The effect of drying rate on viability retention of recalcitrant propagules of *Avicennia marina*

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Recalcitrant propagules of *Avicennia marina* were stored under different relative humidities to achieve both rapid and slow drying. Irrespective of conditions, short-term (4 – 8 days) storage was accompanied by increased rates of protein synthesis and respiratory activity, the initiation of vacuolation and cell division and also by enhanced rates of germination. These data indicate that the germination process is initiated upon shedding. Storage for longer periods resulted in reduced rates of germination and ultimately, in loss of viability. However, propagules dried rapidly retained viability to a lower moisture content than those dried slowly. It is suggested that as germination changes occur during storage, the propagules become increasingly sensitive to desiccation, which might coincide with the degree of vacuolation. Rapidly dried propagules have not proceeded as far along the germination pathway and, at a given moisture content, are not as desiccation sensitive as those dried slowly. Thus viability loss is dependent upon rates of drying rather than on absolute moisture content or storage time, considered independently.
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Weerspannige propagule van *Avicennia marina* is teen verskillende relatiewe vogtigheidswaardes geberg om vinnige sowel as stadige uitdroging teweeg te bring. Ongeag omstandighede, is korttermyn-berging (4 – 8 dae) gekenmerk deur 'n verhoogde tempo van proteïensintese en respiratoriese aktiwiteit, aanvang van selholtevorming en seldeling, asook 'n verhoogde ontkiemingstempo. Hierdie gegewens dui daarop dat die proses van ontkieming ingelei word deur propaguulverlies. Berging vir langer tydperke het gelei tot verlaagde ontkiemingstempo, en uiteindelik tot verminderde kiemkrag. Propagule wat vinnig uitgedroog is, het egter hul kiemkrag tot op 'n laer voggehalte behou as dié wat stadig uitgedroog is. Daar word voorgestel dat die propagule toenemend sensitief word vir uitdroging, soos ontkiemingsveranderinge gedurende berging plaasvind, en dat dit moontlik saamval met die graad van selholtevorming. Vinnig-gedroogde propagule het nog nie so ver gevorder tot ontkieming nie, en by 'n spesifieke voggehalte is hulle nie so droogte-sensitief soos dié wat stadig uitgedroog het nie. Verlies aan kiemkragtigheid is gevolglik eerder afhanklik van uitdrogingstempo as van absolute voggehalte of bergingstyd.
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Keywords: *Avicennia marina*, desiccation, germination, recalcitrance, seed-ageing

Commemorating the 75th Anniversary of the University of Natal

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Introduction

The seeds (propagules) of most species investigated to date undergo a series of developmental events on the parent plant prior to shedding. The event terminating this series of events, maturation drying, is characterized by a great reduction in the seed moisture content (to approximately 15% or less) and a general reduction in metabolism, following which the seed passes into a quiescent, or sometimes dormant, state. Such seeds, termed orthodox (Roberts 1973), can be further dried to very low moisture contents (5 – 7%) without damage and can usually be stored for long periods. In general, maintenance of a low moisture content under a storage regime of reduced temperature and low relative humidity will ensure viability retention for a century or more (International Board for Plant Genetic Resources [IBPGR] 1976).

Another group of plants produces seeds which do not undergo maturation drying on the parent and are shed at relatively high moisture contents. Such seeds, termed recalcitrant, appear to be damaged by subsequent dehydration and thus are unstorable under the conditions recommended for orthodox species (King & Roberts 1979). To date, alternative storage regimes have proved unsuccessful.

Recalcitrant seeds are damaged by low temperatures (some tropical species are susceptible to chilling injury at temperatures as high as 15 °C [Chin & Roberts 1980]) and so low temperature storage is not possible. Warm, moist storage, on the other hand, results in fungal, microbial or viral infection and curtails storage life of the seeds from a few days to, at most, a few months, depending on the species (Chin & Roberts 1980).

Although *A. marina* (Forssk.) Vierh. is a mangrove species it shows no real features of structural viviparity, and indeed, germination is severely curtailed if mature propagules are forcibly removed from the parent tree (unpublished observations). The propagules are shed within the intact pericarp at a moisture content in the range 170 – 190% (dry mass basis), the actual value being consistent within any one season but apparently varying between seasons. The pericarp is rapidly shed in wet conditions, or retained if conditions are not suitable for seedling establishment. In the latter case, the propagules will retain viability for a matter of days only.

