Germination-associated events and the desiccation sensitivity of recalcitrant seeds – a study on three unrelated species

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Abstract. The storage behaviour of recalcitrant seeds was assessed using three diverse species: a gymnosperm, Araucaria angustifolia (Bert.) O. Kuntze; a herbaceous monocotyledon, Scadoxus membranaceus (Bak.) Friis Nordal; and a woody dicotyledon, Landomphia kirki Dyer. Seeds were stored under conditions of high relative humidities that maintained seed moisture content and under low relative humidities that caused drying. At regular intervals moisture content was determined, germinability assessed and the ultrastructure of radicle meristem cells examined. Under storage at high relative humidity, seed moisture content was maintained at the original level and subcellular germination events were initiated in the short-term. Such seeds showed enhanced rates of germination when removed from storage and planted. Long-term storage under these conditions resulted in the initiation of subcellular damage which intensified with time and ultimately resulted in the loss of viability. The rate at which germination events proceeded varied among the three species, and could be directly correlated with the period of viability retention under humid storage conditions. Storage under desiccating conditions resulted in subcellular damage and rapid loss of viability. The rate at which the seeds dried varied among the three species. The proportion of water loss tolerated by the different species before loss of viability, correlated with the rate of drying. The storage behaviour of the seeds of these three species is discussed in terms of a previously described model.

Key words: Araucaria (seed viability) – Germination (recalcitrant seeds) – Landomphia (seed viability) – Scadoxus (seed viability) – Seed (desiccation sensitivity) – Viability (retention/loss)

Introduction

Recalcitrant seeds, unlike orthodox seeds are not tolerant of desiccation. These seeds are shed at high moisture contents and lose viability if they are dried, the amount of water loss tolerated varying among species (Roberts 1973). Furthermore, most recalcitrant seeds are sensitive to chilling injury. For these reasons, such seeds cannot be stored under the conventional low-moisture, low-temperature storage conditions used for orthodox seeds (for a review, see King and Roberts 1979). Storage at ambient temperatures in fully imbibed states usually results in microbial contamination, and even if anti-microbial agents are applied the period of viability remains short, varying from a few weeks to a few months depending on the species. To date, there has been no successful method developed for long-term storage of recalcitrant seeds (Chin and Roberts 1980).

Previous studies (Pammenter et al. 1984; Farrant et al. 1986, 1988) on the recalcitrant seeds of Avicennia marina have shown that subcellular germination events are initiated shortly after shedding and that these continue in storage, for up to 10–12 d, even in the absence of additional water. These events include enhanced mitochondrial organisation and succinic-dehydrogenase activity, increased levels of protein synthesis, endomembrane development, and the initiation of vacuolation and cell division. Consequently, such short-term-stored seeds show enhanced rates of germination when removed from storage and planted out. However, once these germination events have proceeded (at least in the root meristem) to the stage when cell division is initiated and extensive vacuolation occurs, the seeds require additional water to complete the germination process. If water is not supplied,
subcellular damage occurs and viability is lost. This is because, as these germination events proceed, the seeds become increasingly sensitive to desiccation and the amount of water loss that can be tolerated declines (or the minimum lethal water content is raised) until, ultimately, even seeds stored such that there is no decline in their moisture content lose viability as their water content becomes lethal (Farrant et al. 1986, 1988).

Storage under desiccating conditions results in dehydration damage which severely curtails the germination process. Because these seeds become increasingly sensitive to desiccation as germination proceeds, the rate at which they are dried in storage affects their viability characteristics. A slow rate of drying allows the germination process to reach a more advanced stage and, consequently, the seeds become more sensitive to desiccation and lose viability at high moisture contents. If dried rapidly, before the seeds have proceeded to any great extent along the germination pathway, they can be dried to slightly lower moisture contents before viability is lost (Farrant et al. 1985).

Farrant et al. (1986, 1988) have proposed a model, based on the data outlined above, which describes the storage behaviour of A. marina. Those authors suggest that although the initiation of germination on shedding was the basis of the storage behaviour of recalcitrant seeds, variations in detail would exist among different species. The present work tests this hypothesis. The storage behaviour of the recalcitrant seeds of three diverse plant types, a gymnosperm, Araucaria angustifolia (Bert.) O. Kuntze; a dicotyledonous woody angiosperm, Landolphia kirkii Dyer; and a herbaceous, monocotyledonous plant, Scadoxus membranaceus (Bak.) Friis Nordal, were studied. Seeds of each species were stored such that there was no change in their original moisture content (wet storage) and also under dehydrating conditions (dry storage). The effect of these storage conditions on seed viability and ultrastructural status was assessed.

