Post-abscission, pre-dispersal seeds of *Digitalis purpurea* remain in a developmental state that is not terminated by desiccation *ex planta*

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**Background and Aims** Seed quality may be compromised if seeds are harvested before natural dispersal (shedding). It has been shown previously that slow or delayed drying can increase potential quality compared with immediate rapid drying. This study set out to investigate whether or not there is a critical moisture content, below which drying terminates maturation events for seeds harvested after mass maturity but before dispersal.

**Methods** Seeds of foxglove (*Digitalis purpurea*) in the post-abscission pre-dispersal phase were held at between 15 and 95 % RH for 4 or 8 d, with or without re-hydration to 95 % RH for a further 4 d, before drying to equilibrium at 15 % RH. In addition, dry seeds were primed for 48 h at -1 MPa. Subsequent seed longevity was assessed at 60 % RH and 45 °C.

**Key Results** Rate of germination and longevity were improved by holding seeds at a wide range of humidities after harvest. Longevity was further improved by re-hydration at 95 % RH. Priming improved the longevity of the seeds dried immediately after harvest, but not of those first held at 95 % RH for 8 d prior to drying.

**Conclusions** Maturation continued *ex planta* in these post-abscission, pre-dispersal seeds of *D. purpurea* dried at 15–80 % RH at a rate correlated positively with RH (cf. ageing of mature seeds). Subsequent re-hydration at 95 % RH enabled a further improvement in quality. Priming seeds initially stored air-dry for 3 months also allowed maturation events to resume. However, once individual seeds within the population had reached maximum longevity, priming had a negative impact on their subsequent survival.

**Key words:** *Digitalis purpurea*, seed development, post-harvest treatment, priming, longevity, *ex situ* conservation.

**INTRODUCTION**

Seed development begins with the fertilization of an ovule followed by a period of cell division and differentiation (Bewley and Black, 1994). Thereafter, as dry matter transfers from the maternal plant to the seed, moisture content begins to fall, although seed equilibrium relative humidity (eRH) remains high (Bewley and Black, 1994; Hay and Probert, 2000). The formation of an abscission layer marks the end of reserve accumulation and the attainment of mass maturity (Ellis and Pieta Filho, 1992). In species with dry fruits at maturity, a change in moisture status is the critical variable controlling maturation during the post-abscission phase of seed development. There is a significant, often rapid, reduction in seed moisture content. This loss of moisture is thought to trigger a switch from a seed development to a germination programme. Protein expression studies have indicated that drying immature seeds induces a switch from a developmental to a germination mode; upon rehydration, a developmental pattern of protein synthesis does not resume (Dasgupta and Bewley, 1982; Kermode and Bewley, 1986; Kermode et al., 1986). However, it is also apparent that there can be significant increases in seed quality (e.g. longevity) during the post-abscission drying phase (Pieta Filho and Ellis, 1991; Demir and Ellis, 1992; Hay and Probert, 1995; Lima et al., 2005). Indeed in some wild species, seed longevity has been found to continue to increase until the natural dispersal of mature seeds (Hay and Smith, 2003; Probert et al., 2007). For *ex situ* conservation, seeds should therefore be collected as near to the point of natural dispersal as possible. However, the period during which seeds with maximum longevity can be collected may be narrow; seeds collected too early may have sub-optimal longevity, but if collectors arrive too late the seeds may have already dispersed.

There is some evidence that post-harvest treatments might be used to reproduce on-plant conditions such that seed quality (germinability, desiccation tolerance and/or longevity) continues to develop if they are harvested before the point of natural dispersal (e.g. Hay and Probert, 1995; Hay et al., 1997; Hong and Ellis, 1997; Lima et al., 2005; Probert et al., 2007). Post-harvest treatments usually involve retaining seeds within fruits or holding seeds at higher humidity than would be usual in normal seed processing, to delay or slow drying to rates comparable with those which would occur *in situ*. For example, in *Digitalis purpurea*, the species studied here, intact immature capsules placed under field conditions increased in both seed desiccation tolerance (survival at 15 % RH, 15 °C) and longevity (Hay et al., 1997). Whilst seed moisture content decreased during this treatment, it is likely that their eRH remained high and that multi-molecular water (Vertucci and Leopold, 1987) remained within seed tissues, facilitating continued metabolism. Once seeds have...
equilibrated to very low moisture contents (in equilibrium with 
≤20 % RH) and all of the multi-molecular and most of the 
weakly-bound water have been removed, the accepted view 
is that the developmental state of the seeds is ‘fixed’. In seed 
science (other than ecological studies), the behaviour of 
the seeds at this point is often taken as the baseline against 
which the effects of treatments are compared.

