processes within a seed population can abut or overlap. The limit is also close to the lower limit (−2.4 MPa) at which priming can proceed in lettuce (Tarquis and Bradford, 1992).

Similar critical water potentials (−3 to −5 MPa) for the onset of desiccation intolerance have been reported for several other recalcitrant-seeded species, including Q. robur (Pritchard, 1991), Q. robur (Poulsen and Eriksen, 1992) and Araucaria hunsteinii (Pritchard et al., 1995). However, for A. hippocastanum this threshold was shown to be highly dependent on the post-harvest treatment of the seeds. Both moist chilling for 26 weeks (Fig. 3) to near the point of pre-germination and longer-term retention at reduced moisture contents (Fig. 1, Table 1) raised the desiccation tolerance threshold to about −1.5 MPa (Fig. 7). This value is close to the permanent wilting point for some leaf-type material (Kramer, 1983). Thus, the critical water potential for the onset of desiccation injury in the axis of pre-chilled A. hippocastanum seed is close to that of adult plant tissues, even before germination occurs.

The equilibrium relative humidity (RH) equivalent for the onset of desiccation damage in A. hippocastanum embryos was calculated from the above water potential values to be in the range 97.5–99%. By contrast, in the case of Zizania seed, Vertucci et al. (1995) gave a single value for the parameter ‘water activity’ of 0.9, implying an equivalent of about 90% RH for tissue-damage onset in Zizania, a much lower value than that for Aesculus hippocastanum. However, the storage physiology category of Zizania species is in dispute (Aldridge and Probert, 1992; Kovach and Bradford, 1992; Vertucci et al., 1994).

The longevity of pre-chilled seeds appeared to be independent of water potential over the range from −1 to −2 MPa (Figs 6 and 7), and directly dependent on water potential above this range. Earlier data on Araucaria hunsteinii (Pritchard and Prendergast, 1986; Pritchard et al., 1995) imply that, for this species, longevity is changed with reducing water potential down to approx. −3 MPa. We propose that the relationship of longevity to water potential may also change with pre-treatment history of the seed lot, possibly explaining the observed differences between species.

Germination processes in pre-chilled, non-dormant seed were observed down to water potentials of about −1.3 MPa.
as indicated by pre-germination during 16 °C storage (Table 2, Fig. 7). In general agreement, germination proceeds at water potential values above about –2 MPa in various other species with desiccation-intolerant and desiccation-tolerant seeds; examples include sugar beet (Gummerson, 1986), lettuce (Bradford, 1990) and oak (Finch-Savage and Clay, 1994).

We conclude that at least four physiological processes (desiccation maturation, the onset of desiccation tolerance, longevity and germination) can proceed between particular axis water potential limits, the overall range extending down to about –3 MPa. Moreover, the threshold water potentials at which these physiological processes occur appear to be highly dependent on the post-harvest history of the seeds.

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