Materials and methods

Seeds and storage conditions. Mature, newly-shed seeds of each species were collected locally and transported to the laboratory in sealed polythene bags. The surrounding fruit tissue was removed from the seeds of L. kirkii and S. membranaceus prior to their being dusted with a benomyl fungicide (Benlate; Du Pont) and placed in storage. The cones of Araucaria species break up into numerous bract-seed-scale units (Tompsett 1982, 1984). In the case of A. angustifolia, these consist of an outer woody covering, which surrounds the female gametophyte and embryo. These units, which will be referred to as seeds, were stored intact.

Seeds were stored under sterile conditions in single layers on plastic mesh in sealed plastic buckets at 24–28°C. For the wet storage treatment, water was placed in the bottom of the buckets, the seeds being suspended at a height of 10 cm over the water. Dry storage involved suspending the seeds 10 cm above a 10-cm layer of activated silica gel. The silica gel was renewed every 2 d. At regular intervals, seeds were removed from each of the storage regimes and processed as outlined below, the same procedure being carried out on newly-collected material. Seed availability was restricted, this being a problem with many recalcitrant species, because of the typically abbreviated fruiting period. Additionally, sample size was curtailed because of seed size coupled with the necessity for monolayer storage, and the frequency of sampling.

Moisture-content analyses (10 seeds). The moisture content of individual embryonic axes of A. angustifolia and S. membranaceus was determined gravimetrically. Because of the extremely small embryonic axis, moisture contents of individual whole seeds of L. kirkii were determined. Moisture contents were expressed on a dry-mass basis.

Germination assessment (20 seeds). Seeds were germinated in trays of moistened vermiculite in a greenhouse. The outer woody covering of seeds of A. angustifolia was removed prior to planting. This reduced the period required for germination from 35 to 7 d. Germination was assessed daily. The criterion for germination in A. angustifolia and L. kirkii was root growth to a minimum length of 5 mm; the criterion for S. membranaceus was protrusion of the cotyledonary sheath (Wettstein 1962) through the covering structures. Germinability was expressed both as final percent germination and as germination rate. The latter was calculated using the method of Czatator (1962) and is essentially the slope of the line joining the origin to the shoulder of the sigmoid curve representing the time course of germination.

Ultrastructural studies (10 seeds). Embryos were removed from surrounding tissue. The terminal 2 mm, of the radicle in the case of A. angustifolia and L. kirkii, and of the cotyledonary sheath in the case of S. membranaceus, were excised and processed for transmission electron microscopy according to the method outlined by Drennan and Berjak (1982). In each case, the region of the embryo chosen for examination is the first to show germination-associated, ultrastructural changes.

Results

Storage under conditions that maintained the original moisture content

Embryo/seed moisture content

Wet storage of seeds maintained their moisture content at the original level (Fig. 1). Standard deviations were large, a feature that appears to be common in recalcitrant seed material (unpublished protocol – International Seed Testing Association [ISTA] Working Groups on Recalcitrant Seeds, Zurich, Switzerland. This is particularly true when small embryonic axes are used for moisture-content determination, as was the case for S. membranaceus.
Germination characteristics

Short-term storage at high moisture content enhanced the rate of germination in all three species (Fig. 2a). Germination rate increased in a sigmoid fashion in seeds of *A. angustifolia*, reaching a plateau after 28 d in storage. The experiment was terminated after 40 d due to lack of further seed material. Extended investigations are, however, currently in progress. Rates of germination of seeds of *L. kirkii* increased up to 28 d storage and thereafter declined. Germination rate increased gradually in seeds of *S. membranaceus* stored for up to 40 d. After this period, some of the stored seeds had visibly germinated (protrusion of cotyledonary sheath through covering structures) in the storage containers, which resulted in a large increase in the calculated value of germination rate (Fig. 2a). Germination rates declined after storage for 65 d.

Final percent germination remained at the initial high level in seeds of *A. angustifolia* for the 40 d of the experiment (Fig. 2b). Seeds of *L. kirkii* maintained a final germination of 100% during the period of increasing germination rate. After 35 d in storage, however, there was a decline in final percentage germination and by 50 d in storage there was a total loss of viability (Fig. 2b). A similar trend was observed for *S. membranaceus*, where the final germination percentage declined in parallel with the decline in germination rate, the seeds having lost viability after 75 d in storage (Fig. 2b).

