Studies on the Development of the Desiccation-sensitive (Recalcitrant) Seeds of *Avicennia marina* (Forssk.) Vierh.: The Acquisition of Germinability and Response to Storage and Dehydration

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Germinability and responses to storage and dehydration were studied throughout the development of the desiccation-sensitive seeds of *Avicennia marina*. Seeds acquired the ability to produce roots at 55 d after fruit set (DAFS) which is shortly after histodifferentiation, but the capacity for full germinability (seedling establishment) was not attained until 70 DAFS, which is midway through the phase of growth and reserve accumulation. Pre-mature seeds showed a germination lag that was equivalent to the period between harvest and full maturity, but, following short-term storage, this was reduced to that of mature seeds. At no stage, however, would seeds with an intact pericarp germinate.

Once seeds were fully germinable, storage lifespan under non-desiccating conditions was independent of developmental stage, but was considerably reduced by the presence of the pericarp, probably because of fungal contamination. Prior to the acquisition of full germination capacity, the seeds were unable to tolerate any dehydration but became tolerant to slight water loss once they became fully germinable, after which desiccation sensitivity was not influenced by the stage of development. If rapidly dried, excised axes of germinable seeds survived to lower water contents than did axes removed from seeds following slower drying.

**Key words:** Desiccation-tolerance/sensitivity, germination, mangrove, recalcitrant, seed development, seed storage.

**INTRODUCTION**

The differences in storage characteristics between desiccation-tolerant (orthodox) and desiccation-sensitive (recalcitrant) seeds have been documented (for example Chin and Roberts, 1980). Desiccation-sensitive seeds are shed at high water contents and are relatively metabolically active. It has been suggested that their curtailed lifespan is a consequence of the initiation of germination on, or shortly after, shedding (Pammenter, Farrant and Berjak, 1984) and that, in most cases, additional water is required for this process to go to completion (Farrant, Pammenter and Berjak, 1986). The differences in post-shedding behaviour between desiccation-tolerant and -sensitive seeds must arise due to differences in pre-shedding development. There is a considerable body of literature on the development of desiccation-tolerant seeds, but little has been published in this context on desiccation-sensitive seeds. The present contribution is part of a comprehensive study of the development of the recalcitrant seeds of the mangrove, *Avicennia marina* (Forssk.) Vierh.

Immature embryos of many orthodox species can germinate shortly after the completion of histodifferentiation, if excised and placed under suitable culture conditions (Long, Dale and Sussex, 1981; KerMODE and Bewley, 1988). However, intact seeds attain the ability to germinate only considerably later in development, and the proportion of seeds showing normal post-germinative growth increases with seed maturity, often being enhanced by some dehydration (Rosenberg and Rinne, 1986; Ellis, Hong and Roberts, 1987). The stage at which developing orthodox seeds can exhibit desiccation tolerance appears to depend on the rate of drying. Tolerance to slow drying appears to be achieved about midway through development, but tolerance to rapid, forced drying occurs only near the end of reserve deposition (Matthews, 1973; Ellis *et al*., 1987; KerMODE, 1990).

Mature seeds of *A. marina* are highly sensitive to desiccation (Berjak, Dini and Pammenter, 1984; Farrant, Berjak and Pammenter, 1985). In addition, desiccation sensitivity increases as germination progresses (Farrant *et al*., 1986). Studies on seed development in *A. marina* have shown that histodifferentiation takes place up to 55 d after fruit set (DAFS), after which seed growth and reserve accumulation occur until abscission from the parent plant at 83–85 DAFS. Fully-formed seeds of *A. marina* harvested prior to abscission have a lower degree of subcellular organization than naturally-shed seeds that have initiated germination (Farrant, Pammenter and Berjak, 1992). It is possible that such pre-mature seeds may respond differently to storage and desiccation.

In the current study, the developmental stage at which intact seeds, excised embryos and isolated axes (cultured *in vitro*) of *A. marina* become germinable, was determined. The response of whole seeds at different stages of development to storage under conditions which maintained a high water content, was also assessed. As rate of drying can affect desiccation tolerance, this was also tested. It has recently
been shown that extremely rapid (flash) drying of small, isolated embryonic axes of desiccation-sensitive seeds allows drying to much lower water contents than can be achieved by slower drying of whole seeds (Berjak et al., 1990). Thus the response of the relatively large axes of *A. marina* to flash drying was compared with that exhibited by axes during whole seed drying.

**MATERIALS AND METHODS**

The age of developing seeds was assessed and collection undertaken as described by Farrant et al. (1992). Because of the large number of seeds required for other studies, the experiments reported in this paper were conducted only once at each developmental stage. However, germinability assessments were performed over three seasons and storage and desiccation experiments were repeated over two seasons. As no differences in the patterns of responses were evident, the data for the final season only are presented.

