Effect of desiccation and storage on the seeds of two related species in the Amaryllidaceae from the western Cape Province.

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Abstract

This paper reports results on the first detailed physiological study undertaken on potentially recalcitrant seeds from the western Cape. Germination and dehydration behaviour of two related monocots *Boophane flava* Barker ex Snijman and *Amaryllis belladonna* L. (Amaryllidaceae) were studied to determine whether these seeds are indeed recalcitrant, and if so their degree of tolerance, and to gain an understanding of the reproductive strategies of these species through their seeds behaviour. Seeds were stored under mildly desiccating conditions, moisture content was determined at regular intervals and the ultrastructure of the hypocotyl apex tissue was examined. Both species are shed at high moisture content in a metabolically active state, and are able to tolerate considerable loss of this water (420-120% for *B.flava*) before water contents became lethal. Nevertheless, seeds were killed at high water contents a characteristic typical of recalcitrant seeds. *A.belladonna* seeds showed increased levels of subcellular organization during storage despite low viability scores. *B.flava* seeds showed hypocotyl extension and in a few instances roots and shoots were produced in storage, they began to lose viability after 8 weeks in storage at a moisture content below 120%. *B.flava* is a moderately recalcitrant seed species, with a slow germination rate. Although hypocotyl extension occurs in store, the onset of cell division and axis differentiation is delayed and occurred only occasionally in store. This is interpreted as being a strategy to halt subcellular events which increase susceptibility to water loss. Seed maturity of *B.flava* is suggested to be polymorphic in response to an unreliable seasonal rainfall pattern.

Introduction

The regeneration of plants from seed hinges on the seed being at the right place at the right time in the appropriate physiological state to germinate and establish seedlings. Physiological conditions for germination depend on the maturation behaviour of the seed. Seeds can be categorised into two broad guilds according to their maturation and storage behaviour: orthodox and recalcitrant (Roberts 1973). Orthodox seeds are shed from the adult plant at low moisture contents, having undergone maturation drying prior to this event. Typically these seeds suffer no injury when moisture contents in the range of 1-5 %, furthermore they can withstand sub-zero temperatures (Chin and Roberts, 1980). Orthodox seeds are characteristic of plants inhabiting temperate and arid regions, where the growing season in interrupted by a cold winter. In these conditions seeds are able to resume growth when conditions suitable for germination occur, remaining in a dry state over winter.

Recalcitrant seeds are hydrated and metabolically active on dispersal, and unlike orthodox seeds are not desiccation tolerant (Roberts 1973). When dried they gradually lose viability and the amount of water lost before injury depends on the species (Berjak, Farrant and Pammenter 1989). These seeds are have been shown to initiate subcellular germination on or shortly after shedding, which accounts for their desiccation sensitivity (Farrant, Berjak and Pammenter 1985; Farrant, Pammenter and Berjak 1986, 1989 & 1992). Plants which produce recalcitrant seeds have a tropical/ subtropical/ wetland distribution, with a reproductive strategy aimed at immediate seedling establishment. The rate and timing of germination commencement differs between species (Farrant et al 1989). It has been shown that as germination proceeds, the seeds become increasingly sensitive to drying (Farrant et al. 1986). This could account for the differences in desiccation tolerance observed between different species i.e those seeds which initiate and rapidly proceed along the germination pathway

being more sensitive to drying than those which are shed relatively more quiescent and the initiate germination more slowly (Berjak et al.1989).

To date their has been little classification of seed type in the winter rainfall region of the Cape Province. Based on canopy (Bond, 1985) and soil seed bank data (Le Maitrre and Midgley, 1992) it would appear that many fynbos species would seem to produce orthodox seeds. This suggestion is substantiated by the observation that many appear to be shed at the start of the dry summer and remain dormant until germination is triggered by smoke (De lange and Boucher, 1990), heat (Brown and Dix, 1985), charate or indirect microclimate alterations following fire (see Le Maitre and Midgley, 1992).

In the winter rainfall region of Namaqualand germination is linked to the availability of water (Van Rooyen and Grobbelar, 1982). Most seeds in this semi-arid environment appear to be orthodox remaining dormant through the harsh summer. However a few species of the Amryllidaceae produce fleshy fruits, in which seeds have high moisture contents. It may be possible that these species fall into the recalcitrant category. This is supported by the fact that most seeds of Amaryllid species are produced at the start of the rainy season and would be able to germinate immediately and grow to a sufficient size in winter, to survive the following summer. *Boophane flava* Barker ex Snijman is a species in the Amaryllidaceae native to Namaqualand (Snjiman, 1983) (Fig 1), which has a hysteranthous habitat (flowering and leafing temporally separated). That these seeds and this species might be recalcitrant is further supported by the observation of hypocotyl protrusion occurring when the seeds are still attached to the dried umbel (fruiting and dispersal structure) (Pers obs).

This study attempted to determine whether the seeds of this spp which are produced at the

start of the rainy season are indeed recalcitrant, if so what are it's limits to desiccation tolerance and how is this related to probable reproductive strategies. If the seeds do not display typical recalcitrant characteristics, then the question of how to classify the seeds will be addressed.

A simple test for recalcitrant seeds is their inability to withstand drying. Thus storage under desiccating conditions and the assessment of seed viability will indicate whether these seeds are truly recalcitrant. In order to assess the subcellular process associated with the desiccation and storage of these seeds, microscopic studies of the hypocotyl ultrastructures were undertaken during desiccation. This tissue was selected as it is the first to protrude from covering structures, and thus becomes exposed to environmental conditions.

A pilot study of a similar nature was done on a related Amaryllid spp *Amaryllis belladonna* L., which is endemic to fynbos communities of the southwestern Cape. This species inhabits sites which remain moist throughout the year, and thus might also show recalcitrant characteristics. However its reproductive strategy is likely to differ from *B.flava.* Unlike *B.flava* hypocotyl extension is not observed on the fruiting structures in this species (Johnson pers comm.

Materials and Methods

Materials

Inflorences containing *B. flava* seeds were acquired from the Karoo National Botanical Gardens (these seeds were originally collected from Grootvlei west of Kamieskroon). Seeds of *A.belladonna* were collected from a population near Kommetjie on the Cape Peninsular.