Ultrastructure

Microscopical studies showed that all three species displayed the same general evidence of increasing metabolic activity during short-term storage. However, due to certain differences in ultrastructural organisation, each species will be treated separately. Table 1 gives the results of the ultrastructural effects described below that are suitable for quantification.

*Araucaria angustifolia*. Figure 3 depicts the subcellular detail typical of radicle meristem cells in newly-collected seeds. Cells were compact and characterised by having relatively large nuclei and abundant storage reserves. The latter included protein, in the form of protein bodies, starch (contained in plastids) and lipid bodies. There was little endomembrane development, and few mitochondria.

With storage, meristem cells appeared increasingly metabolically active. Storage products were progressively utilised (Figs. 4, 5, Table 1). The removal, presumably by digestion, of protein re-
Table 1. Quantitation of the ultrastructural changes occurring during short-term wet storage of the seeds of *Araucaria angustifolia, Landolphia kirkii* and *Scadoxus membranaceus*. Area fractions were obtained using a transparent grid overlay on at least 20 different micrographs at a standard magnification. Means ± SD with the number of replicates in parentheses

<table>
<thead>
<tr>
<th>Time in wet storage</th>
<th>Mitochondria</th>
<th>Plastids</th>
<th>Vacuoles</th>
<th>Protein bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No./cell in cross section</td>
<td>% electron-transparent area</td>
<td>% plastids with starch</td>
<td>% starch in plastid</td>
</tr>
<tr>
<td><em>A. angustifolia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly-shed</td>
<td>10 ± 4 (30)</td>
<td>25 ± 6 (300)</td>
<td>100 ± 0 (500)</td>
<td>0 (30)</td>
</tr>
<tr>
<td>28 d</td>
<td>45 ± 9 (20)</td>
<td>25 ± 6 (900)</td>
<td>95 ± 21 (500)</td>
<td>39 ± 22 (500)</td>
</tr>
<tr>
<td>40 d</td>
<td>36 ± 7 (20)</td>
<td>22 ± 8 (792)</td>
<td>0 (500)</td>
<td>0 (500)</td>
</tr>
<tr>
<td><em>L. kirkii</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly-shed</td>
<td>8 ± 2 (50)</td>
<td>44 ± 10 (400)</td>
<td>3 ± 1.2 (50)</td>
<td>-</td>
</tr>
<tr>
<td>40 d</td>
<td>11 ± 2.2 (40)</td>
<td>-</td>
<td>23 ± 9 (40)</td>
<td>-</td>
</tr>
<tr>
<td><em>S. membranaceus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly-shed</td>
<td>9 ± 3.1 (20)</td>
<td>22 ± 4.2 (180)</td>
<td>25 ± 10 (20)</td>
<td>-</td>
</tr>
<tr>
<td>60 d</td>
<td>8.2 ± 2.1 (30)</td>
<td>-</td>
<td>42 ± 13 (30)</td>
<td>-</td>
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</tbody>
</table>

° % of cross-sectional area of plastids occupied by starch grains
° ° % of cross-sectional area of cell occupied by vacuoles

...resulted in the formation of several vacuoles and plastids became devoid of starch. There was an increase in the occurrence of mitochondria (Fig. 4, Table 1) and in the extent of endoplasmic- reticulum development (inset, Fig. 5).

*Landolphia kirkii*. Newly shed seeds of *L. kirkii* did not have a well-defined radicle meristem region. Where present, the meristematic cells were compact, non-vacuolated and had small quantities of starch and lipid. The mitochondrial matrices had electron-transparent regions and there was little evidence of endomembrane development (Fig. 6).

Short-term storage resulted in the formation of a large, well defined radicle meristem. These cells appeared increasingly metabolically active and by 28 d in storage the cytoplasm appeared dense, plastids stained darkly and there was an increase in vacuolation (Fig. 7, Table 1). Mitochondria appeared highly active, having well developed cristae. Cell division had occurred, and cytoplasmic polysomes, rough endoplasmic reticulum and Golgi bodies were evident (Fig. 8).