The ultrastructural changes that occur during *short-term* storage of the propagules of *A. marina* are indicative of enhanced subcellular activity, similar to changes occurring during early germination of these propagules, and this has led to the suggestion that germination-type changes are initiated on or shortly after abscission (Pammenter, Farrant & Berjak 1984). Also, it has been shown that the changes that

occur during *desiccation* of these recalcitrant propagules are not analogous to those associated with maturation drying of orthodox types, but rather, appear to be similar to the deleterious events which take place on dehydration of imbibed, germinating, orthodox seeds (Berjak, Dini & Pammenter 1984). *Long-term* storage, however, irrespective of the conditions used, results in deteriorative subcellular changes and a loss of viability. What is not immediately clear is whether this loss of viability is time- and/or moisture-related.

The present investigation was initiated to elucidate the events occurring during storage (short and longer term) of these propagules in an attempt to establish the cause(s) of viability loss.

Materials and Methods

In an attempt to differentiate between the effects of time and moisture content on the viability of the propagules, an experiment was designed in which propagules were stored under conditions of different relative humidity such that different, reproducible drying rates could be achieved.

Propagules were stored on five 5-mm grid, stainless steel, horizontal shelves evenly spaced in a Perspex container, 180 mm high, 425 mm wide and 210 mm deep. Perspex plates with several regularly spaced 5-mm holes at the back and front of the container acted as air diffusers. Air (flow rate approximately 9 dm^3 min^{-1}) was passed through two columns (40 mm diameter, 700 mm length) in parallel, combined and passed horizontally through the container over the propagules. Filling the columns with water generated a relative humidity of 80% and filling them with silica gel produced a relative humidity of 10% (at 22 °C) in the containers, giving rise to a slow and a relatively rapid drying rate, respectively.

Propagules were removed from the rapid drying treatment every second day and from the slow drying treament every fourth day and were processed according to one of the procedures outlined below, the same procedure being carried out on newly-abscissed propagules.

Moisture content analysis

Moisture content of 10 propagules was determined gravimetrically (separately for cotyledons and embryonic axes) on an individual basis. As moisture content is probably highly critical over a narrow range, it has been expressed on a dry mass basis; this allows for a more direct comparison than does the expression of water content on a fresh mass basis. The moisture contents of the cotyledons and axes differed considerably and those quoted (unless otherwise stated) are for the embryonic axes only.

Germination assessment

Twenty propagules were soaked in water for 24 h prior to being placed in trays of moistened vermiculite in a greenhouse.

Germination assessments were performed daily, the criterion for germination being active root growth to a minimum length of 5 mm. As the final percentage of germination need not accurately reflect propagule vigour (since it does not account for the rate of germination), the germination index (GI) of Czabator (1962) was calculated.

$$GI = MDG \times PV$$

MDG (mean daily germination) is a measure of the totality of germination and is calculated as the final per cent germination achieved in the test divided by the length of the test (15 days in this case). PV (peak value) is a measure of the rate of germination. It is calculated as the maximum value of per cent germination on any day divided by the number of days taken to achieve that percentage. It is, in effect, the slope of the line joining the origin to the shoulder of the sigmoid curve representing the time course of germination.

The PV component of the index was found to be more useful when expressing the changes occurring during storage of these propagules (especially in short-term storage) and was used independently of MDG where indicated.

Biochemical studies

Determination of rate of protein synthesis in embryonic axes

The embryonic axes were excised from three propagules, the cut surfaces sealed with vaseline and incubated in 10 cm^3 of water containing 100 mm^3 ^{3}H-leucine, of specific activity 0,93 Ci mg^{-1} and radioactive concentration 1 mCi cm^{-3}. A further three axes were placed in 10 cm^3 of water containing cold leucine as a control. Following incubation in a shaking water bath at 30°C for 4 h, the embryos were blotted dry, the cut surfaces removed (to remove wound sites) and the mass determined. Material was then ground in 10 cm^3 of Tris-HCl buffer (pH 8,0) and 50-mm^3 aliquots of each homogenate were spotted onto three 15-mm-diameter discs of Whatmann 3MM filter paper. The discs were further processed by the technique of Mans & Novelli (1960) to remove all material except protein and were then counted in a Beckman LS 75 000 scintillation counter.

Determination of succinic dehydrogenase activity in embryonic axes and cotyledons

Cotyledons and axes were separated and 0,5 g of each was ground in 10 cm^3 extraction buffer (50 mmol dm^{-3} Tris-HCl; 4 mmol dm^{-3} NaCl; 3 mmol dm^{-3} CaCl; pH 7,0). The homogenate was centrifuged for 30 min at 25 000 g, after which the supernatant was retained and assayed for succinic dehydrogenase activity (Hiatt 1961).

Microscopical studies

In a second experiment, propagules were dried at different rates by storing them over layers of silica gel of different depths.