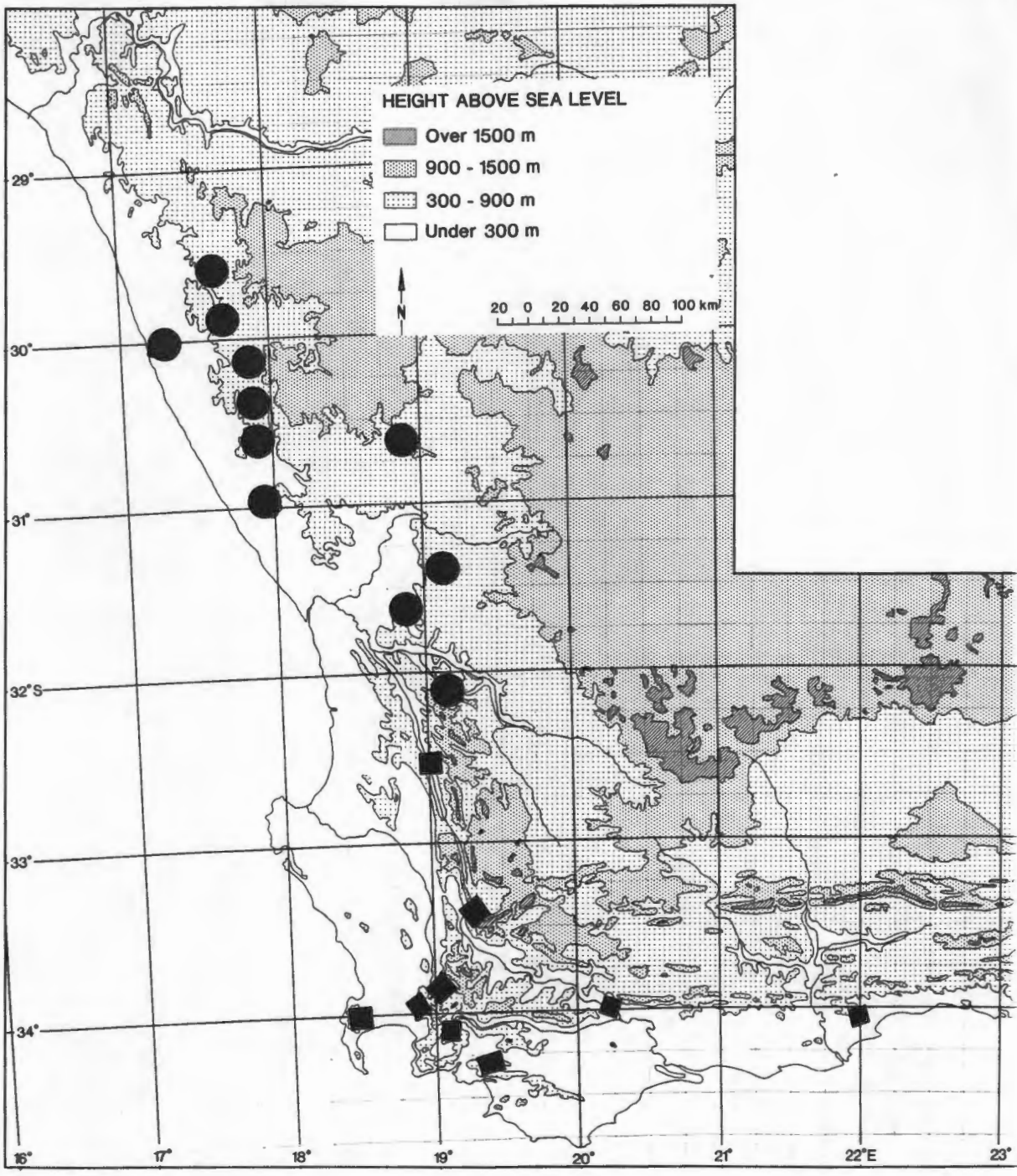


Fig. 1. Distribution of *A. belladonna* (■) and *B. flava* (●).

In the laboratory the seeds were removed from the umbel (Fig 2) and stored on a metal grid above activated silica gel (the desiccation agent), in opaque plastic buckets for the duration of the experiment. The silica gel was replaced regularly, to maintain it's hygroscopicity. Seeds of *B.flava* were sampled every week for the first six weeks and thereafter every 2-3 weeks, and were assayed for moisture content and viability as discussed below. Due to low seed availability seeds of *A.belladonna* were sampled only every four weeks. Ultrastructure studies were performed every four to five weeks.

Moisture-content analyses

The moisture content of embryonic axes and endosperm (separately) of *B.flava* and *A.belladonna* were determined gravimetrically, from 10 individual seeds. All seeds were oven dried at 100° C for two days, and cooled in a desiccator prior to weighing. Endosperm moisture contents of B. flava were taken until the endosperm was utilized by the embryo, after which only embryonic moisture contents are expressed. The moisture contents are expressed on a dry-mass basis.

Viability assessment

Seeds (10) were germinated in trays containing riversand (1/3) and peat (2/3) (G. Duncan Pers comm), and placed in a glass house (average temperature 25 °C) for the duration of the experiment. The seeds were checked each week day and the numbers germinated recorded. Two germination criterion were used for *B.flava*: 1) hypocotyl protrusion to a length of 2 mm, and 2) appearance of shoots. This was done as many seeds extended the hypocotyl while still in the bucket, not all of these retained the ability to form seedlings. Thus the latter assessment was necessary for an accurate assessment of viability. *A.belladonna* did not extend a hypocotyl in store, and hypocotyl extension appeared to be an

accurate reflection of viability in this spp. Hence only the first criterion was used for scoring Viability is expressed as the final percent germination. The cut off time for assessment was 51 days for hypocotyl protrusion and 61 days for leaf extension (*B.flava*). Hypocotyl viability was also assessed for *B.flava* as a germination rate, using Czabator's (1962) method.

Procedures for transmission electron microscopy (TEM)

The embryo was removed from the surrounding tissue and the hypocotyl apex detached. The growing tip was split longitudinally. Individual apex halves were treated as follows:

1) Fixed in 2.5 % glutaraldehyde in sodium cacodylate buffer (pH 7.2) for 12-18 hours.
2) Washed with six ten-minute changes of sodium cacodylate buffer (pH 7.2) to remove excess fixative.
3) Post fixed in 1% osmium tetroxide for 2 hours at room temperature.
4) Washed with three ten-minute changes of sodium cacodylate (pH 7.2) buffer to remove excess osmium.
5) Dehydrated in graded ethnol series:

   25 % - 10 min; 50 % - 10 min; 70 % -10 min; 95 % -10 min; 100 % - min.

6) Infiltrated with Spurs (1969) epoxy resin by the following procedure:

propylene oxide	10 min.
fresh propylene oxide	10 min.
1:1 propylene oxide: Spurs resin	4 hrs.
pure Spurs resin	45 min.
fresh Spurs resin	10 - 12 hrs.

The fixed and stained hypocotyl tips were then placed in moulds half full with fresh spurs resin and orientated under a dissecting microscope. The moulds were completely filled with resin and left to stand in a fume cupboard. Afterwards, remaining air bubbles in the resin were drawn to the surface. The resin was then polymerised at 60 C for 16 hours.

Sections of 120-160 nm were cut using a Reichent OM U2 microtome. These were collected on copper grids and post-stained with lead citrate, using the following procedure:
A few drops of lead citrate were pipetted onto a piece of dental wax, placed on a layer of filter paper, inside a Petri-dish, containing many 1.0 M sodium hydroxide pellets. The copper grids were floated section-side down on the drops for 45 minutes. They were then washed in 0.02 M sodium hydroxide, and delicately rinsed in distilled water. The grids were allowed to air-dry and were stored in Petri-dishes lined with filter paper.

Results

Boophane flava

The embryo moisture content of *B.flava* did not change significantly during the first 8 weeks of storage, although there was considerable variation in the moisture content of seeds stored for the same period (Fig 3). Between week 8 and week 11, the embryo moisture content declined from an average of 244 % on week 8 to 104 % on week 11, after which the moisture content of the embryo gradually declined to around 28 % by week 18 (Fig 3).

The moisture content of the endosperm showed a gradual decline until the 8th week in storage, after which it dropped considerably (11th week) (Fig 3). After 13 weeks in storage the moisture content of the endosperm could not be determined as it had been utilized by the

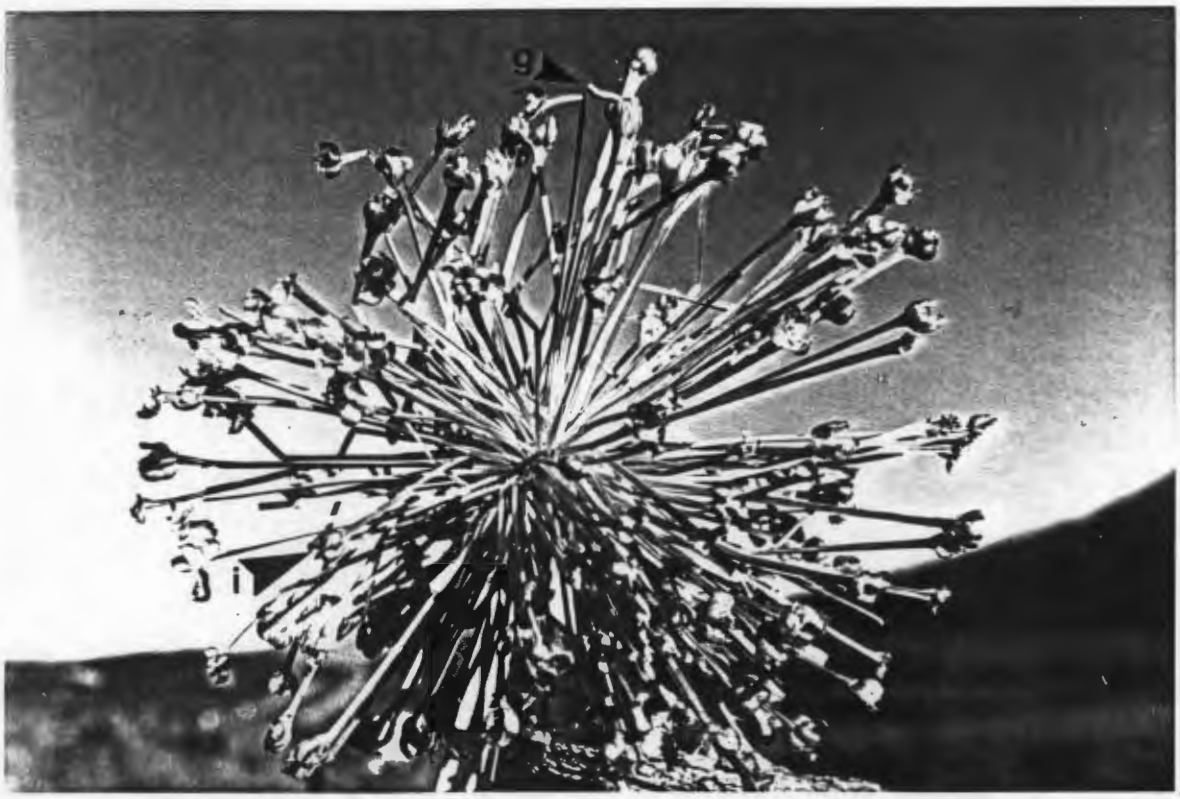


Fig. 2a . Dried inflorescence (umbel) of *B.flava*. Note than seeds in different stages of germination are on this umbel (some seeds have long since protruded the hypocotyl g , while others remain relatively inactive i).