Seeds stored for longer than 30 d showed increasing signs of subcellular deterioration. Mitochondria appeared to be less organised than before and plasmalemma withdrawal occurred in places (Fig. 9). Total cell collapse accompanied loss of viability.

Fig. 2a, b. Effect of storage time on germination rate (a) and final percentage germination (b) of seeds stored at their initial moisture contents
Fig. 3. Subcellular aspects of radicle meristem cells of newly-collected seeds of *A. angustifolia*. Note presence of storage reserves. *l*, lipid; *pb*, protein body; *s*, starch. × 4500

Figs. 4, 5. Subcellular detail of seeds of *A. angustifolia* stored at the initial moisture content for 28 d (Fig. 4: × 7280) and 40 d (Fig. 5: × 5000; inset showing endoplasmic reticulum; × 12000). Note the frequency of mitochondria (*m*) and formation of vacuoles (*v*) by digestion and/or removal of protein

Fig. 6. The typical ultrastructural appearance of radicle meristem cells of newly-collected seeds of *L. kirkii*. *l*, lipid; *n*, nucleus; *s*, starch. × 6000

Figs. 7, 8. Radicle meristem cells of seeds of *L. kirkii* stored for 28 d at the initial moisture content. Note vacuolation (*v*), darkly staining plastids (*p*) (Fig. 7: × 12700) and cell division, rough endoplasmic reticulum, polysomes (*arrows*) and Golgi body (*G*) (Fig. 8: × 30000)

*Scadoxus membranaceus*. The cells of the cotyledonary sheath of newly-collected seeds were vacuolated and contained little storage reserves. There was little endomembrane development and mito-
chondria had relatively electron-transparent matrices (Fig. 10).

With increased time in storage, starch accumulated in the plastids (Fig. 11, Table 1), mitochondria became highly active in appearance, having well defined cristae and there were signs of Golgi bodies and endomembrane development (inset

Fig. 9. Radicle meristem cells of seeds of L. kirkii stored for 40 d at the original moisture content. Note invagination of the plasmalemma and compaction of the cytoplasm. ×14100

Fig. 10. Subcellular detail of cotyledonary sheath cells of newly-collected seeds of S. membranaceus. The cells are vacuolated (v) and mitochondria have electron-transparent matrices. ×2250

Fig. 11. Subcellular detail of cotyledonary sheath cells of seeds of S. membranaceus stored for 21 d at the original moisture content. ×3640. Note accumulation of starch (s) in plastids and appearance of mitochondria and Golgi bodies (inset; ×10150). er, endoplasmic reticulum; v, vacuole

Fig. 12. Mitosis occurs in the cotyledonary sheath cells of S. membranaceus once it has protruded through covering tissues. ×4500

Fig. 13. Subcellular deterioration occurs in S. membranaceus after storage for 75 d at the original water content. Note the collapsed cell wall (arrowhead), n, nucleus; s, starch. ×2930

Fig. 11). Mitosis occurred in the cells of the cotyle-
donary sheath, once it had protruded through covering structures (Fig. 12).

Accompanying the rapid decline in both rate and percentage germination (more than 65 d in storage), there was a marked deterioration in sub-
cellular organisation. Vacuolar and plasma mem-
Table 2. Proportion of total water lost (%) after dry storage of recalcitrant seeds for various time periods

<table>
<thead>
<tr>
<th>Days in storage</th>
<th>Species</th>
<th>A. angustifolia</th>
<th>L. kirkii</th>
<th>S. membranaceus</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>8</td>
<td>31</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>14/15</td>
<td>12</td>
<td>42</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>20</td>
<td>46</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>26</td>
<td>87</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>30</td>
<td></td>
<td>49</td>
<td></td>
</tr>
</tbody>
</table>

Branes lost their integrity and cell walls collapsed (Fig. 13).

Storage under desiccating conditions

Seed/embryo moisture content

Changes in moisture content during storage over activated silica gel are shown in Fig. 1. In A. angustifolia, moisture content declined gradually from 102% (newly-collected) to 70% over 35 d. The moisture content of L. kirkii seeds declined rapidly, reaching 10% after 28 d in storage. Embryonic axes of S. membranaceus became dehydrated from a moisture content of 342% to 120% over 75 d in dry storage. The rate of dehydration, expressed as a proportion of the total water initially present, is given in Table 2.