Newly abscissed propagules were placed in a single layer on plastic mesh supported at a height of 100 mm over a layer of newly dried silica gel in a sealed container. To achieve a rapid drying rate a 100-mm layer of silica gel was used, while a slower drying rate was achieved by using a 20-mm layer of silica gel. The silica gel was changed each day.

Twenty propagules were removed from the containers at regular intervals. One superficial root primordium (of which there are several) was excised from each of five embryos and processed for transmission electron microscopy by a standard glutaraldehyde-osmium procedure which has been used previously for this material (Berjak *et al.* 1984). The remainder of each axis (separated from the cotyledons) along with those of five other propagules was used for moisture content determination; that of the cotyledons was assessed separately.

Results

Effect of dehydration on germination

Figure 1 shows the rate of drying of the propagules with the two treatments and Figure 2a gives the effect of storage under these conditions on the germination index. In each case, irrespective of the treatment, there was an initial enhancement of germination. Considering the contribution of the individual

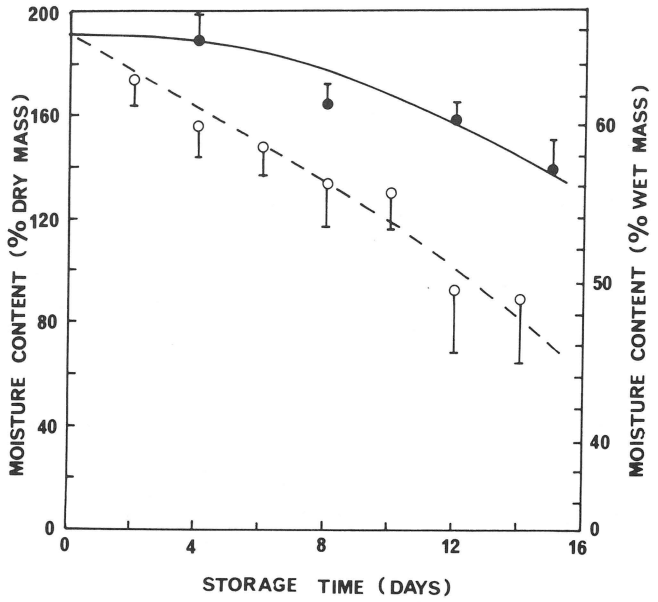


Figure 1 Rate of drying of embryonic axes of *A. marina* stored at 80% relative humidity (●——●) and 10% relative humidity (○– –○).

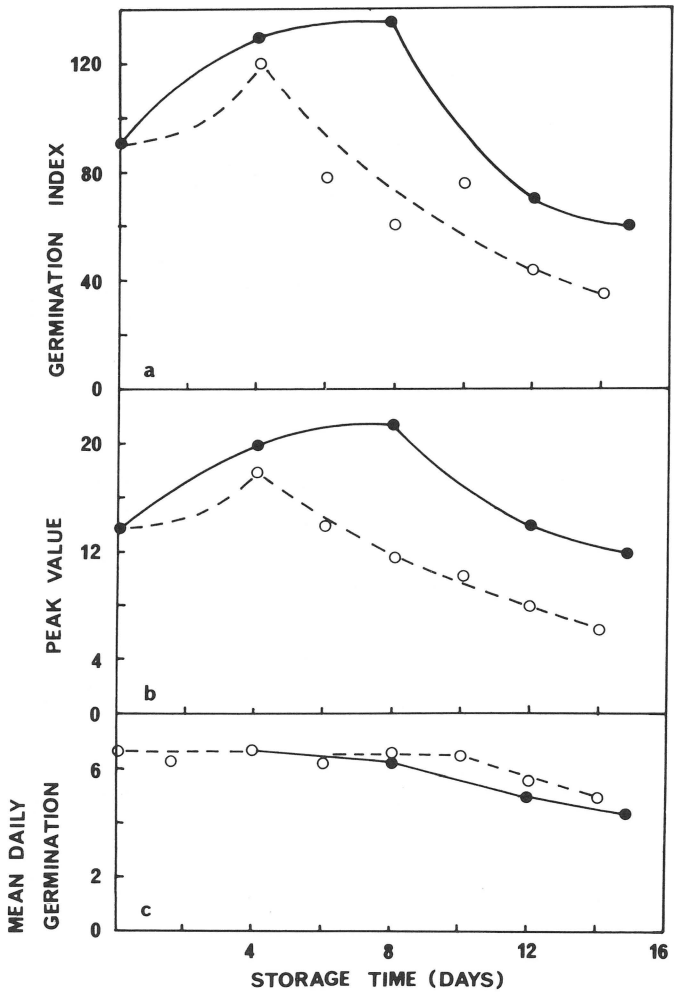


Figure 2 Effect of storage under conditions of 80% (●——●) and 10% (○– –○) relative humidities on (a) germination index, (b) peak value (rate of germination) and (c) mean daily germination (totality of germination).