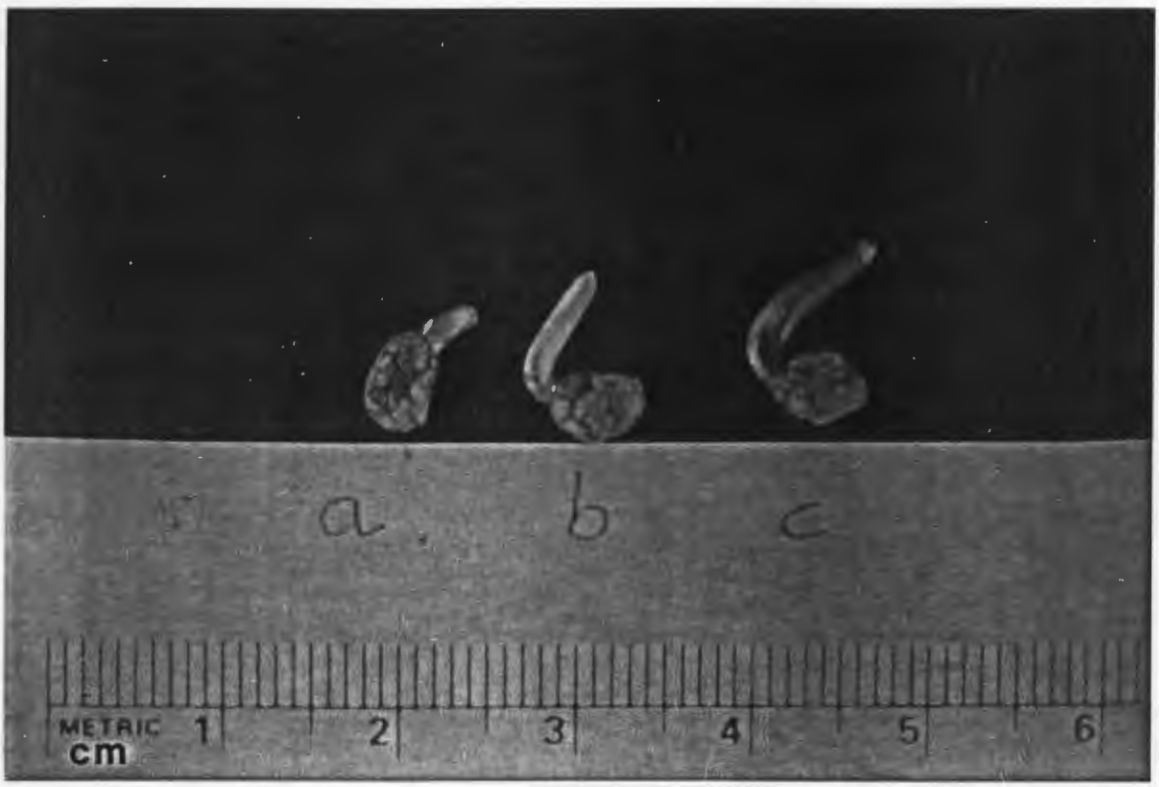


Fig. 2b. Seeds of *B.flava* stored for 6 weeks, at various stages of germination. (**a** & **b** hypocotyl extension, **c** shoot formation)

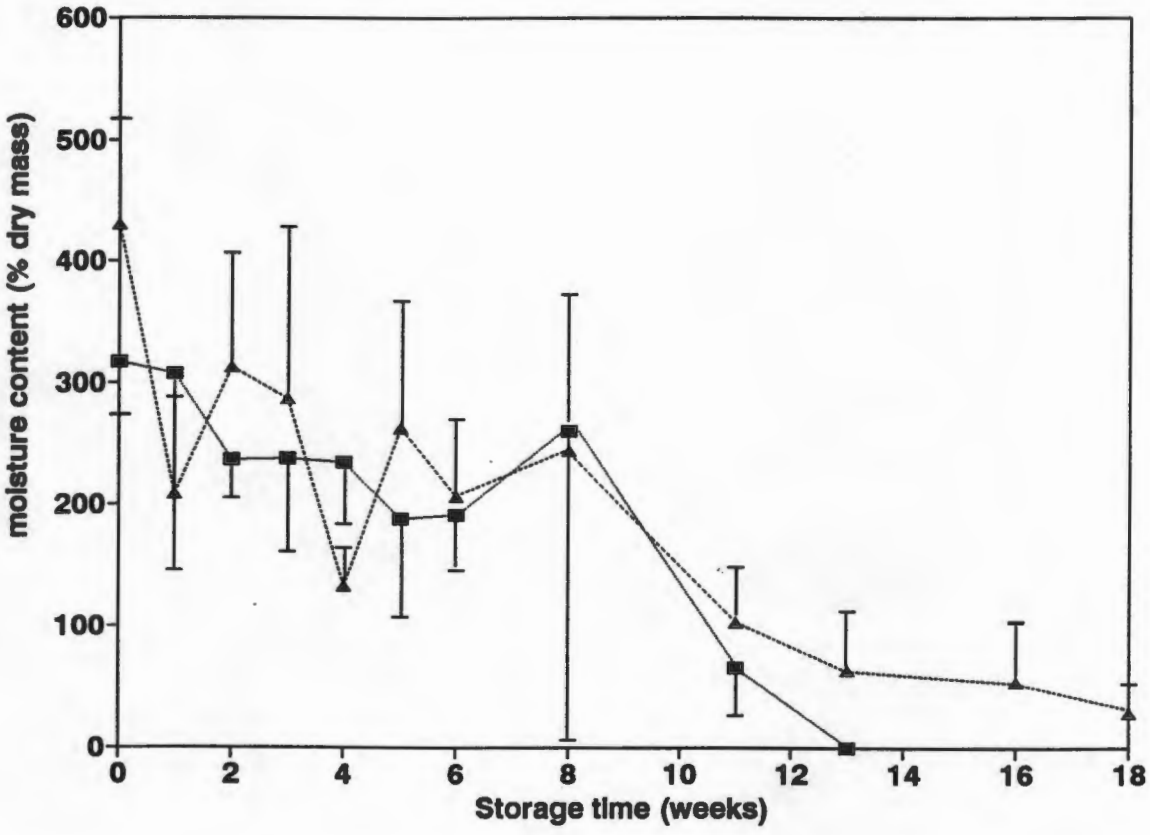


Fig. 3. Effect of dry storage on the moisture content of the seeds of *B.flava* (■ endosperm; ▲ embryo). Values are means +/- SD.