In the natural environment, particularly in temperate 
climates, naturally dispersed (mature) seeds may experience 
such low moisture contents only briefly, if at all, before 
arriving on the soil and regaining a high moisture status. Assuming 
the seeds are dormant, if they are close to or fully imbibed and 
oxygen is available, they can maintain viability for consider-
able periods, presumably because they can metabolize and 
repair damage (Villiers and Edgcumbe, 1975; Ibrahim and 
Roberts, 1983). These repair processes are impaired at low 
water potentials (Vertucci and Farrant, 1995; Pammenter and 
Berjak, 1999) and do not operate below a critical moisture 
content. This cessation of metabolism upon drying in mature 
seeds contrasts with the continued improvement in the 
quality of developing seeds as they dry. Furthermore, it is 
when seeds are at intermediate RH (e.g. 50–75 % or more) 
that ageing of ‘air-dry’ seeds occurs most rapidly (Roberts 
and Ellis, 1989). Even though it is possible or even probable 
that developing seeds accumulate damage during maturation 
drying, evolutionary theory would suggest the benefits of 
this drying must outweigh the detrimental effects of any 
damage that occurs.

Seed priming, a conditioning treatment used by the seed 
industry to improve the rate of germination and seedling 
vigour, involves rehydrating seeds to just below full imbibition 
(Heydecker and Gibbins, 1978). The improvement in these 
seed quality parameters is usually attributed to the activation 
of repair mechanisms that normally commence during the 
imbibition stage of germination (Burgass and Powell, 1984; 
Sivritepe and Dourado, 1995). Priming has been shown to be 
both beneficial (Georgiou et al., 1987; Probert et al., 1991) 
and detrimental (Argeric et al., 1989; Tarquis and 
Bradford, 1992) to longevity. In seeds of cauliflower 
(Brassica oleracea var. botrytis), seed longevity increased 
following priming only if the seeds had been aged previously; 
longevity was impaired if the seeds had not been aged 
(Powell et al., 2000). Thus, whilst there is usually correlation 
across a range of seed quality parameters, including longevity, 
during seed development (Demir and Ellis, 1993) and for dry 
seed lots (Ellis and Roberts, 1981), this is not always the case 
following a priming treatment.

This study was a physiological investigation to find out (a) 
whether developmental and maturation events were accelerated 
and/or switched off by holding post-abscission and pre-
dispersal phase seeds at 80, 65, 50, 30 or 15 % RH and (b) 
whether or not seeds could resume development upon rehydra-
tion at 95 % RH. Also the effects of a re-hydration treatment 
applied immediately after harvest or after a short period of 
drying were compared with a priming treatment given to simi-
larly dry seeds a few months after harvest. The null hypothesis 
was that seed quality development is terminated by desicca-
tion. Foxglove, Digitalis purpurea, was chosen as the study 
species because its seed development has previously been 
studied in detail (Hay, 1997). It is a biennial or short-lived 
perennial species, native to the UK, commonly found 
growing in open places, particularly woodland clearings, on 
acidic soils (Stace, 1997). Plants produce one or more linear 
inflorescences of many flowers, each, when pollinated, 
giving rise to a capsule containing hundreds of seeds. The 
seeds are small (mean 1000 seed weight 0.1 g) and have an 
oil content of approx. 40 % dry weight basis (Liu et al., 
2008). Therefore, in the quantities and conditions used in the 
experiments, seed moisture content would be expected to 
equilibrate rapidly with the atmospheric relative humidity. 
Increases in germinability, desiccation tolerance and longevity 
have been observed in immature foxglove seeds when either 
slow-dried (Hay and Probert, 1995) or held within intact 
detached capsules under ambient conditions (Hay et al., 1997).

MATERIALS AND METHODS

Seed collection

Flowers from a wild population of foxglove (Digitalis 
pupurea L.) in the Loder Valley Nature Reserve (OS Grid 
Reference TQ 336300) were tagged as they were just 
opening on 7 June 2005. The tags were thin strips of electrical 
wire marker tape (W. H. Brady & Co., USA) wrapped loosely 
around the pedicel. Seed development and maturation was 
monitored by determining seed moisture content on samples 
of seeds harvested at regular intervals. Guided by data from 
Hay and Probert (1995), seed capsules were harvested on 17 
July, 40 days after flowering (DAF) when their moisture 
content was expected to be just below 50 % (on a fresh 
weight basis). At this value, seeds would be expected to 
have achieved maximum desiccation tolerance but maximum 
longevity would not yet have been acquired.