components of the germination index (Figures 2b & c), it is apparent that this initial enhancement was due to an increase in the PV component of the index; that is, short-term storage resulted in an enhanced rate of germination. Following this, longer term storage was accompanied by first a decline in the rate and later in the totality of germination, the combined effect resulting in a lowered germination value (Figure 2a). What is also apparent is that there was a difference between the treatments in both the extent of the initial rate enhancement and the storage time at which the peak of germination index occurred. The rate of germination increased, to peak on day four and day eight for rapidly dried and slowly dried propagules, respectively, with the enhancement being greater in the latter.

The effect of moisture content on the rate of germination is shown in Figure 3a. The initial enhancement of the rate of germination occurred at higher moisture contents and was greater in the slowly dried than in the rapidly dried propagules. However, once the rate of germination started to decline this trend tended to be reversed, and at any *one moisture content* the rate of germination was higher in the rapidly dried propagules. This effect was even more pronounced when the totality component was included (Figure 3b).

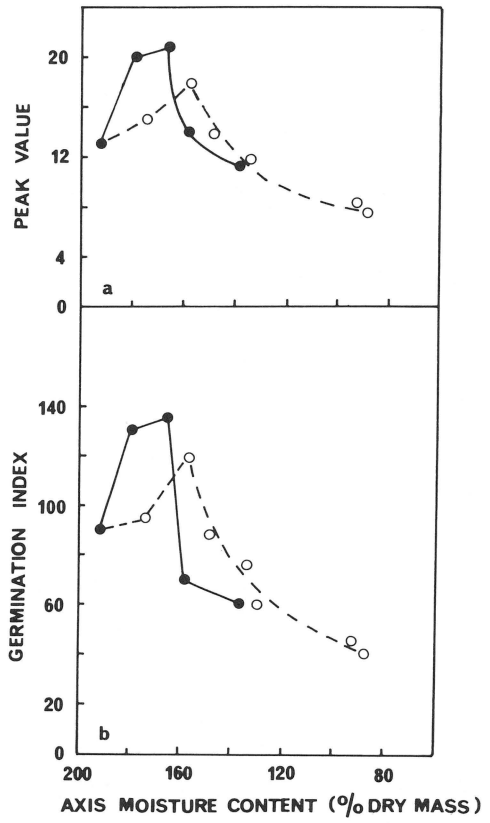


Figure 3 Effect of axis moisture content on (a) peak value and (b) germination index. ●——● — storage at 80% relative humidity; ○– –○ — storage at 10% relative humidity.

Biochemical studies

Results from biochemical studies followed trends similar to those shown in the germination studies. Succinic dehydrogenase activity in both embryonic axes and cotyledons increased from low levels in newly abscissed propagules, to peak after four and eight days of storage in the rapidly dried and slowly dried propagules, respectively, the peak activity being greater in the slowly dried propagules (Figures 4a & b). The rate of protein synthesis in the embryonic axes followed a similar pattern (Figure 5). Again, the extent of enhancement was greater in the slowly dried than in the rapidly dried propagules.