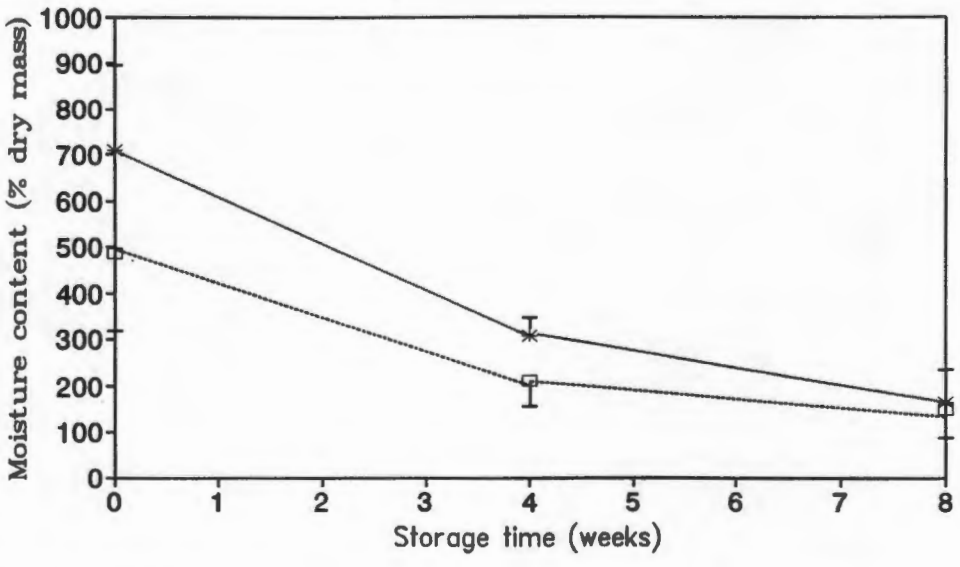


Fig. 4. Effect of dry storage on the moisture content of the seeds of *A.belladonna* (* endosperm; □ embryo). Values are means +/- SD.

embryo. The endosperm moisture content is generally lower than the embryos, but on week's 1 and 4 there are inversions (Fig 3).

Amaryllis belladonna

The moisture content declined more steeply during the first four weeks of storage compared to four to eight weeks (Fig 4). The endosperm moisture content was higher than in the embryo for the first four weeks.

Germination characteristics

Boophane flava

Seeds of the spp appears to be polymorphic, with seeds showing hypocotyl extension at different time intervals on the fruiting structure (Fig. 2a). The hypocotyl extended up to 1cm before axis differentiation took place. Shoot formation slightly proceded the initiation of roots in storage (Fig 2b). Most seeds which were stored for longer than 8 weeks extended hypocotyls in the bucket. The rate of hypocotyl extension increased rapidly until the 8th week, there after it declined (Fig.5) as water became limiting. The final percent of hypocotyl extension remained relatively the same until the 8th week in storage, after which the ability to produce a hypocotyl declined rapidly. (Fig. 6). After 18 weeks in store 40 % of the seeds had extended a hypocotyl (Fig.6) however none of these seeds were viable, if assessed by ability to produce shoots (Fig 7).

Seeds show 90 % final germination at a moisture contents between 370-128 % after 8 weeks in store. Viability begins to decline after 8 weeks in storage. Moisture content below 110 ± 10% (8th-11th week) appear lethal to these seeds (Fig 6).

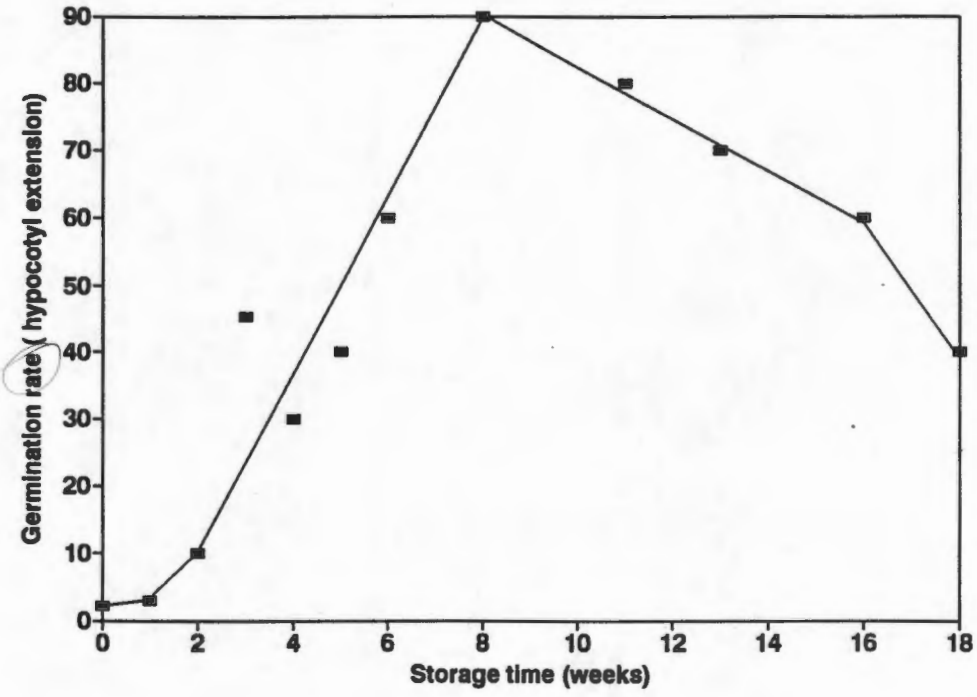


Fig. 5. Effect of storage time on the rate germination (hypocotyl extension) of *B.flava* seeds.

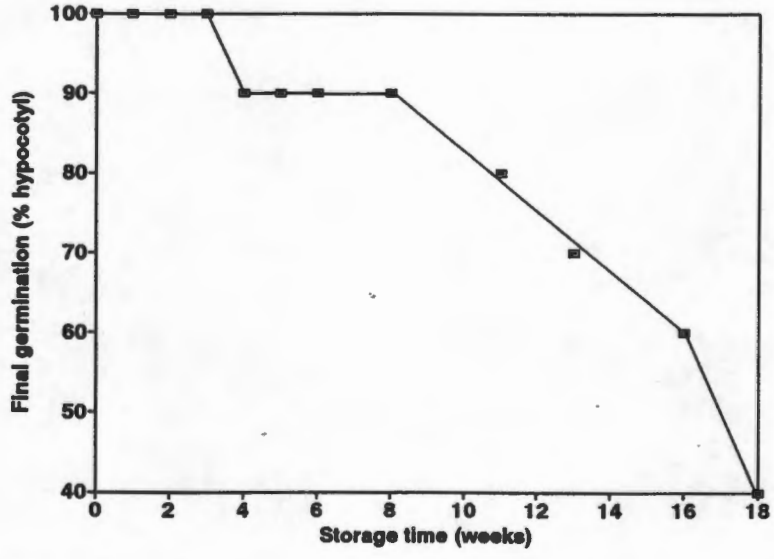


Fig. 6. Effect of dry storage on the final percent hypocotyl extension of *B.flava* seeds.

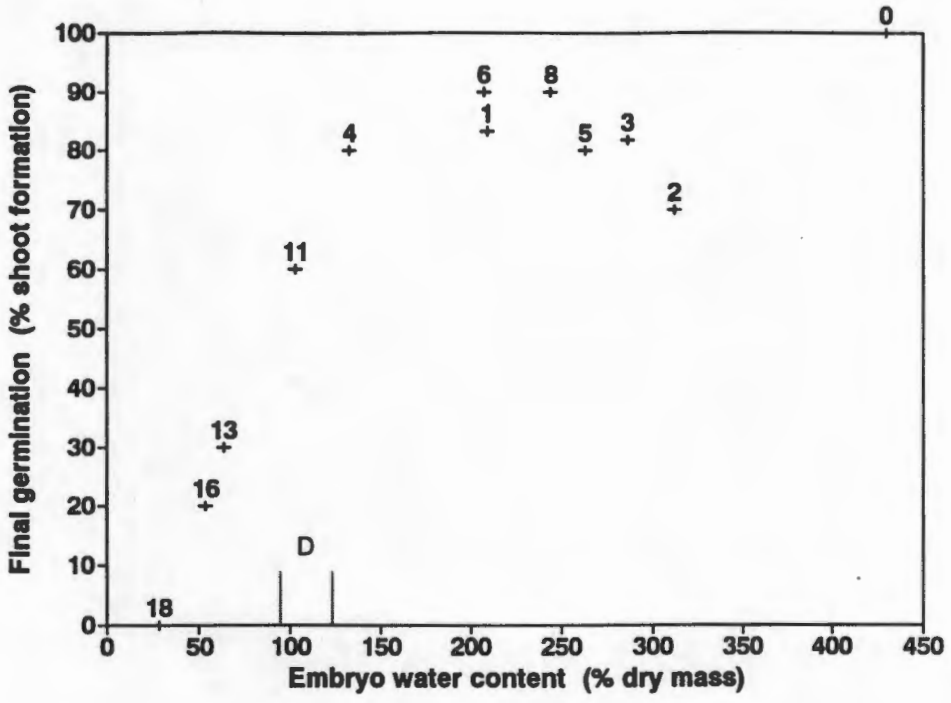


Fig. 7. Effect of dry storage on the final percent germination (shoot formation) of *B.flava* seeds (numbers above indicate weeks in storage). **D** is the lethal water content zone below which seeds lose viability.