Germination characteristics

In both A. angustifolia and S. membranaceus there was a slight increase in germination rate for seeds stored for 7 and 15 d, respectively, and final percent germination remained at the initial high level during this period (Fig. 14). Storage for periods longer than these resulted in a decline in both germination rate and in final percentage germination. While rate of germination declined rapidly in seeds of L. kirkii, final percentage germination remained at the initial high level for the first 7 d, after which it declined (Fig. 14). For all species there was no relationship between either absolute moisture content or the proportion of total water lost and germination rate or final percentage germination (compare Fig. 1 and Table 2 with Fig. 14; see also Table 3).

Ultrastructure

 Araucaria angustifolia. Over the first 7 d of storage, during which the germination rate increased and moisture content declined only slightly, subcellular organisation resembled that of newly-shed materi-

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Fig. 14a, b. Effect of storage time on germination rate (a) and final percentage germination (b) in seeds stored under desiccating conditions

Table 3. Relationship between germinability, proportion of water lost (%) and rate of drying of recalcitrant seeds

<table>
<thead>
<tr>
<th>Species</th>
<th>Proportion of water lost when:</th>
<th>Rate of drying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Germination initially declines</td>
<td>50% final germination</td>
</tr>
<tr>
<td>A. angustifolia</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>S. membranaceus</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>L. kirkii</td>
<td>31</td>
<td>42</td>
</tr>
</tbody>
</table>
Fig. 15. Subcellular detail of radicle meristem cell in embryonic axes of *A. angustifolia*, moisture content 82% (21 d storage). Note the electron transparent spaces in the mitochondrial matrices, and invagination of the plasmalemma (pl). ×17150

Fig. 16. Ultrastructural appearance of meristem cells in seeds of *A. angustifolia* with a moisture content of 75% (28 d storage).

Fig. 17. Total deterioration accompanies viability loss in seeds of *A. angustifolia* (moisture content 70%, 35 d storage). ×4200

Fig. 18. Radicle meristem cells of *L. kirkii*, moisture content 59%. Most cells appear undamaged, although some have deteriorated (arrow). ×6900
al. However, once moisture content declined to 82%, (a loss of 20% of the total water content Table 2), 21 d storage, radicle meristem cells showed signs of subcellular deterioration. Mitochondrial matrices became increasingly devoid of internal detail, and plasmalemma invagination occurred in places (Fig. 15). After storage for 28 d (75% moisture content, 26% of total water lost), withdrawal of the plasmalemma from the cell wall occurred, accompanied by compaction of the cytoplasm (Fig. 16). Total cell deterioration accompanied viability loss (Fig. 17).

*Landolphia kirkii.* After the first 7 d in storage (31% total water lost [Table 2]), the majority of radicle meristem cells of *L. kirkii* appeared undamaged, but some showed signs of deterioration (Fig. 18). With increased time in storage, and further water loss, a larger proportion of cells became increasingly deteriorated (Fig. 19). After 28 d in storage (88% total water lost [Table 2]), total cell collapse occurred (Fig. 20).

*Scadoxus membranaceus.* There was little change in subcellular organisation over the first 15 d in storage. However, after 28 d (40% total water lost [Table 2]) cotyledonary sheath cells became increasingly vacuolated (Fig. 21) and organelles, such as mitochondria (inset, Fig. 21) showed signs of internal deterioration. Initially, this damage occurred in only a few cells, but with increased water loss the extent of deterioration increased and became more widespread. By the time 49% of the total water had been lost the cotyledonary sheath cells were all highly deteriorated (Fig. 22).

**Discussion**

The seeds of all three species examined exhibited typical recalcitrant storage behaviour. In accor-

dance with the original definition of recalcitrance (Roberts 1973), these seeds were shed at high moisture contents, the exact moisture content varying among the species (Fig. 1), and were highly sensitive to desiccation, viability declining as water was lost (Fig. 14). In addition, their storage behaviour was in accordance with that predicted by the model of Farrant et al. (1986, 1988).

Storage under conditions that maintained the original seed moisture content resulted, in the short-term, in the initiation of metabolic events typical of early germination. There was increased mitochondrial organisation, indicating increased respiratory activity; increased levels of cytoplasmic and membrane bound polysomes, implying increased protein synthesis; and the appearance of Golgi bodies, indicating increased subcellular activity in general. Reserve mobilisation occurred in seeds of *A. angustifolia* and nuclear and cell division occurred in *S. membranaceus* and *L. kirkii*. Such ultrastructural changes are characteristic of the early stage of germination in both orthodox (see Ching 1972, and Bewley 1979) and recalcitrant seeds (Farrant et al. 1985, 1986, 1988) and indeed, in germinating material of these species (Farrant et al. 1987). As a consequence of the initiation of these subcellular germinative events, seeds stored for short periods showed enhanced rates of germination when removed from storage and planted out, compared with the newly-shed seeds (Fig. 2).