These results, expressed in terms of embryo moisture

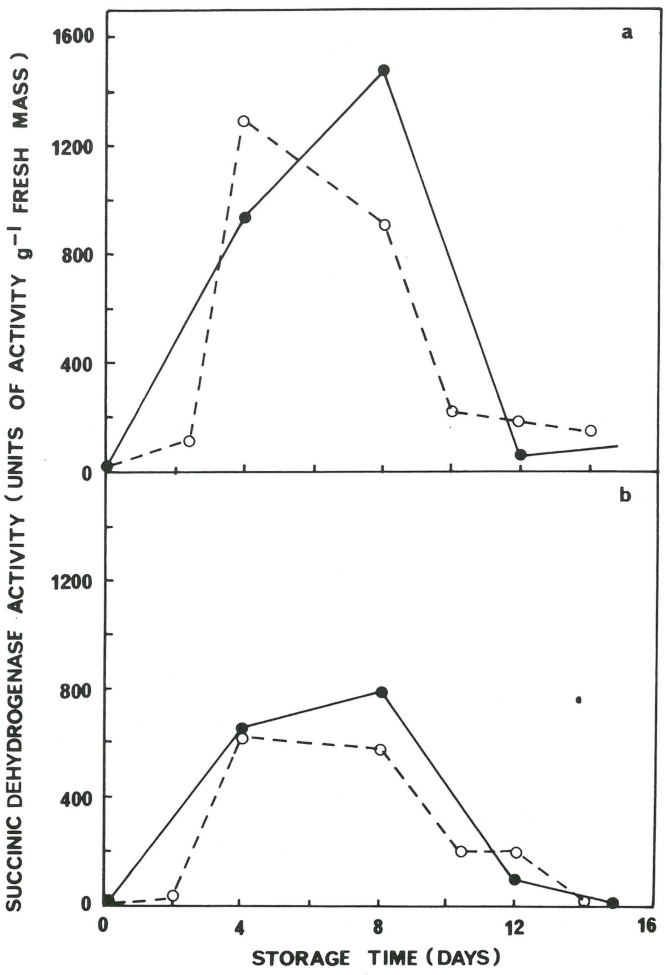


Figure 4 Effect of storage on levels of succinic dehydrogenase activity in (a) embryonic axes and (b) cotyledons. ●——● — storage at 80% relative humidity; ○– –○ — storage at 10% relative humidity.

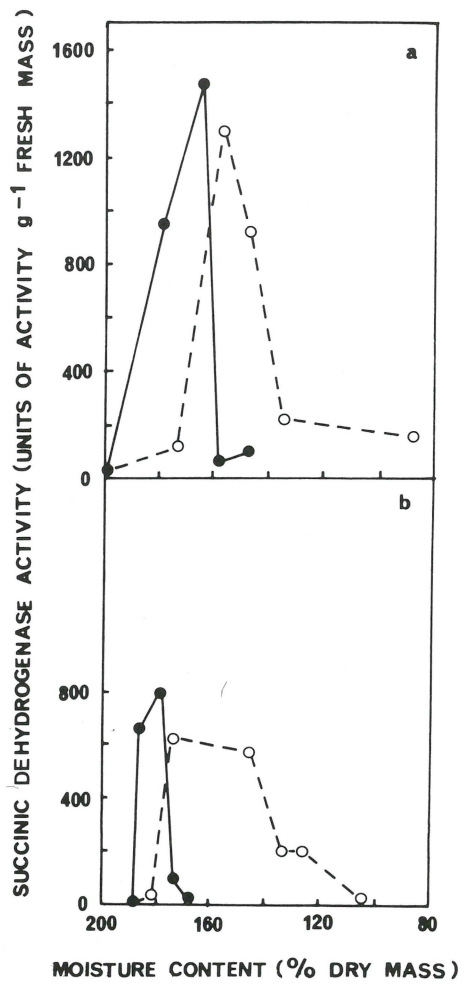


Figure 6 Changes in levels of succinic dehydrogenase activity in relation to tissue moisture content in (a) embryonic axes and (b) cotyledons. ●——● — storage at 80% relative humidity; ○– –○ — storage at 10% relative humidity.

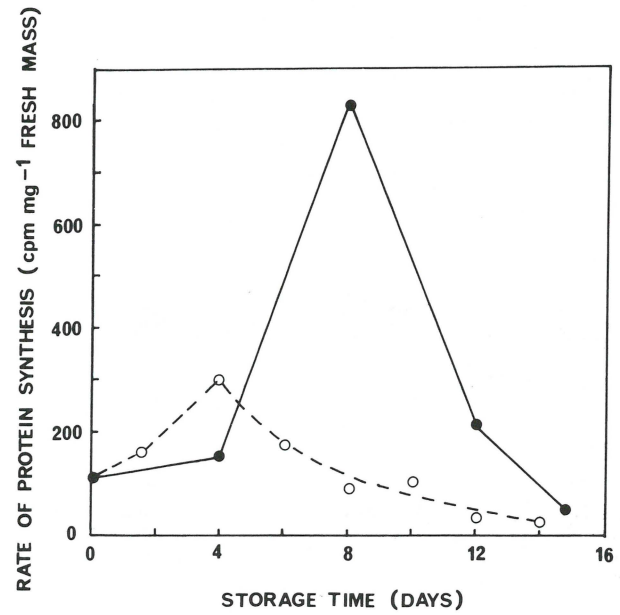


Figure 5 Effect of storage on rate of protein synthesis in embryonic axes. ●——● — storage at 80% relative humidity; ○– –○ — storage at 10% relative humidity.