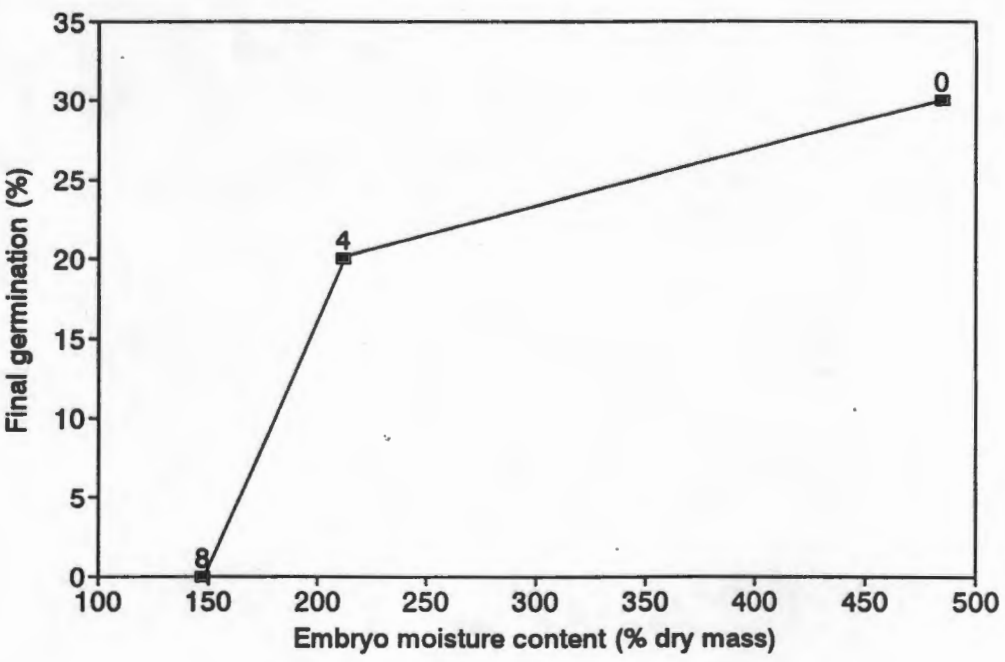


Fig. 8. Effect of dry storage on the final percent germination of *A.belladonna* seeds (numbers above indicate weeks in storage).

Amaryllis belladonna

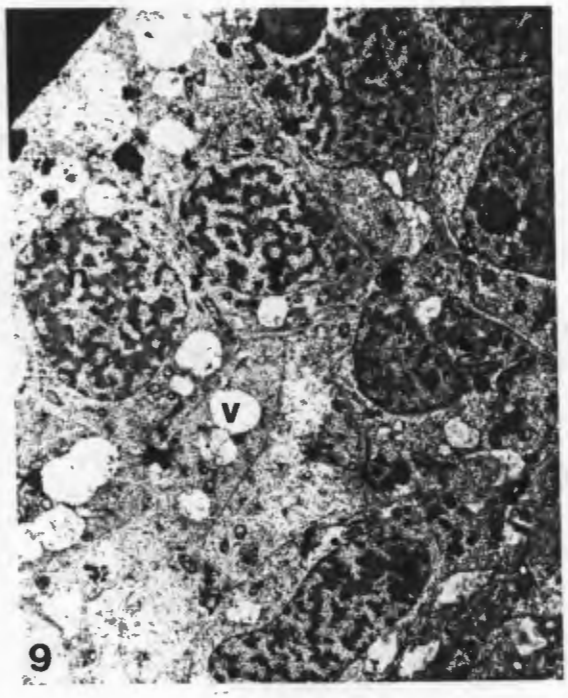
None of these seeds germinated in storage and only 30 % of newly shed seeds eventually germinated (Fig. 8). The viability of newly shed seeds did not differ significantly from those seeds stored for four weeks. Seeds lost viability between the 4th and 8th week, the critical moisture content is between 160-200 % (Fig. 8).

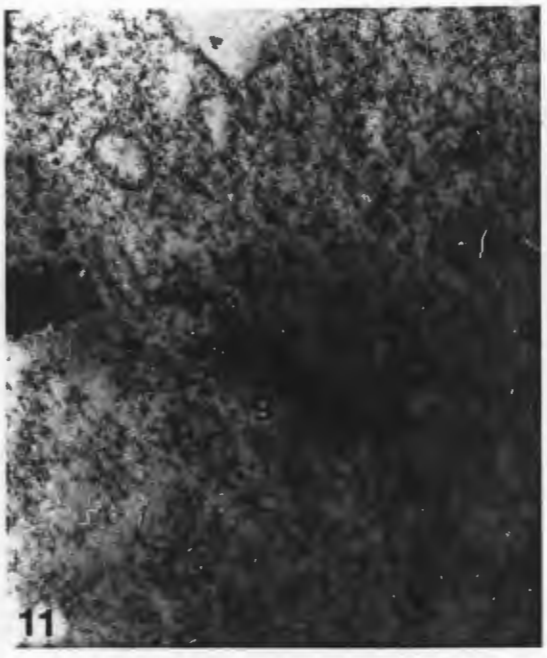
Ultrastructure

Boophane flava

The ultrastructure of hypocotyl tissues from newly collected (control) seeds is typical of comparatively metabolically active tissue (Figs 9 & 10). This is indicated by the relatively electron dense matrixes of most mitochondria (Fig. 10), short endoplasmic reticulum profiles (Fig.10), and some Golgi activity (Fig. 11). The degree of vacuolation is slight (Fig. 9). Few storage reserves such as lipophillic bodies and starch filled plastids are present at this stage (Fig. 9).

After 11 weeks of storage seeds appeared to show a marginal increase in cellular activity. Increased levels of cellular metabolism are evident by increased density of cristae in the mitochondria (Fig. 12) indicating enhanced mitochondrial activity, the accumulation of starch in plastids (Fig. 13), and increase in the numbers of polysomes in the cytoplasm (Figs 12 & 13). However by 11 weeks, some tissues of the stored seeds show evidence of damage (Figs 14-16). Figures 15 and 16 reveal cells lacking in subcellar detail and cytoplasmic clumping. Further evidence of subcellular abnormalities are the accumulation of dark staining substances in the vacuoles, while other vacuoles fused (Fig. 14). Extreme cellular damage is exemplified by extensive vacuolar and plasma membrane dissolution (Fig. 14). Despite this mitochondria in these tissues remain intact and retain their active appearance (dark matrix;





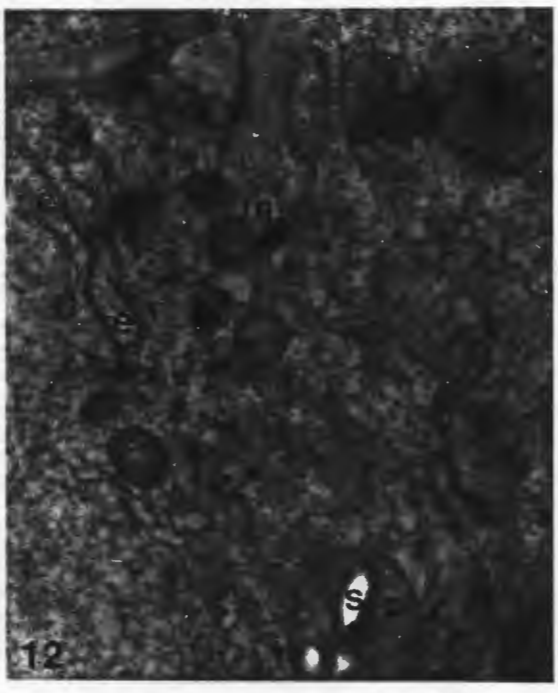
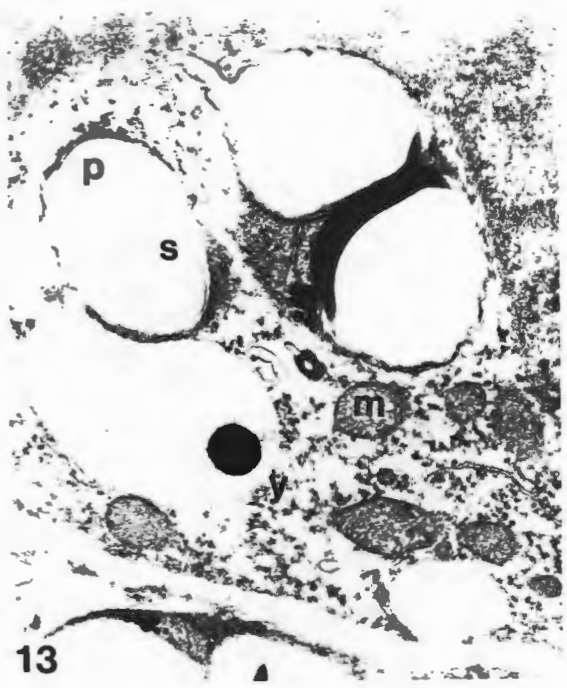
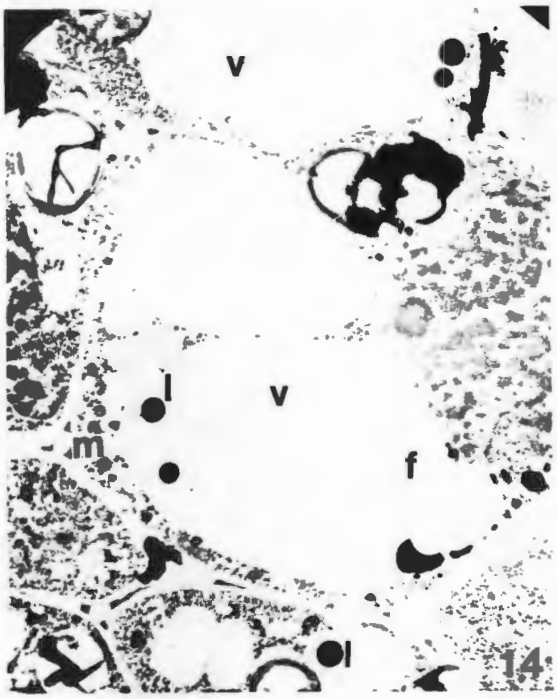