With increased time in wet storage, however, subcellular damage occurred in *S. membranaceus* and *L. kirkii* (Figs. 9, 13) and there was a concomitant decline in the rate of and ultimately the final percent germination (Fig. 2). The results for *A. angustifolia* are not yet available. The moisture content of germinating seeds of both *L. kirkii* (98%) and *S. membranaceus* (80%) were higher than the moisture contents of wet-stored seeds (86% and 330% respectively). This implies that the completion of the germination process requires a higher water content than that of freshly shed material, leading to the eventual death of the wet-stored material. Such a suggestion is in accordance with the model describing the storage behaviour of recalcitrant seeds of *Avicennia marina* proposed by Farrant et al. (1986, 1988). In both *L. kirkii* and *S. membranaceus*, viability declined shortly after the onset of nuclear and cell division, and it would appear that in these species at least, these events coincide with the requirement for additional water, as is the case with *A. marina* (Farrant et al. 1986).

The rate at which subcellular germination events proceeded to the point at which additional water was required varied and appeared to deter-

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**Fig. 19.** Subcellular damage accompanies dehydration to 21% (21 d storage) in seeds of *L. kirkii.* Note withdrawal of plasmalemma (arrow) and compaction of cytoplasm in central cell, and deterioration of surrounding cells. × 1500

**Fig. 20.** Total cell collapse accompanies loss of viability in seeds of *L. kirkii.* × 8300

**Fig. 21.** Dehydration of embryonic axes of *S. membranaceus* to 200% (28 d storage) results in an increase in vacuolation (e) and deterioration of mitochondria (inset; ×11000). × 2800

**Fig. 22.** Subcellular deterioration accompanies dehydration of axes of *S. membranaceus* to 170% (35 d storage). × 15000
mine the longevity of seeds maintained under wet storage conditions. The subcellular germination events which accompanied short-term storage of seeds of *L. kirkii*, proceeded relatively rapidly and cell division occurred after approx. 28 d in storage. These seeds could thus be stored for only one month before viability declined. Subcellular germination events proceeded at a slower rate in seeds of *S. membranaceus*. Between 40 and 55 d the cotyledonary sheath protruded through the covering structure of many of the seeds. Once protrusion had occurred, mitosis and cell division were noted to occur in the cotyledonary sheath. The seeds of *S. membranaceus* could thus stored for approximately two months, after which viability was rapidly lost.

Storage under desiccating conditions resulted in the initiation of subcellular damage, the extent of which increased as moisture content decreased. Initially, these deteriorative events appeared to occur to a small extent in only a few cells. Presumably, such damage was repaired when the seeds were set out to germinate. This would account for the decline in the rate of germination whilst final percentage germination remained high. With further moisture loss, the extent of deterioration increased and a larger proportion of cells was affected, resulting in a further decline in rate of germination and also in totality of germination. Ultimately a critical proportion of cells was damaged and total loss of viability occurred (Figs. 14a, b). Similar deteriorative events have been shown to accompany dehydration in the recalcitrant seeds of *A. marina* (Berjak et al. 1984; Pammenter et al. 1984; Farrant et al. 1985, 1986, 1988), *Podocarpus henkelii* (Farrant and Berjak 1983; Dewar et al. 1987), *Hovea brasilensis* (Chin et al. 1981), and *Theobroma cacao* (Hor and Hill 1980).

The absolute water contents upon shedding, the rate of water loss and the amount of water loss tolerated varied amongst the species. However, a comparison of Tables 2 and 3 indicates there may be a relationship between the rate at which water was lost under dry storage conditions and the desiccation tolerance of the seeds of the different species. Seeds of *L. kirkii* dried the fastest but tolerated the greatest water loss; those of *A. angustifolia* dried the slowest but were the most desiccation sensitive. Although the model of Farrant et al. (1986) was proposed to describe the storage behaviour of a single species under different conditions, this observation of the desiccation tolerance of seeds of different species being related to the rate of dehydration, is in accordance with the predictions of the model.

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**References**


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