Post-harvest treatments

Capsules were taken to the laboratory and seeds removed 
quickly by shaking or using a small paintbrush. Samples of 
these seeds were placed in glass Petri dishes at 20 °C with 
30, 50, 65, 80 or 95 % RH. The RH treatments were created 
using non-saturated solutions of lithium chloride (LiCl; Hay 
et al., 2008) in polyethylene sandwich boxes (160 × 115 × 
60 mm), sealed with Nescofilm (Bando Chemical Ind. Ltd, 
Japan). After 4 d half of the seeds at each RH were 
removed, split into two sub-samples and then either placed 
at 95 % RH for 4 d before being transferred to a dry-room 
maintained at 15 °C and 15 % RH, or transferred directly to 
the dry-room. This was repeated after 8 d with the remainder 
of the seeds. A control sample (no post-harvest treatment) 
was dried immediately at 15 °C and 15 % RH. Sub-samples 
of these seeds were re-hydrated at 20 °C and 95 % RH for 
4 d after 4 or 8 d and then returned to 15 °C and 15 % RH. 
Treatments are summarized in Table 1. Seed moisture 
content and ability to germinate were monitored throughout 
the post-harvest treatments and after 10 or 14 d at 15 °C and 
15 % RH. Seeds were held in the dry-room for between 14 
and 33 d before storage experiments. Under the conditions 
of the above post-harvest treatments and the experimental 
storage conditions and seed priming treatments described 
later, the availability of oxygen to seeds was not impaired.
Table 1. Moisture content during post-harvest treatment of D. purpurea seeds collected in 2005 at 40 DAF, with a harvest moisture content of 43.9 ± 3.42 % (f.w.b.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration in post-harvest treatment (d)</th>
<th>Moisture content (mean; % f.w.b.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>95 % RH</td>
<td>4</td>
<td>17.0±c–17.2±c</td>
</tr>
<tr>
<td>80 % RH</td>
<td>4</td>
<td>17.2±c–17.3±c</td>
</tr>
<tr>
<td>80 % RH</td>
<td>8</td>
<td>17.3±c–17.4±c</td>
</tr>
<tr>
<td>65 % RH</td>
<td>4</td>
<td>17.0±c–17.3±c</td>
</tr>
<tr>
<td>65 % RH</td>
<td>8</td>
<td>17.1±c–17.3±c</td>
</tr>
<tr>
<td>50 % RH</td>
<td>4</td>
<td>17.3±c–17.4±c</td>
</tr>
<tr>
<td>50 % RH</td>
<td>8</td>
<td>17.4±c–17.5±c</td>
</tr>
<tr>
<td>30 % RH</td>
<td>4</td>
<td>16.7±c–16.8±c</td>
</tr>
<tr>
<td>30 % RH</td>
<td>8</td>
<td>16.6±c–16.8±c</td>
</tr>
<tr>
<td>15 % RH</td>
<td>4</td>
<td>15.3±c–15.5±c</td>
</tr>
<tr>
<td>15 % RH</td>
<td>8</td>
<td>15.5±c–15.6±c</td>
</tr>
</tbody>
</table>

Seeds were held at a range of different post-harvest RHs for 4 or 8 d with and without re-hydration at 95 % RH for 4 d.

Values given are the mean of five replicates; those with different superscript letters are significantly different (P < 0.05).

Seed moisture content determination

Seed moisture contents were determined gravimetrically using five replicates of approx. 30 seeds each and calculated on a fresh weight basis. Seeds were dried for 17 ± 1 h in an oven at 103 °C (Hay and Probert, 1995).

Seed germination

All germination tests were carried out using four replicates of 25 seeds each. Seeds were sown on 1 % dH2O agar (Fisher Scientific, UK) containing 1 mM KNO3 (to improve rate of germination) in 50-mm-diameter Petri dishes and incubated at 25/10 °C (8/16 h, respectively, with light provided in the warm phase). Lateral illumination with a flux density of 50–85 W m⁻² was provided by 30-W cool white fluorescent tubes. Seeds were scored as germinated when the radicle had emerged by at least 2 mm. Germination tests were terminated after seeds had been incubated for 56 d. In order to identify potential differences in seed quality during the post-harvest treatments, germination was recorded every 1–3 d and germination rate (GR) calculated for each Petri dish using the equation

\[
GR = \frac{\Sigma n_i}{\Sigma t_i n_i}
\]

where \( t_i \) is the duration (in days) since sowing, and \( n_i \) is the number of seeds germinating on day \( t_i \) (Bewley and Black, 1994).