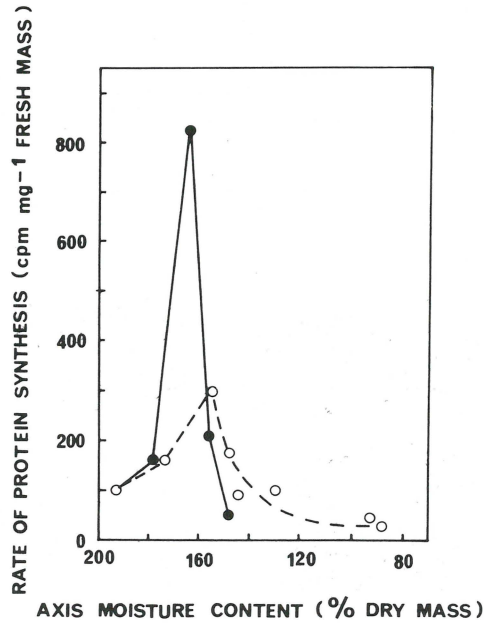
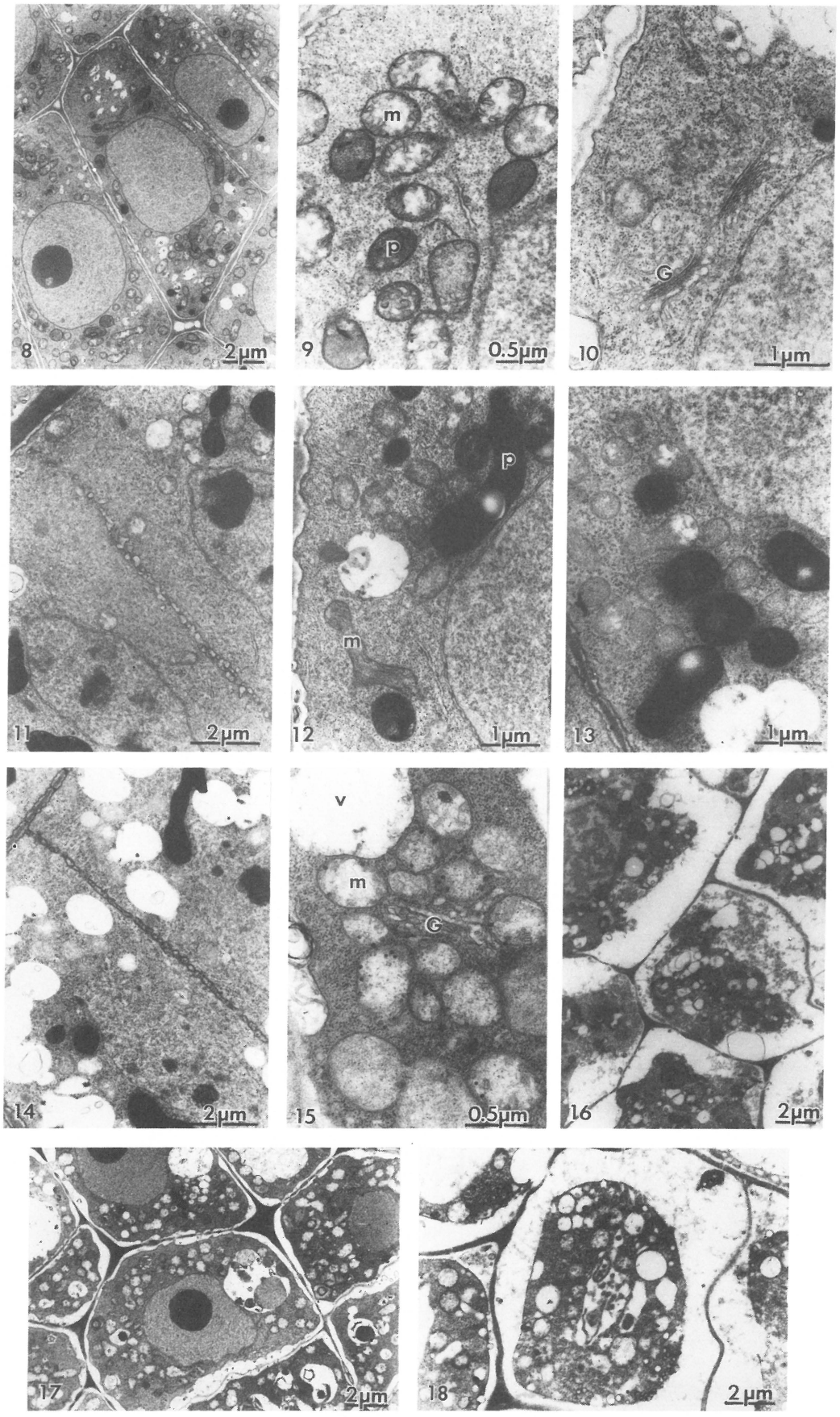


Figure 7 Changes in rates of protein synthesis as a function of axis moisture content ●——● — storage at 80% relative humidity; ○– –○ — storage at 10% relative humidity.

content show that the peak in activity occurred at a higher moisture content in the slowly dried propagules (Figures 6a, b & 7), and, for any *given moisture content* these propagules initially showed a greater amount of activity than the rapidly dried propagules. This trend, however, reversed after the peak in activity in the slowly dried propagules had been reached. These results compare with the germination data relative to moisture content.