**Attainment of germinability**

Germinability assessments were performed on seeds at 10 d intervals from 10 to 50 DAFS and at 5 d intervals from 55 DAFS until the seeds were shed. Seeds were planted in moistened vermiculite and maintained in a greenhouse under natural diurnal temperature regimes (18–30 °C). Mature seeds were immersed in water for 15 min during which natural pericarp sloughing occurred. The pericarp was manually removed from pre-mature seeds prior to planting. Ten seeds were used in each age group for each treatment. All responses (root protrusion, root growth and seedling establishment) were noted.

The *in vitro* germination response of isolated axes was tested only from the completion of histodifferentiation (55 DAFS) to maturity. The following medium was used for all *in vitro* experimentation: Murashige and Skoog (1962) nutrients and vitamins, 15 mM sucrose, 1.0 μM naphthalenacetic acid, 1.4 μM gibberellic acid, 0.1% casein hydrolysate, 4% activated charcoal and 0.8% agar. The pH was adjusted to 5.7. Prior to culture, excised axes (ten per age category) were surface-sterilized for 20 min in 2% sodium hypochlorite containing 0.02% Tween 20. After five rinses in sterile distilled water, axes were aseptically transferred to the culture vessels and placed in a 16 h photoperiod at 200 μmol m⁻² s⁻¹ at 25–27 °C. All responses were noted.

**Seed storage**

These experiments were performed only on seeds that had developed the capacity for normal post-germinative growth. Seeds were stored at 25 °C in a monolayer, on plastic mesh grids which were suspended over water containing 1% sodium hypochlorite, in sterile, opaque plastic buckets. In order to assess the effect of the pericarp on storability, seeds were stored with and without this covering. Seeds were sampled every 2 d. Moisture content was determined gravimetrically for the individual seed components from ten separate seeds, and viability assessed using a further ten seeds planted out in moistened vermiculite after pericarp removal (see above). The criterion for germination was root growth to a minimum length of 4 mm. As final germination totality does not give an indication of a characteristic such as the lag period before the onset of visible root growth, or of seed vigour, these parameters were assessed independently. The lag was expressed as the period elapsed before the first seed had germinated. The rate of germination was calculated as the slope of the germination curve from the end of the lag period to 80% germination. Seed vigour was assessed using the germination index (GI) of Timson (1965) which is calculated by summing the percentage germination for successive days, a total of 30 d being used in the present study.

**Drying treatments**

These were carried out on whole seeds and axes, starting from the developmental stages at which root protrusion was first possible, and continuing to maturity. All drying treatments were performed at room temperature (approximately 25 °C) and utilized ten seeds or axes for viability assessment and a further ten for water content determination.

**Whole seed drying.** Seeds were dried in an air stream [approximately 50% relative humidity (RH)] as described by Farrant et al. (1985). Seeds were sampled and treated as for those stored at high RH. For comparison with flash-drying experiments, the water contents of the embryonic axes alone, are reported.

**Flash drying.** Excised axes were surface sterilized and then rinsed with sterile water as described above. Surface moisture was removed by blotting with sterilized filter paper prior to flash drying. Axes were then dried as described by Pammenter, Vertucci and Berjak (1991). The embryonic axes of *A. marina* are very large compared with those of many other recalcitrant species, thus flash drying for periods of up to 12 h was necessary. As the axes increased in size with maturity, differential drying rates were achieved. Thus axes of young seeds (less than 70 DAFS) were sampled hourly and older axes every 2 h. Following flash drying, axes were aseptically cultured as described above. While all responses were noted, the criterion used for survival was root growth.

**RESULTS**

Pre-mature seeds were unable to slough the surrounding pericarp spontaneously when placed in water. Such seeds, planted with an intact pericarp, did not germinate and rapidly lost viability. There was concomitant microbial, particularly fungal, proliferation. Germinability of pre-mature seeds was thus assessed after removing the pericarp. At 55 DAFS, although root protrusion through the enclosing hypocotyl tissue occurred, there was little further root growth. By 60 DAFS some root growth did take place, and by 65 DAFS primary shoot elongation occurred in several of the embryos. However, these never produced leaves and ultimately became necrotic. From 70 DAFS onwards, all embryos produced normal seedlings. These seeds germinated faster and more uniformly than did those at 65 DAFS (Fig. 1). The lag time was approximately the
same as the period between the age (DAFS) at which the
seeds were harvested and the age at which they would have
been naturally shed (mean 83 DAFS) (Table 1).

Embryonic axes became germinable in vitro at the same
developmental stage as that at which seeds were able to
germinate when planted out. Similarly, although root
growth took place in some axes 65 DAFS, apparently
normal establishment occurred from only 70 DAFS on-
wards. A germination lag similar to that displayed by whole
seeds occurred in axes regenerated on a supplemented
medium (Table 1).