Experimental storage

One hundred seeds were counted into each of 12 glass Wheaton vials (2 mL; Fisher Scientific). The open vials of seeds were equilibrated at 20 °C and 47 % RH, in a 300 × 300 × 102 mm sealed plastic electrical enclosure box (Ensto, Finland) over a non-saturated solution of LiCl to increase seed moisture content (Hay et al., 2008). After at least 14 d equilibration, seeds were transferred to 45 °C and 60 % RH (in sealed plastic boxes over LiCl as before). Vials were removed at intervals of 2–7 d, up to a maximum of 78 d, and the seeds tested for germination. Seed moisture content was monitored during storage to ensure that conditions were stable. Results were subject to probit analysis using GenStat for Windows 8th edition (VSN International Ltd, 2005) to estimate \( \beta_0 \) (half-viability period or the time taken for viability to fall to 50 %) and fit seed survival curves to the data using the equation

\[
v = K_i - \frac{p}{\sigma}
\]

where \( v \) is the viability of a seed lot which has been stored for period \( p \) (days), \( K_i \) is initial seed lot viability [normal equivalent deviates (NED)] and \( \sigma \) is the standard deviation of the distribution of seed deaths in time, i.e. the time for viability to fall by 1 NED (Ellis and Roberts, 1980).

Seed priming

Additionally, seeds that had either been dried at 15 % RH immediately after harvest or held at 95 % RH for 8 d prior to drying, were removed from the dry-room after approx. 3 months. Seeds were equilibrated at 20 °C and 47 % RH as described previously. After 14-d equilibration seeds from each treatment were divided into two sub-samples; half of the seeds were primed for 48 h in the dark at 20 °C in 90-mm-diameter Petri dishes on two layers of filter paper (QL 100; Fisher Scientific) saturated with a solution of −1 MPa PEG 6000 (Fisher Scientific) and sealed with Nescofilm. Petri dishes were wrapped in aluminium foil to exclude light and inhibit germination. The water potential of the PEG solution was confirmed using a WP4 Dewpoint PotentiaMeter (Decagon Devices, Inc., USA). After 48 h priming, seeds were washed in distilled water and excess water was removed using a vacuum pump. Seeds were then dried for 24 h at 20 °C and 47 % RH (i.e. equilibration conditions), before being transferred to experimental storage (45 °C and 60 % RH). Seeds that were not primed were transferred to experimental storage at the same time as primed seeds.

Automated isotherm determination

Moisture sorption isotherms were determined using an IGA sorp (Hiden Analytical, UK). Before loading, seeds were dried to a low moisture level by holding them at 20 °C over silica gel. The IGA sorp was run at 20 °C, with humidity set points of 0, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 92 and 95 % RH for two cycles of adsorption and desorption. Moisture content (% fresh-weight basis, f.w.b.) was calculated by assuming that the weight of seeds at 0 % RH (before the first absorption) was equivalent to seed dry weight. This analysis was carried out on seeds that had been:

(a) dried at 15 % RH immediately after harvest;
(b) held at 95 % RH for 8 d after harvest prior to drying at 15 % RH;
for seeds placed at lower RH for similar periods after harvest (Fig. 2). The maximum $p_{50}$ observed was approx. 42 d as seen in seeds given 80 % RH for 4 or 8 d with or without rehydration or seeds given 65 % RH for 4 or 8 d followed by rehydration. This is a near doubling in longevity compared with seeds immediately placed and held at 15 % RH (Fig. 2).

Effects on seed germination rate. There was a significant linear relationship ($P < 0.001$) between the equilibrium moisture content of seeds initially placed at between 15 and 80 % RH for 4 or 8 d and their germination rate (GR; Fig. 3A). Within this relationship, GR was similar in seeds held at the same RH for 4 or 8 d, since the moisture content of the seeds did not change significantly in this period (Table 1). The GR of seeds placed at 95 % RH for 4 d after harvest was higher than that for seeds placed at ≤65 % RH for 4 or 8 d. However, the maximum GR observed overall was in seeds that had been at 95 % RH for 8 d, with GR increasing from approx. 0.115 to 0.127 seeds d$^{-1}$ between 4 and 8 d at 95 % RH, when seed moisture content fell from 22.5 to 17.0 % f.w.b. (Fig. 3A and Table 1). Whether held for either 4 or 8 d at relative humidities ranging from 15 to 80 %, the linear relationship between GR and the moisture level to which seeds had equilibrated was maintained when seeds were transferred to 95 % RH. For the seeds placed at 15, 30 or 50 % RH initially there were also further significant increases in GR (Fig. 3A).