Figures 8 – 18 **(8 & 9)** Root primordia of newly abscissed propagules. The state of organization within the cells is typical of what might be expected in an hydrated meristematic plant tissue. Vacuoles that do occur are small and inconspicuous. m — mitochondrion; p — plastid. **(10, 11 & 12)** Root primordia of material that had undergone short-term storage. Note development of Golgi bodies (10) and mitochondria (12). Deposition of starch and dense material within the plastids has also occurred (12), and cell division has taken place in storage (11). G — golgi body; m — mitochondrion; p — plastid. **(13 & 14)** Root primordia of fresh material set out to germinate for 24 h. Note similarity of subcellular organization to that of short-term stored material, including the occurrence of cell division (14). **(15 & 16)** Root primordia of material stored for longer periods. There is a general deterioration of subcellular structure. m — mitochondrion; v — vacuole; G — Golgi body. **(17 & 18)** Root primordia of rapidly dried material [12 days, moisture content 71% (17)] and slowly dried material [29 days, moisture content 68% (18)]. Note the far less deteriorated state of the fast dried material compared to that dried slowly.

Ultrastructural studies

Figures 8 & 9 depict the ultrastructural status of the superficial, meristematic root primordia of newly abscissed propagules. The cells were compact and characterized by only a few, very small vacuoles. Mitochondria had clearly defined cristae and relatively electron transparent matrices. There was little polysome formation, and plastids did not contain much storage material.

By contrast, root primordia of material that had been stored for a short period showed signs of enhanced intracellular activity (Figures 10, 11 & 12). The mitochondrial matrices were considerably denser, an indication of enhanced activity over the newly abscissed condition, and ribosome aggregation to form cytoplasmic and membrane-associated polysomes was evident. There were signs of enhanced Golgi activity and a dense material, as well as starch, had accumulated in the plastids. The occurrence of cytolysome formation suggests the onset of vacuolation. In addition, there is evidence of cell division having occurred during storage (Figure 11).

These changes are very similar to those which occurred during early stages of germination in these propagules. Figures 13 & 14 depict the ultrastructural status of propagules which, immediately after shedding, were set out in conditions conducive to germination for a period of 24 h. Mitochondria appeared to be active, polysomes had formed, Golgi bodies were numerous and appeared to be active, storage material was present in plastids and the onset of vacuolation and cell division was evident.

Longer term storage resulted in the onset of ultrastructural deterioration, which increased with increasing time in storage (Figures 15 & 16). Mitochondria appeared to be less organized than before, ribosomes existed mostly as disassociated monosomes and there was a marked increase in the extent of vacuolation. Deterioration increased with longer storage periods and generally with decreasing moisture content, the actual value of which depended on the drying rate, until ultimately, total viability was lost (Figure 16).

There is, however, a significant difference between the ultrastructural status of propagules which were rapidly dried to a given moisture content and those taking a longer time to reach a similar moisture content (compare Figures 17 & 18). The level of subcellular organization of the rapidly dried propagules was considerably greater than that of the slowly dried propagules at any one moisture content.

Discussion

The ultrastructural, biochemical and germination studies support the earlier suggestion that germination-type changes are initiated on, or shortly after, the abscission of the propagules of *A. marina* from the parent plant (Berjak *et al.* 1984; Pammenter *et al.* 1984).

The ultrastructure of newly abscissed propagules shows the root primordium cells to be well organized, but also suggests that they are relatively quiescent compared with such cells in germinating material. This is indicated by the electron transparency of the mitochondrial matrix; the ribosomes exist largely as monosomes and there is a lack of reserve build-up in the plastids. These features suggest low levels of respiratory activity, protein synthesis and reserve synthesis, or its importation from the cotyledons.

Biochemical evidence supports these observations. The activity of the respiratory enzyme, succinic dehydrogenase, was low in the embryonic axis and undetectable in the cotyledons, and the rate of protein synthesis, as measured by the rate of incorporation of a radioactive label, was low. Also, preliminary unpublished observations indicate that only a low level of α-amylase activity occurred in both cotyledons and axes of freshly abscissed material.

By contrast, after a short period in storage, there was markedly enhanced subcellular activity. Both biochemical and ultrastructural evidence indicate increased respiratory activity and an enhanced rate of protein synthesis, changes which are associated with early germination in orthodox seeds (Ching 1972; Bewley 1979). The similarity of the ultrastructural changes occurring during short-term storage and those occurring during early germination of these propagules supports the suggestion that germination-associated changes are initiated shortly after abscission and continue in storage. The occurrence of cell division in root primordia during short-term storage provides convincing evidence that germination is proceeding. This would account for the apparent enhancement in the rate of germination of propagules that have been stored for short periods (once they are set out to germinate), as the germination process has been initiated during storage. However, longer term storage results in a decline in germination rate and ultimately a decline in the final percentage of germination.