Seeds stored over water maintained a high moisture
content, which increased after 8 d in storage, coincident
with the visible presence of pericarp-associated fungi (results
not shown). The germination index of stored seeds increased
during the first 8 d of storage and thereafter decreased. The
decline in germination index was more precipitous and loss
of viability occurred earlier in less mature seeds (Fig. 2).

Storage without the pericarp extended longevity from 12 to
20 d in 70 DAFS embryos and from 16 to 36 d in older
embryos (Fig. 2). After 2 d in storage, the germination index
of all pre-mature seeds was very similar, the seeds behaving
essentially as a single population until the germination index
started to decrease. The initial increase in the index was
primarily due to the decrease in germination lag from the
original values to that equivalent of mature, naturally-shed
seeds, rather than to a change in the rate of germination
(Table 2). There were no marked changes in the ultra-
structure of root meristem cells of pre-mature harvested
seeds during storage for up to 8 d (data not shown) that
could account for the marked decrease in the germination
lag brought about by short-term storage.

The decline in germination index of seeds stored with
intact pericarps was due to a decline in the proportion
retaining the ability to germinate. The onset of this decline
was coincident with visible fungal growth on the pericarp of
some of the seeds within the storage container. In those
seeds retaining the potential to germinate, the lag in the
onset of this process remained the same until total loss of
viability of the seed sample had occurred. There was little
visible evidence of microbial contamination in embryos
from seeds stored without the pericarp. The decline in
germination index in these seeds appeared to result from a
decline in germination vigour [both an increase in lag and
decrease in germination rate (data not shown)] and
ultimately in the proportion of seeds with the ability to
germinate. The ultrastructure (not shown) of embryos at all
developmental stages just prior to loss of viability in
storage, showed evidence of subcellular damage similar to
that described previously for stored mature seeds (Farrant
et al., 1986).

Young seeds and axes dried considerably faster than more
mature individuals, presumably because they were smaller.
Table 2. Germination lag and rate of germination of seeds of Avicennia marina of different developmental stages (DAFS) after 4 d of storage under high humidity conditions.

<table>
<thead>
<tr>
<th>DAFS</th>
<th>Lag (d)</th>
<th>Rate (% d⁻¹)</th>
<th>Rate (% d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>2</td>
<td>13.6 ± 1.2</td>
<td>15.7 ± 1.8</td>
</tr>
<tr>
<td>75</td>
<td>2</td>
<td>12.5 ± 1.2</td>
<td>8.9 ± 1.3</td>
</tr>
<tr>
<td>80</td>
<td>2</td>
<td>13.6 ± 1.2</td>
<td>16.0 ± 1.9</td>
</tr>
<tr>
<td>ns</td>
<td>2</td>
<td>19.0 ± 3.6</td>
<td>20.0 ± 2.0</td>
</tr>
</tbody>
</table>

ns, newly shed mature seeds. For comparison the rates of germination of seeds of the same developmental age set out to germinate immediately after harvesting are also shown. Rate of germination was calculated as the slope of the germination time course up to 80% germination, ± s.e. of the estimate of the slope.

Fig. 3. Drying time course for whole seeds dried slowly (A), and excised embryonic axes flash dried (B). (◇) 65; (●) 70; (▲) 75; (■) 80 DAFS; (●) newly shed mature seeds. Standard deviations of water contents were approximately 0.15 g g⁻¹ dry weight.

Fig. 4. Germination response to drying of whole seeds (open symbols) and excised axes (closed symbols): (●, ○) 70; (▲, △) 75; (■, □) 80 DAFS; (●, ○) newly shed mature seeds.

Developmental age did not influence the desiccation sensitivity of either the seeds or the excised axes (Fig. 4). The drying rate strongly influenced viability characteristics. Slow drying of whole seeds resulted in a decline in viability once the axis water content had been reduced below 1.0 g H₂O g⁻¹ dry mass (g g⁻¹) and total viability loss at approximately 0.8 g g⁻¹. Flash drying allowed survival to considerably lower water contents: axes exhibited 80% viability at 0.55 g g⁻¹ and total loss of viability occurred at approximately 0.35 g g⁻¹ (Fig. 4).

Discussion

Root growth and seedling establishment occur in mature A. marina seeds only after the pericarp has sloughed. Similarly, in pre-mature seeds, germination cannot occur without pericarp removal. This suggests that the pericarp must have an important role in the prevention of precocious germination.