More seed lots achieved the apparent maximum GR of approx. 0.127 seeds d$^{-1}$ when dried at 15 % RH following their initial RH treatments compared with seed lots sown after extended periods (8 or 12 d total treatment period) at the highest RHs without subsequent drying at 15 % RH (cf. Fig. 3A, B). There were significant linear relationships between the GR after equilibration at 15 % RH of seeds not rehydrated and their moisture content after 4 or 8 d at between 15 and 80 % RH ($P < 0.05$). These relationships, as with the response in seed longevity, were not maintained for seeds initially placed at 95 % RH (Fig. 3B). For seeds which were transferred to 95 % RH after the initial RH treatment, there were again linear relationships between moisture content after the initial RH treatment and GR (with the same relative effect of moisture content); however, it was not sustained for seeds rehydrated after initial treatment at >50 % RH (dashed lines, Fig. 3B). Those given just 4 d at the initial RH generally had a GR that was approx. 0.008 seeds d$^{-1}$ lower than those given 8 d at the same initial RH, with or without 4 d rehydration at 95 % RH. The fastest GR, however, was observed for seeds given 4 d at the initial RH followed by 4 d rehydration at 95 % RH.

Effects on seed water relations. The moisture absorption and desorption isotherms show the sigmoid shape typical of orthodox seeds (Fig. 4). Hysteresis was observed at RH values <70 % through two cycles of absorption and desorption (Fig. 4 inset); seeds that were losing water had a higher moisture content at a given RH than those gaining water. At RHs above approx. 70 % RH, seeds dried immediately after harvest or held at 95 % RH for 8 d after harvest had higher moisture contents than seeds held at 65 % RH for 4 d or seeds held at 65 % RH for 4 d followed by 95 % RH for a further 4 d (Fig. 4). The data presented in Table 1, when

(c) held at 65 % RH for 4 d after harvest prior to drying at 15 % RH;
(d) held at 65 % RH for 4 d, then re-hydrated to 95 % RH for a further 4 d before drying at 15 % RH.

RESULTS

Maturity of experimental seed material

Seeds were harvested at 40 DAF when their moisture content was 43.9 ± 4.2 % f.w.b. Whilst some capsules were green and intact, others were turning brown and starting to split. There was no indication that seeds had begun to disperse. Comparison with Hay and Probert (1995) shows that these characteristics occur when all seeds have achieved mass maturity but are some 10–16 d before natural shedding. It is concluded that the experimental seeds had abscised from the maternal vascular tissue and were in the post-abscission phase of their development and maturation.

Post-harvest treatments

Effects on seed moisture content. After 8 d, seeds had equilibrated to 17.0, 9.4, 8.0, 6.9, 5.3 or 4.7 % moisture content (f.w.b.) at 95, 80, 65, 50, 30 or 15 % RH, respectively (Table 1). Loss of moisture between 4 and 8 d was only significant in seeds at 95 % RH ($P < 0.05$), but there was no further change between 8 and 12 d. This suggests that seeds had fully equilibrated by 4 d at all RHs between 80 and 15 % and by 8 d at 95 % RH. There was no significant difference in moisture content between seeds placed at 95 % RH for 8 or 12 d or those equilibrated to lower RH and re-hydrated to 95 % RH for 4 d ($P > 0.05$). An exception occurred in seeds held at 15 % RH for 8 d which had a significantly lower moisture content ($P < 0.05$) when re-hydrated.

Effects on seed longevity. Seeds initially placed at 30–95 % RH before transferring to 15 % RH had much greater longevity than seeds dried at 15 % RH immediately after harvest (Figs 1 and 2). This was expressed as a longer log phase before viability loss was observed (Fig. 1). In general, the subsequent distribution of seed deaths was more uniform in seeds given a post-harvest treatment compared with those immediately placed at 15 % RH. This is reflected in the reduction of the time for viability to fall by 1 NED (e.g. from 84.1 down to 50 %) from 8.3 to approx. 6.7 d. For seeds initially placed at 30, 50 or 65 % RH, rehydration to 95 % RH further increased their longevity, again, by extending the period before rapid loss of viability (Fig. 1B–D). There was little difference between seeds treated for 4 or 8 d at 30–80 % RH, either with or without the 95 % rehydration (Figs 1B–E and 2). Regardless of whether or not the seeds were subsequently placed at 95 % RH, the higher the RH of the post-harvest conditions, the greater was their longevity (half-viability period; Fig. 2). Seeds initially placed at 15 % RH for 4 or 8 d and then rehydrated at 95 % RH, also followed this trend. However, this relationship breaks down for seeds placed at 95 % RH after harvest. The improvement in longevity after 4 or 8 d at 95 % RH before drying was not as great as...
FIG. 1. Survival curves, fitted by probit analysis, for *Digitalis purpurea* seeds harvested in 2005 at 40 DAF and in experimental storage at 45 °C and 60 % RH (5.5 ± 0.09 % moisture content f.w.b.). The data for dry control seeds (dried immediately at 15 % RH) is repeated (grey squares) on all graphs. (A) Seeds dried initially at 15 % RH and re-hydrated at 95 % RH after 4 (squares) or 8 d (triangles); (B) seeds dried initially at 30 % RH for 4 or 8 d; (C) seeds dried initially at 50 % RH; (D) seeds dried initially at 65 % RH; (E) seeds dried initially at 80 % RH. Seeds were dried for 4 or 8 d at the initial RH and then either dried at 15 % RH (closed squares or triangles for 4 or 8 d, respectively, and continuous lines) or re-hydrated at 95 % RH for a further 4 d before drying at 15 % RH (open squares or triangles for 4 or 8 d, respectively, and dotted lines). (F) Seeds dried initially at 95 % RH for 4 (squares), 8 (triangles) or 12 (circles) d before drying at 15 % RH. For all treatments individual lines of best fit are shown, although all curves in (A), (C) and (E), excluding the control in (C) and (E), could be constrained to a common slope ($P > 0.05$).
plotted as an isotherm, gives a similar plot to that shown in Fig. 4. Comparison suggests 95 % RH lies in Region III (multi-molecular water present; Vertucci and Leopold, 1987), whilst 80, 65, 50, 30 and 15 % RH cover the full range of Region II (with decreasing amounts of weakly-bound water).