The rate of drying affected the extent of initial enhancement of the germination rate. In slowly dried propagules, the initial enhancement was greater and extended for a longer period than in the rapidly dried propagules (Figure 2b). From this it may be inferred that germination-associated changes were able to proceed further during storage in slowly dried propagules than in those dried rapidly. The biochemical evidence supports this view, as the increase in the levels of respiratory activity and of protein synthesis extended for longer periods and was higher in the slowly dried than in the rapidly dried propagules (Figures 4 & 5).

Although the initial enhancement of the rate of germination occurs at a higher moisture content in the slowly dried material, the decline in rate also occurs at a higher moisture content compared with the rapidly dried propagules. In fact, the decline in germination rate of propagules dried slowly occurs at a moisture content above that at which the rapidly dried propagules attain their maximum germination rate (Figure 3b). This can be explained in terms of initiation of germination of the propagules in storage. It is well documented that imbibed, germinating orthodox seeds become sensitive to subsequent desiccation, the onset of desiccation-intolerance coinciding with the initiation of vacuolation and of cell division (Bewley 1979). A similar situation could prevail in recalcitrant propagules: Since it appears that the changes occurring in storage are germination changes, it is suggested that as these changes occur, and because of them, the propagules become increasingly sensitive to desiccation. The further a propagule has proceeded along the germination pathway, the less the water loss that it will tolerate.

At any one time during drying, the rapidly dried propagules were at a lower moisture content than those dried slowly (Figure 1). After four days in storage there was a rapid decline in the germination rate of the rapidly dried material. Thus, although the propagules had not proceeded far along the germination pathway, moisture content had presumably become limiting and repair may have been necessary, therefore retarding germination when the propagules were removed from storage and set out to germinate. In the slowly dried propagules, on the other hand, the moisture content remained higher and the germination-associated changes could proceed for longer during storage. This gave rise to a greater apparent enhancement of germination later during storage (Figure 2b).

After eight days of slow drying, propagule moisture content became limiting and there was a decline in the rate of germination. This decline occurred at a higher moisture content in the slowly dried than in the rapidly dried propagules because they were further along the germination pathway and thus more sensitive to desiccation.

In material that had been stored to the point at which germination rates were declining, at any given moisture content, propagules that had been dried rapidly had a higher germination index than those dried slowly (Figure 3b). Rapidly dried propagules would not have had the time to proceed as far along the germination pathway as the slowly dried propagules. The slowly dried material would thus have reached a more desiccation-sensitive stage and so at any given moisture content would have been more adversely affected than the propagules that had been dried rapidly. Subcellular structure was better conserved in rapidly dried propagules than in those dried slowly to the same moisture content (Figures 17 & 18). The primordium cells of the rapidly dried propagules were vacuolated to a far lesser extent than those of the slowly dried propagules. It is likely that in these recalcitrant propagules vacuolation plays an important role in the increasing sensitivity to desiccation as germination proceeds, as is the case for germinating orthodox seeds. The rapidly dried propagules had not yet reached a stage of substantial vacuolation and were thus not as sensitive to desiccation. It is well established that most desiccation-tolerant tissues, including embryos of orthodox seeds, have no, or only a few, very small vacuoles (Bewley 1979; Levitt 1980).

Nevertheless, whatever the drying rate, there comes a stage when moisture content becomes limiting. Further water loss results in, first, a decrease in the rate of germination (accompanying which is a decline in the respiratory activity and protein synthesis), followed by a decline in the final level of germination. By this stage several deteriorative subcellular events have been initiated, which become more extensive with increasing water loss, until ultimately there is a total loss of viability.

In summary, it is proposed that germination-associated changes are initiated at, or shortly after, shedding of the propagules of *A. marina*. These changes will continue in storage up to a point (determined by the drying rate), and give rise to an enhanced rate of germination when the propagules are subsequently afforded conditions conducive to the completion of the germination process. However, since they are germinating, these propagules, like orthodox ones, are sensitive to subsequent dehydration and become even more sensitive as germination proceeds. The further along the germination pathway the propagules are, the greater is the water requirement. Thus, irrespective of rate of dehydration, there will come a stage when additional water is required for the completion of germination. Once this stage is reached, unless additional water is supplied, damage will occur which must be repaired on the subsequent addition of water, thus leading to lower rates of germination. If too much damage is done prior to the addition of water, propagules will lose viability.

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