Embryos of A. marina were able to protrude roots at 55 DAFS, which corresponds to the completion of histodifferentiation (Farrant et al., 1992), although the ability to establish seedlings was attained by both whole embryos and isolated axes only at 70 DAFS. By this latter stage, approximately 30% of the cotyledonary reserves had been accumulated and axis development was essentially complete (Farrant et al., 1992). However, the resultant seedlings were stunted and those from older seeds grew more vigorously. This is similar to the situation described for seeds of other species (for example, Kermode and Bewley, 1985). It appears, therefore, that full morphological development of the axis of A. marina (which has occurred by 70 DAFS) is necessary for the attainment of germinability, and further accumulation of cotyledonary reserves is necessary for optimal seedling survival.

However, the germination lag of harvested pre-mature
seeds of *A. marina* (which cannot accumulate further reserves) was similar to the time required for the completion of the developmental processes on the parent tree. A similar lag occurred in isolated axes on supplemented medium (Table 1) and *in vitro* axis germination was poor without the addition of a carbon source and plant growth regulators to the culture medium. This implies that completion of some developmental event(s) may be necessary for the initiation of germination and that these processes are under the control of the seed itself, rather than under maternal control. This is in contrast to the suggestion of Hughes and Galau (1991) that in cotton a maternal maturation factor sustains the maturation programme whilst the seed is attached to the parent plant.

There was an increase in the germination index, consequent upon a decline in the germination lag, of harvested pre-mature seeds stored for two or more days (with or without the pericarp), compared with that of similar seeds immediately set out to germinate (compare Tables 1 and 2). It is not known what caused this reduction in germination lag, although changes in levels of growth regulators are a possibility. This decrease in lag can be compared with the situation in many orthodox seeds where delaying the initiation of excised immature axes permits or enhances germination, especially if a very slight degree of desiccation has occurred (Rosenberg and Rinne, 1986; Bewley, Kermode and Misra, 1989; reviewed by Kermode, 1990). Although no desiccation occurred during the storage of the *A. marina* seeds, neither was there the increase in water content (from approximately 24 to 30 g g⁻¹) associated with setting the seeds out to germinate.

Storage longevity was not markedly related to seed age after 70 DAFS, but rather to the presence or absence of the pericarp (Fig. 2). Storage within the pericarp severely curtailed longevity and seed death was associated with microbial contamination. With ongoing storage, despite the application of topical mycotoxins, seeds became increasingly encased within a covering that was a proliferating mass of fungal mycelium rather than the original pericarp. A similar situation has been previously documented for recalcitrant seed species during wet storage (Mycock and Berjak, 1990). The differences in storage survival between the seeds stored with, and without, the pericarp can probably be largely explained by this severe contamination.

Developing *A. marina* seeds are not able to tolerate any drying until 70 DAFS when they attain the full capacity to germinate. After 70 DAFS, once the seeds become tolerant of some dehydration, developmental age has little effect on the degree of water loss tolerated (Fig. 4). There are few studies in which the response of developing recalcitrant seeds to desiccation has been tested. Finch-Savage (1992) has shown that the desiccation tolerance of *Quercus robur* L. increases with increasing developmental stage, once the seeds have become germinable. Hong and Ellis (1991) have suggested that developing seeds of *Acer pseudoplatanus* L., once germinable, become increasingly tolerant of drying until a month prior to shedding, after which the response to desiccation remains unchanged. In contrast, Berjak, Vertucci and Pammenter (1992) have shown that rapidly-dried, excised axes of *Landolphia kirkii* Dyer are less desiccation sensitive than are mature axes. To resolve these apparent differences, desiccation responses require to be evaluated at several unequivocally-defined stages during development for a variety of desiccation-sensitive seeds from a wide range of natural habitats.

Flash drying, which applies exclusively to excised axes, resulted in much more rapid removal of water (Fig. 3) and allowed survival to considerably lower water contents compared with axes from seeds of *A. marina* dried intact, irrespective of developmental age from 70 DAFS onwards (Fig. 4). It has been suggested that flash drying permits survival to lower water contents in axes of *L. kirkii* and *Camellia sinensis* (L.). O. Kuntze, because water is removed sufficiently rapidly to prevent any significant desiccation-associated damage from occurring (Berjak et al., 1990; Berjak, Farrant and Pammenter, 1989). Although the size of the *A. marina* axes necessitated flash drying over a period of hours, as opposed to minutes for the species described by those authors, this is sufficiently rapid to allow a similar conclusion to be drawn. As the axes were immediately rehydrated (in culture) after drying, no time was allowed for the accumulation of damage in the (partially) dehydrated state. Rate of drying does not appear to affect desiccation sensitivity of *Q. robur* seeds (Finch-Savage, 1992). Although rapidly-dried isolated axes did survive to lower water contents than those dried within intact seeds, the effect was ascribed to the removal of the influence of the cotyledons. Nothing can presently be said about the cotyledonary influence in this context in *A. marina*, a possibility that needs to be investigated.

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**LITERATURE CITED**