**Priming**

**Effects on seed moisture content.** Seed moisture content after 48 h priming did not differ between seeds dried at 15 % RH immediately after harvest (31.0 ± 0.10 %) and those first held at 95 % RH for 8 d before drying (31.2 ± 0.92 %; P > 0.05).

**Effects on seed longevity.** Priming improved the longevity of seeds that were dried at 15 % RH after harvest by almost 50 %; half-viability periods were estimated as 20.5 d (± 0.34 s.e.) and 29.6 d (± 0.35 s.e.) for control and primed seeds, respectively (Fig. 5). Priming did not improve the quality of seeds from the same harvest that had been held at 95 % RH for 8 d before drying; $p_{50}$ values were 30.6 d (± 0.36 s.e.) and 31.3 d (± 0.31 s.e.) for control and primed 95 % RH-treated seeds, respectively. Seeds dried immediately after harvest and primed were of similar longevity to seeds held at 95 % RH for 8 d after harvest but not primed (29.6 and 30.6 d, respectively).

**Effects on individual seed life spans.** Survival curves for 15 % RH control, 15 % RH primed and 95 % RH control seeds could be constrained to a common slope without significantly increasing residual deviance (P > 0.05). Priming (2 d) 15 % RH seeds or placing them at 95 % RH after harvest (8 d) resulted in increases in $K_i$ and no significant change in $\sigma$ (eqn 2). Hence, all seeds within each population appeared to benefit equally from either treatment and there was no change in the rate of viability loss following priming. However, the survival curve for the 95 % RH primed seeds could not be constrained to the same slope (P < 0.05). Priming 95 % RH seeds resulted in an increase in $K_i$ and a decrease in $\sigma$. Thus, the shorter-lived seeds benefited from the priming treatment whilst priming reduced the life spans of the longer-lived seeds. Although significant, these differences were relatively small.
DISCUSSION

Is seed development switched off by drying below a critical level?

Desiccation, or partial desiccation, has been described as a necessary part of seed development (Le Deunff and Rachidian, 1988) and has been shown to promote the ability of seeds removed prematurely from the maternal plant to germinate (Kermode and Bewley, 1985a,b; Welbaum and Bradford, 1989). Holding immature but desiccation tolerant seeds at high humidity after harvest has also been shown to improve subsequent longevity compared with immediate drying at low RH (≤20%) as is reported here for post-abscission-phase seeds (Figs 1 and 2) and as has been reported elsewhere (Hay and Probert, 1995; Hong and Ellis, 1997; Probert et al., 2007). In mature seeds at eRHs within Region II of the moisture sorption isotherms (which would have included the 15, 30, 50, 65 and 80% RH treatments), the resistance to ageing and thus the preservation of quality in air-dry storage, measured for example by $P_{50}$, would be expected to increase as the moisture content reduced. However, for the current results with immature seeds the opposite appears true. Seed quality was lowest in seeds dried at 15% RH, whilst drying at ≥30 % resulted in progressively greater seed quality (Figs 2 and 3). Thus, the present results...

Fig. 4. Desorption isotherms determined at 20 °C using an IGA sorp for Digitalis purpurea seeds harvested in 2005 at 40 DAF and dried at 15 % RH immediately after harvest (closed triangles); held at 95 % RH for 8 d before drying (closed squares); held at 65 % RH for 4 d before drying (open triangles) or held at 65 % RH for 4 d and re-hydrated at 95 % RH for 4 d before drying (open squares). Moisture content was calculated by assuming that the weight of seeds at 0 % RH was equivalent to seed dry weight. Inset figure shows two cycles of absorption (closed symbols) and desorption (open symbols) isotherms determined for seeds held at 65 % RH for 4 d.