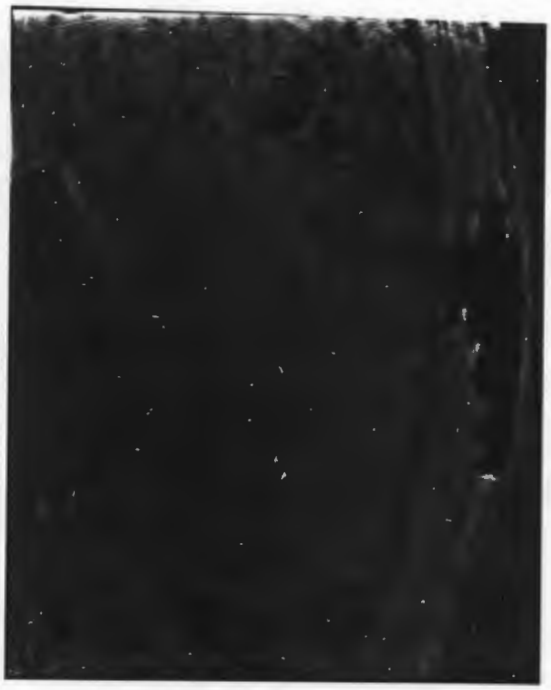
Fig. 9. Subcellular detail of newly shed *B.flava* seeds, tissue taken from hypocotyl. Note healthy appearance of the cells, and presence of lipophillic bodies l., nucleus n. (2500 Mag.)

Figs 10 & 11. Typical ultrastructural activity of newly shed seeds of *B.flava*. Note endoplasmic reticulum e., plastids p. with starch s. and electron transparent mitochondria m. (Fig. 10; 12000 Mag.). Golgi g. activity indicates more active tissue (Fig.11; 25000 Mag).

Fig 12. Subcellular aspects of *B.flava* hypocotyl tissue stored for 11 weeks. Note accumulation of lipophillic bodies l. (17000 Mag.).







Figs 13 & 14. Micrographstaken from *B.flava* hypocotyl tissue stored for 11 weeks. Note: Plastids have accumulated starch s., mitochondria m. have dark cristae and polysomes are more clustered y. (Fig.13; 12000 mag.). extensive vacuolation, vacuolar fusion f. and active mitochondria m. (Fig 14: 3000 Mag.). (vacuoles v.)

Figs 15 & 16. Cellular deterioration of *B.flava* hypocotyl tissue after 11 weeks in storage. Note absence of cytoplasmic detail, clustered organelles o, dark staining mitochondria m. (Fig. 15: 10000 Mag.) (Fig. 16; 5000 Mag.).

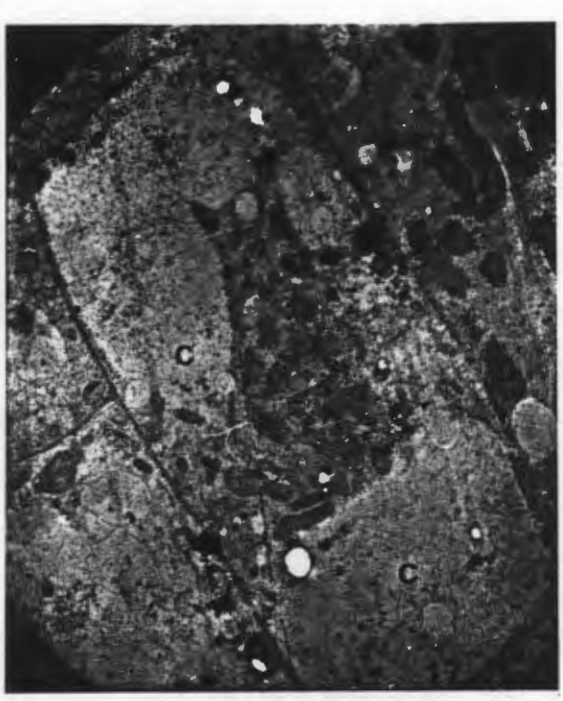
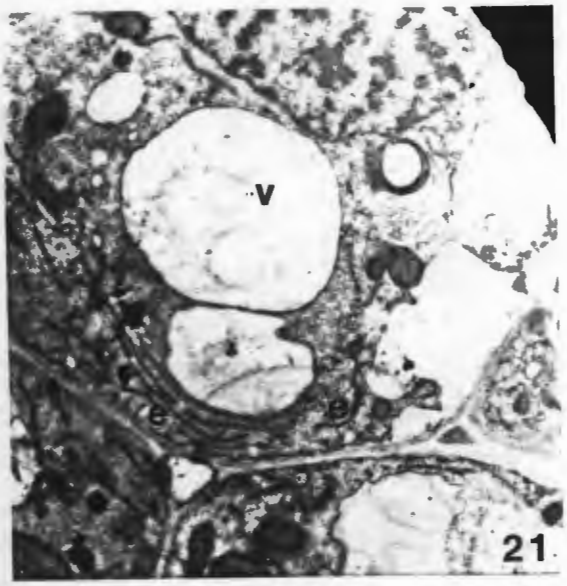
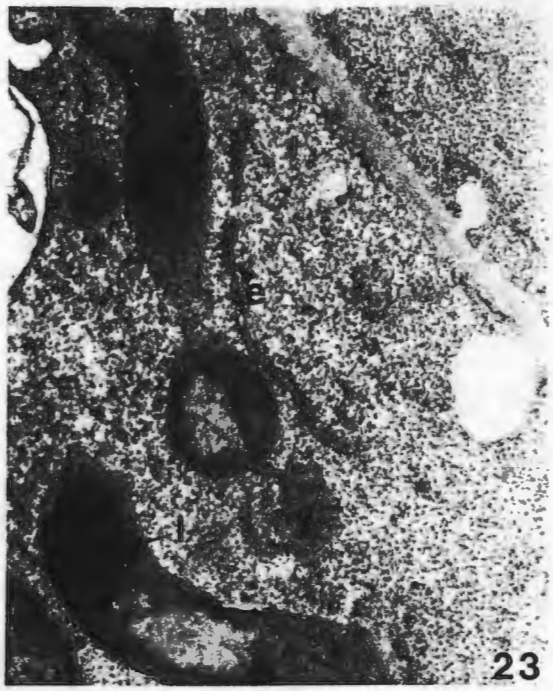