Fig. 5. Survival curves fitted by probit analysis for Digitalis purpurea seeds harvested in 2005 at 40 DAF and experimentally stored at 45 °C and 60 % RH. After harvest, seeds were either held at 95 % RH for 8 d (triangles) before drying to 15 % RH or dried immediately (squares). Seeds were then either primed using −1 MPa PEG for 48 h at 20 °C and dried for 24 h before experimental storage (open symbols) or stored without priming (closed symbols). For all treatments individual lines of best fit are shown, although 15 % RH control, 15 % RH primed and 95 % RH control treatments could all be constrained to a common slope ($P > 0.05$).
demonstrate that beneficial maturation events continue whilst developing seeds are at, or equilibrating to, intermediate moisture contents (corresponding to Region II of the isotherm and expected to be damaging to air-dry seeds), before the moisture content of the seeds falls below a critical level. The idea of a critical moisture content below which maturation events cease has been suggested previously (Welbaum and Bradford, 1989; Leprince et al., 1993). The plasticity in response reported here suggests a number of critical moisture contents below which drying initiates a cascade of different events.

It has also been suggested that, during the post-abscission drying phase, seeds are on a programmed maturation sequence and only acquire their maximum potential quality if they experience progressively lower humidity over a sufficient period of time, as is likely to be the case in situ (e.g. Hong and Ellis, 1997). The present results, however, demonstrate that seeds are not necessarily restricted to the drying-maturation course once it has commenced. Even if this programme is interrupted, the sequence can resume when the seeds are taken back above these critical moisture contents. This can be seen in this study when the seeds were placed at 95 % RH after experiencing lower RH: further increases in longevity and GR were observed (Figs 1–3). There may be benefit at the high humidity as well as the stimulation of other maturation events upon subsequent re-drying. Furthermore, the additive benefits of rehydration are limited if the seeds have initially been placed at high humidity (seen here for GR after drying for seeds placed at 65 or 80 % RH; Fig. 3B). Improvements in seed quality do not appear infinite and development, which occurs when seeds are at high humidity, will reach a conclusion. Although 95 % RH returns seeds to Region III of moisture sorption isotherms where metabolic turnover and repair is expected (Vertucci and Leopold, 1987), increases in longevity have also been observed in seeds dried to, for example, 65 % RH for 4 d and then transferred to a humidity only a little higher (72 %: Butler, 2007). In mature seeds, by contrast, rate of loss of viability would be expected to be greater. In the natural environment, seeds are likely to be exposed to fluctuating ambient RH: a maturation programme, such as that described here, where increases in humidity have more beneficial than detrimental effects on subsequent seed quality would be selected as advantageous.

Since foxglove seeds are small their moisture content would be expected to equilibrate rapidly in response to change in relative humidity (although we note that equilibration at 95 % RH was delayed; Table 1). Thus it is difficult to ascribe the effects of the various RH treatments to differences in the resultant drying rates per se. Furthermore, increases in the subsequent germination rate of seeds occurred between 4 and 8 d at each of 15, 30, 50 and 65 % RH, which cannot be explained by the difference in water content when the seeds were sown since the increases were also apparent in dry seeds (Fig. 3). Rather, higher water contents increase the rate of maturation processes resulting in greater seed quality and so, for example, it was observed that seeds initially placed at 80 % RH subsequently provided the greatest estimate of P50.

The biochemistry or gene expression within the seeds during these post-harvest treatments has not been examined, although earlier research has shown that biochemical changes occur in this phase of development ex planta (Hong et al., 2000). Similarly, the moisture sorption isotherms give an indication that there is a change in the molecular composition of the seeds (Fig. 4) that could be reflected in both of these areas. In contrast with other studies which have demonstrated changes within Regions I and II of moisture sorption isotherms between different seed lots (e.g. Welbaum and Bradford, 1989; Sun et al., 1997), a change in Region III of water binding (i.e. at > 80 % RH) was observed. Seeds which were held at 65 % RH for 4 d (with or without subsequently being placed at 95 % RH) took up less water at high RH compared with seeds that were either immediately dried at 15 % RH or immediately placed at 95 % RH after harvest. This latter treatment is the closest to an ‘on-plant’ control. Thus, this seed lot and the rapidly-dried (15 % RH) seed lot would be expected to behave similarly. In contrast, it was the period at intermediate RH (65 %) that affected the moisture sorption properties of the seeds. Nonetheless the differences in the isotherms and their expected changes in the molecular composition of the seeds do not appear to correlate with either GR or longevity.

Are the effects of high humidity and priming similar?