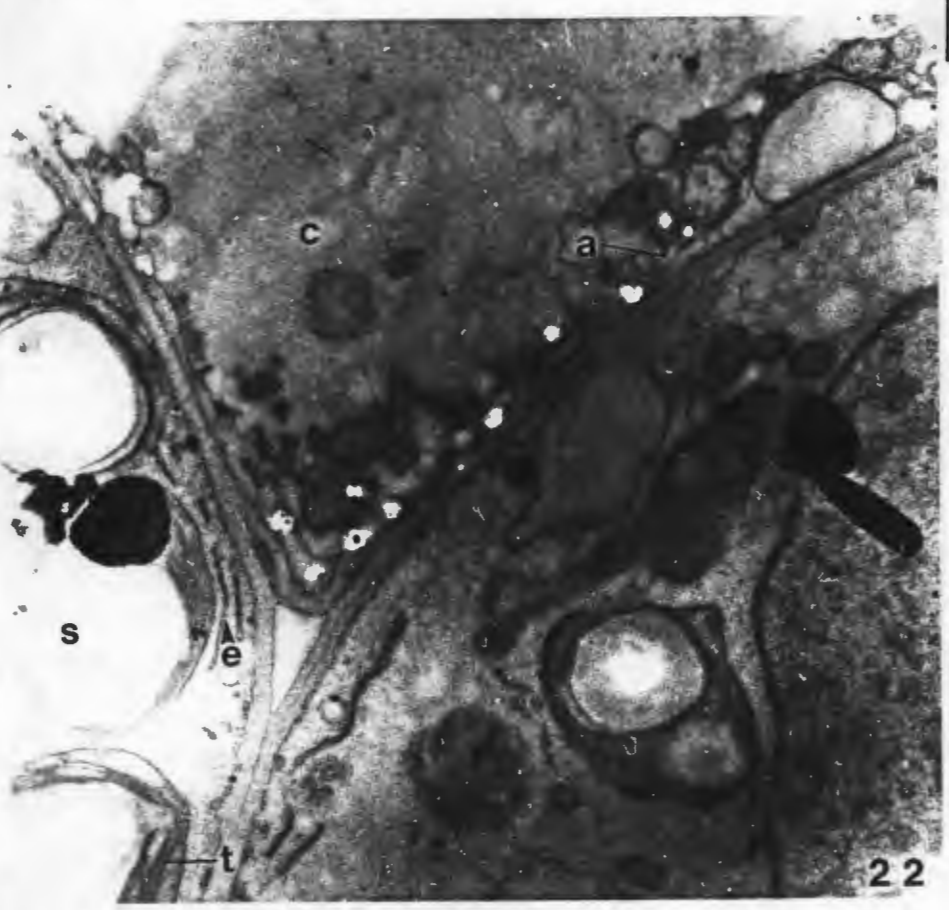
Fig. 17. Typical ultrastructural activity of newly shed seeds of *A.belladonna*. Note plastids p. electron transparent cytoplasm c., indicates relatively inactive cytoplasm (2500 Mag).

Figs 18 & 19. Subcellular details of fleshly collected *A.belladonna* hypocotyl tissue. Note short e.r profiles lycophililic bodies l., nucleus n. vacuoles v. (Fig. 18: 4000 Mag.). (Fig.19; 12000 Mag.).

Fig. 20. Subcellular aspects hypocotyl tissue of *A.belladonna* stored for 4 weeks. Note formation of inner plastid membranes ↱. (Fig. 20: 15000 Mag).







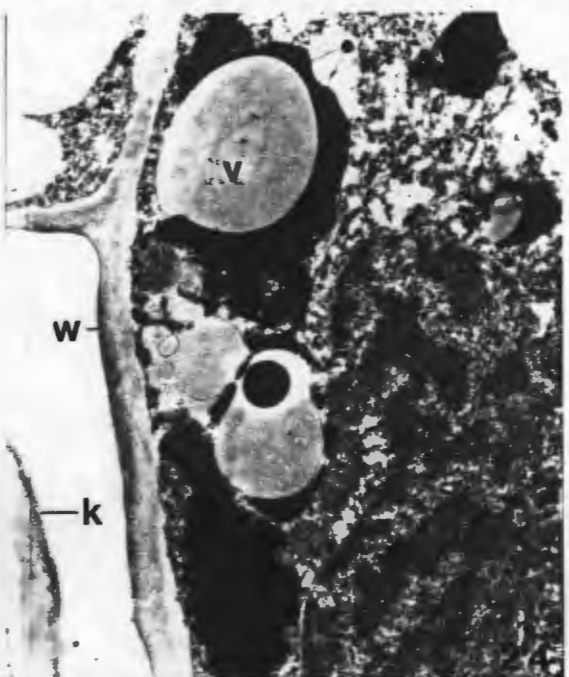


Fig. 21. Ultrastructural detail of hypocotyl tissue of *A.belladonna* stored for 4 weeks. Note; numerous e.r profiles e. and increased vacuolation (Fig. 21: 6000 Mag.)

Figs 22, 23 & 24. *A.belladonna* hypocotyl tissue after dry storage for 4 weeks. Note: accumulation of starch and presence of thylakoid membranes in plastids t. in left and right cells and damage a. to the plasmalemma in the upper cell (Fig. 22: 16000 Mag.), presence dense clusters of polysomes y. in the cytoplasm c. and the accumulation of lipophillic bodies (Fig. 23: 25000 Mag.), build up of electron dense substances in and around the vacuoles, and plasmalemma withdraw k from the adjacent (left) cell wall w. (Fig. 24: 10000 Mag)

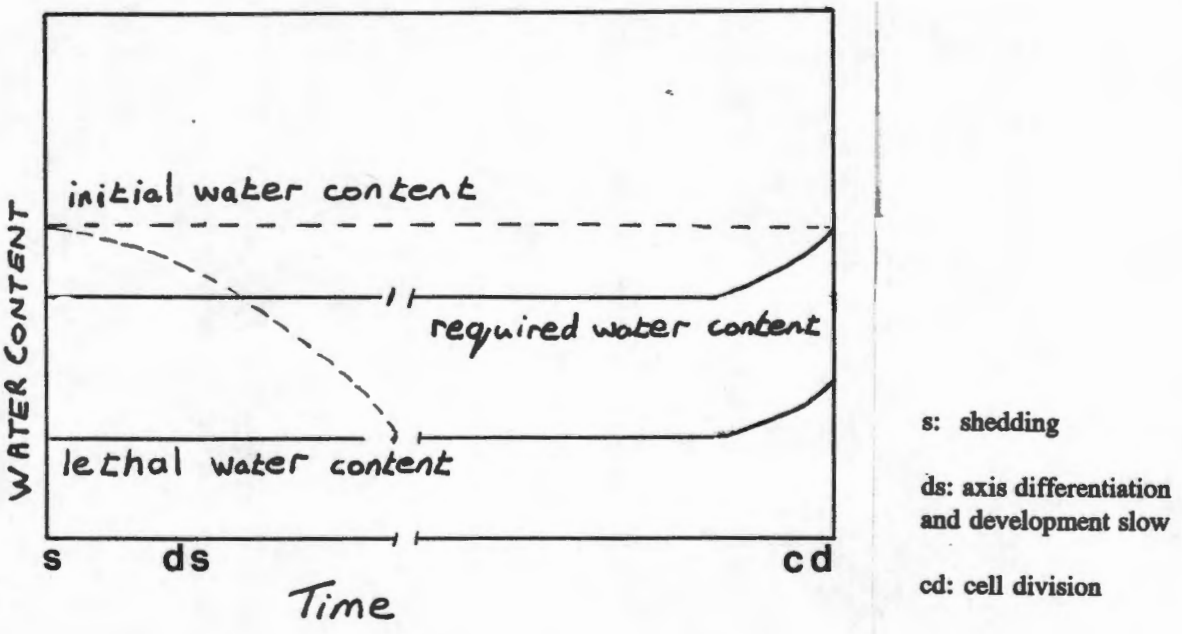


Fig. 25. Relationship between drying rate and the the timing of germination-related processes of *B.flava*. Note that the rate of germination is very slow. (adapted from a model (c) showing differing degrees of recalcitrant seed behaviour from Berjak et al.(1989).

(Fig. 15 & 16).

Amaryllis belladonna

Hypocotyl tissue of newly collected (control) seeds were in a state typical of metabolically active tissue, but did not show high levels of metabolism. The cytoplasm was not electron dense (Figs 17,18,19), endoplasmic reticulum (e.r) profiles are short (Fig. 19), monosomes are prevalent (Fig. 19), plastids are numerous (Figs 17, 18, 19), and although they don't contain starch there is evidence of internal membranes (Fig. 20). In general the hypocotyl tissues contain no storage reserves, except for a few scattered lipophillic bodies (Figs 17 & 19).

After four weeks in storage hypocotyl tissues of *A.belladonna* seeds show enhanced metabolically activity indicative of subcellular progression to germination (Figs 21 & 22), with an increasing abundance of e.r profiles (Figs 21 & 22), a build up of starch reserves in plastids (Fig 22), and dense clusters of polysomes in the cytoplasm (Fig 23). However the loss of water (Fig 8) has caused some cellular deterioration. Plasma membrane damage is illustrated in the upper cell in figure 22. The electron transparent cytoplasm of the upper cell in figure 22 indicates the loss of organelles and polysomes. Cellular deterioration is more evident in cells where the plasmalemma had withdrawn from the cell wall (Fig 24). The accumulation of lipophillic bodies and other electron dense substrates in the cytoplasm's of damaged cells are perhaps a sign of a response to desiccation injury (Fig.24).

Discussion

The seeds of *B. flava* and *A.belladonna* exhibit typical recalcitrant storage behaviour, being shed at high moisture contents (Figs 3 & 4) in a metabolically active state (Fig 9-24), and

as water was lost viability declined. Total loss of viability occurred at relatively high moisture contents (Fig 7 & 8). Furthermore seeds of *B.flava* showed hypocotyl extension and in some instances root and leaf formation in dry storage (Fig. 2b), a character often displayed by recalcitrant seeds. The low viability score of newly collected *A.belladonna* seeds suggests that inbreeding depression may be taking place in the small sample population (Johnson pers comm).

Effects of storage

Hypocotyl tissues of seeds of *A. belladonna* that had been stored for 4 weeks showed signs of increased subcellular activity relative to the newly shed condition (Fig. 17-19). The increase in endoplasmic reticulum profiles (Fig. 20) and the denser polysome clusters (Fig.23) are evidence of increased protein and possibly membrane synthesis, and the increased density of mitochondrial matrices indicate increased respiratory activity.

Farrant et al. (1985,1988,1989) have shown subcellular changes during storage of several different recalcitrant seeds types. These changes were accompanied by increased rates of germination in these stored seeds, the above authors have suggested that the subcellular changes were germination related. Although there was no recorded increase in the rate of germination of *A.belladonna* seeds in the present study it is suggested that the enhanced sub-cellular changes were also germination related, and that the progression to germination is the underlying cause of desiccation sensitivity in this seed.

Effects of damage

The seeds of *B.flava* show increased rates of hypocotyl extension in store until the 8th week (Fig. 5). Despite this there is only slight evidence of increased subcellular activity in this

tissue over 11 weeks of store (Figs 12,13,14). Slight increases in the density of mitochondrial cristae and the increase in polysome profiles, together with increased level of starch might indicate a very slow progression towards germination.

Interpreting subcellular germination events in recalcitrant seeds is far from straight forward, as reference to the literature indicates that marked differences exist in germination behaviour among recalcitrant seed species (Chin and Roberts 1980; Farrant et al. 1989). This prompted Farrant et al.(1988) and Berjak et al. (1989) to explain the variation found between recalcitrant species by modelling the rates of germination against the seeds' tolerance to water loss. *B.flava* seed germination and storage behaviour appears to fit into one of the models proposed by Berjak et al (1989) and described in figure 25. I suggest that during store, axis differentiation occurs which ultimately leads to hypocotyl extension. This continues until the endosperm has been utilized. The response of the seeds to desiccation, during these events, are discussed below. The low viability and limited seed numbers preclude making predictions, with respect to the model the seeds of *A.belladonna* might follow (see comment on inbreeeding above). Extended rates of axis differentiation accompanied by slow germination has also been observed in another amaryllid *Scadoxus membranaceus* (Bak.) Friis Nordal (Farrant et al 1989), suggesting this trait is common in fleshy amaryllids.

Effect of desiccation

A.belladonna seeds appear to be less tolerant of drying than *B.flava*, suffering extensive subcellular damage after 4 weeks in storage at moisture contents between (200-160%) (Fig.8), compared to the 120-100% (Fig.7) in *B.flava*. Although some cells in the hypocotyl of *A.belladonna* stored for 4 weeks are apparently healthy, Others showed considerable

damage accumulating electron dense substances in and around the vacuoles and the plasmalemma has withdrawn from the cell wall, furthermore the break down of organelles in the cytoplasm has released lytic enzymes causing further cellular deterioration (Fig. 22). Similar subcellular damage occurs in hypocotyl tissues *B.flava* stored for 11 weeks at moisture content below 120% (Figs 14,15). The subcellular deterioration associated with desiccation of the embryonic axes of the white mangrove *Avicennia marina* (Forssk.) Vierh. are associated with the accumulation of similar dark staining substances in the vacuoles and cytoplasm (Farrant et al.1986), extensive vacuolation, coalescence of vacuoles, breakdown of internal membranes of plastids of mitochondria and ultimately the dissolution of vacuolar and plasma membranes. Similar types of damage are seen in both *A.belladonna* and *B.flava*; different cells showing variations in degrees of damage. It is suggested that once a significant no of cells become damaged viability will be lost.

B.flava seeds can tolerate a fair amount of water loss, from an initial level of 420%, moisture content dropped below 120% (Fig. 3). Possibly why these why these seeds do not show a rapid decline in viability despite hypocotyl extension (Fig 6) may be due to their ability to slow the germination processes and to delay the formation of a meristem for root/shoot protrusion. Furthermore they might be able to attain the ability to repair considerable damage. This is suggested by highly active mitochondria in cells exhibiting extensive vacuolation (Fig 14) and cytoplasmic destruction (Fig 15).

A.belladonna seeds on the other hand are more sensitive to drying. The embryonic tissues of these seeds appear to progress more rapidly along the germination pathway. (reviewed above this would account for their increased susceptibility to drying)

Reproductive strategies

The rate and timing of germination events are related to seedling establishment. Species with highly recalcitrant seeds such as *A. marina* germinate rapidly and establish as seedlings almost immediately, due to the availability of water and a steady substrate (Farrant et al.1992). Most moderately recalcitrant seeds are produced mainly by species in subtropical habitats and are suggested to germinate more slowly (Farrant et al 1988).

B.flava is endemic to the northwestern Cape (Fig.1) a semi-arid region where annual rainfall varies between 100-200 mm, within 25-30% reliability (Shultze and Mcgee 1975). It would appear risky to produce recalcitrant seeds which have no germination block, in this water stressed environment, unless their reproductive strategy is closely linked to the availability of water. I suggest that the seeds on one fruiting structure are polymorphic with respect to developmental status when shed (Fig 2a); some being more advanced than others (Fig. 2b). The advanced seeds protrude hypocotyls shortly after shedding and those would be ready to produce shoots and roots should precipitation events occurs early in winter. If not these seeds might die due to the loss of water, or utilization of the endosperm. However, as there are less developed seeds on the same umbel partially protected by a dry fruiting capsule, these take longer to reach the stage of hypocotyl extension and endosperm utilization. These in turn may encounter a rainfall event which would facilitate their germination and establishment as seedlings. This strategy may be an evolutionary adaption to highly variable seasonal rainfall, with seeds from one plant germinating at different time intervals and thus increases the probability that some seeds will germinate around precipitation events. Flowering is also affected by water availability, with profuse flowering observed after heavy autumn rain, with irregular flowering during dry years (Snijman 1983).

In contrast seeds of *A.belladonna* are shed into an environment where they are able to establish as seedlings almost immediately, flowering in March and producing seeds at the same time as the arrival of the winter frontal systems bringing rain. This species exhibits slightly more advanced rate of subcellular germination after shedding, but this results in a lower tolerance to desiccation.

Further research

From this study it is not possible to know cell whether cell division is accompanied by an increase in both required and lethal water content, as no co-ordinated measures were taken of seed water content at the onset of cell division, this would be an interesting question to address in future studies. To fully explain seed behaviour using models proposed by Berjak et al.(1989), it is necessary to obtain more precise measurements of desiccation tolerance at specific stages during germination. To determine if *B.flava* does exhibit polymorphic germination behaviour in response to the species reproductive strategies, further studies on the developmental status of seeds from the same individual could be done in the field to minimise population variations in seed set and relate germination to climatic events. Perhaps temperature delays the onset of germination ? A more holistic understanding of physiological events under germination progression in different species can be enhanced if the physiologist mind the biology of the species.

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