After a 3-month period in the dry-room (15 % RH) at 15 °C, seed lots from two of the initial treatments, the 15 % RH control and the 8 d 95 % RH-treated seeds, were primed. As observed in the storage experiments carried out earlier (Fig. 1F), the increases in longevity achieved by placing seeds at 95 % RH for 8 d compared with seeds immediately dried at 15 % RH remained after this much longer period of dry storage (Fig. 5). These increases were consistent with what might have been expected to occur, based on earlier observations in situ (Hay and Probert, 1995). A similar increase in longevity was also observed in seeds dried at 15 % RH and primed at a higher RH (less negative water potential) for 2 d. The seeds although ‘fixed’ in the post-abscission maturation sequence for a much longer period at low moisture content were able to resume and complete these developmental events during priming. Unfortunately, the seeds at 95 % RH for 8 d were not also ‘primed’ to see if the exact effect of this ‘post-harvest’ treatment could be replicated.

The effect of the post-harvest treatment and of priming on 15 % RH control seeds appeared to be uniform for all the seeds in the population. In contrast, when the post-harvest treated seeds were primed, there was a starting point-dependent response, manifest as a decrease in σ (eqn 2). Individuals in the population that were originally shorter-lived increased in longevity whilst those that were longest-lived, lost longevity. If, in this instance, it is assumed that the point during storage at which seeds die is correlated with relative maturity, then the youngest seeds in the population mature during the priming treatment whilst the oldest seeds age. Priming or holding seeds at high humidity post-harvest can improve seed longevity through allowing developmental events to occur if the post-abscission maturation sequence has not reached completion. However, if the sequence is complete, then ageing may occur or seeds may switch to a germination programme whereby desiccation tolerance and hence longevity is impaired when seeds are subsequently dried and/or stored.
Practical implications

Seed collectors are currently advised that if an immature collection is made then seeds should be kept at high RH (>90%; Probert, 2003). Mature collections, on the other hand, should be dried at 15% RH as soon as possible. Where low relative humidity drying facilities are not available, then subsequent handling decisions should take into account both the ambient conditions as well as the moisture status of the seeds. If ambient RH is >50% it is advised that seeds are kept moist and well ventilated; if ambient RH is <50% it will be possible to dry seeds to a ‘safe’ moisture level (Probert, 2003). However, the present research shows that if Digitalis purpurea seeds are collected after mass maturity but before dispersal, then leaving them at almost any intermediate RH, at suitable temperatures in the presence of oxygen, for a few days before drying at 15% RH is unlikely to be detrimental to seed quality. In fact, it was shown that drying such seeds at a wide range of RHs (between 30 and 95% RH, at which seeds reached equilibrium moisture contents of between 5–3 and 17.0% after 8 d) was beneficial to seed quality. If seeds are initially dried slightly too quickly for the maximum benefit in quality to be gained, then re-hydrating after up to 8 d (and possibly longer) can allow maturation to progress further.

The present results suggest that post-abscission pre-dispersal seeds do not respond to moisture levels in the same manner as mature, dried seeds. In fact, the RHs at the upper end of Region II of the isotherm which are most damaging to mature seeds can even be beneficial to such immature seeds. Conditions where ambient RH fluctuates will not be detrimental to developing seeds. This seed behaviour, if confirmed in other species, may allow collectors greater flexibility when handling seeds in the field, where it is not possible to hold seeds under controlled conditions. Post-harvest treatments may be particularly appropriate for species with long flowering periods. Rather than two trips to collect from both early- and late-flowering individuals (Smith, 1995), this research suggests that a single collecting trip could suffice, reducing time spent in the field and expedition costs. Such an approach would involve separate collections being made from plants with mature and immature seeds followed by a post-harvest treatment being applied to the immature collection. Alternatively, if a collection of immature seeds has been made and completely dried, ready for long-term storage, the seeds’ quality may still benefit from a high humidity or priming treatment.

In conclusion, the hypothesis that seed quality development is terminated by desiccation was not proven; when post-abscission pre-dispersal seeds of Digitalis purpurea were dried, no critical moisture content could be found below which seed developmental events were terminated. Re-hydration of seeds from a low moisture level allowed the resumption of the maturation sequence involved in maximizing longevity. This capacity of seeds to resume maturation remained undiminished over several months of dry storage. There is a clear difference in the way developing and mature dried seeds respond to being held at moisture contents within Region II of the isotherm, with developing seeds increasing in quality during time spent at those moisture contents at which mature seeds would be expected to accumulate damage most rapidly.

In maturing seeds, germination rate and longevity are still linked though not as tightly as in mature seeds. We believe that the variable response of seed longevity to priming treatments reported in the literature may be due to variation in the maturity of the seed populations examined as well as the range of maturities between the seeds within each population.

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