Deteriorative changes in embryos of long-stored, uninfected maize caryopses

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The subcellular changes occurring in the embryonic root tip and which accompany the deterioration of maize grain stored for varying periods up to 12 years are described. The ultrastructurally visible events which ultimately involve virtually all the intracellular components, are initiated by deterioration of mitochondria and the occurrence of chromatin clumping at a stage when germination has not declined. This sequence of deteriorative events is similar in all major respects to that which has previously been described for maize grains subjected to an accelerated ageing regime. The results are discussed in the context of the possibility of particularly mitochondria and chromatin having a higher water content than other subcellular locations, and the consequent concentration of free radical mediated events at these sites.

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Die subsellulêre veranderinge wat in die punt van die radikula plaasvind en die gepaardgaande agteruitgang van mielie-graanvruggies wat vir verskillende tydperke oor 'n 12 jaar-periode geberg is, word beskryf. Die gebeure wat op ultrastruktuurvlak sigbaar is, en wat uiteindelik al die intrasellulêre komponente betrek, begin met agteruitgang van mitochondria en die voorkoms van chromatien-klontvorming op 'n tydstip wanneer ontkieming nog nie afgeneem het nie. Hierdie reeks van degenerasie-stappe is in hooftrekke soortgelyk aan dié wat voorheen beskryf is ten opsigte van mielie-graanvruggies wat aan 'n versnelde verouderingsregime blootgestel is. Die resultate word bespreek aan die hand van die moontlikheid dat veral mitochondria en chromatien 'n hoër waterinhoud as ander subsellulêre gebiede het, en die gevolglike konsentrasie van gebeure veroorsaak deur vrye radikale by hierdie gebiede.

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Introduction

Storage of most non-dormant, air-dry seeds for any period exceeding that separating the end of one growing season and the start of the next is an entirely artificial situation, considering the behaviour of orthodox seeds in the field. Viewed in this context, it is not surprising that such seeds rapidly deteriorate when stored air-dry under ambient conditions for any length of time. However, with the necessity of seed storage for conservation of genetic resources, and to ensure high quality planting stocks, as well as for the provision of food and feed, it is essential that long-term maintenance of uninfected seed of high viability and genetic integrity be possible. The ideal approach, although expensive and impractical for the average seed producer, involves the selection of high quality, undamaged seed, reduction of the moisture content to the optimum for storage and the use of sub-zero temperatures (Roberts 1983).

However, as most seed storage facilities fall far short of the ideal and seeds are known to deteriorate in storage, it is important to understand the changes which lead to loss of seed viability in the long term. Additionally, accelerated or artificial ageing techniques, which involve holding seed of known moisture content at a temperature somewhat elevated over ambient, are widely used in seed testing to ascertain the vigour of seed lots. This approach leads to rapid loss of seed viability — in a matter of days or weeks — the time taken depending on seed moisture content and storage temperature. It remains to be ascertained whether or not such approaches genuinely bring about speeding up of the ageing process by acceleration of the same deleterious events which occur during long-term storage (Bass 1984).

Thus far much of the experimental work describing the deteriorative pattern in embryos with time, comes from studies on seeds which have been subjected to an accelerated ageing regime, the experiments on viability loss in stored maize emanating from this laboratory being no exception (Berjak & Villiers 1972a; 1972b; 1972c). This is mainly because of the difficulty in obtaining seed of one variety and of the same provenance, which has been collected from different harvests over the years, and stored under similar conditions. More recently, papers have begun to appear which indicate that research workers now have sources of long-stored seeds, the history of which is known. However, the results of such investigations are equivocal in themselves, and in the comparison between seeds aged in the long term, and those which have been subjected to various accelerated ageing regimes. This is especially pertinent concerning the findings involving analyses aimed at revealing the implication — or not — of free radical

mechanisms in seed deterioration (e.g. Priestley & Leopold 1979; 1983; Priestley *et al.* 1980; Stewart & Bewley 1980; Buchvarov & Gantcheff 1984).

The present work was made possible by the storage of small samples of inbred maize seed of one particular variety from several harvests since 1968, and involves the results of an ultrastructural study on some of this material. It was aimed at ascertaining whether or not the subcellular degeneration pattern in this material was similar to that obtained when maize seeds were subjected to an accelerated ageing regime. The accompanying necessary biochemical analyses are currently in progress.

Materials and methods

Maize caryopses (seeds) of an inbred, white variety (M162W) were regularly harvested at the University Research Farm, Ukulinga, Pietermaritzburg. Small samples, which were immediately placed in hermetic storage at a relative humidity in the range 45 – 50%, from the harvests made in 1968, 1971 and 1975, as well as 5-month-old material from the 1983 harvest (which had been maintained in open storage) and fresh material from the 1984 harvest, were used in the present study. The hermetically sealed cans, which were completely full, were kept at 4°C, while the 5-month-old material had been maintained in an air-conditioned laboratory. Before analysis, the material was categorized as uninfected or infected, on an individual seed basis.

Determination of individual seed status

The dry seeds were surface-sterilized by brief immersion in 1% sodium hypochlorite. A small section of the pericarp overlying the embryo was then removed from each individually numbered seed. Under sterile conditions pericarp segments were placed, inner side downwards, on the surface of potato dextrose agar (PDA) plates, which were then incubated at 25°C for 4 days. Following sampling of the pericarp, seeds were immediately imbibed for 12 h, embryo-side down, on the surface of moist filter paper, one seed per Petri dish, after which half the radicle tip was excised and prepared for electron microscopy.

Immediately after excision of the half-radicle tip, the remainder of the seed was separated into pericarp, endosperm and embryo components, each of which was placed on the surface of a PDA plate which was then incubated as described above. Only material which was ascertained to be free of infection as a result of both tests was designated as infection-free. It was also possible to categorize those seeds harbouring a single species of storage fungus and these were then designated as fungal-infected, a preliminary account of which is given elsewhere (Dini *et al.* 1984).

Moisture content determination

Moisture contents were determined gravimetrically on two replicates (10 seeds each) immediately after opening the storage containers. Seeds were dried down at 70°C until a constant mass was obtained and moisture content calculated on a wet mass basis.

Assessment of germination and seedling establishment

Twenty-five seeds from each sample were surface-sterilized and set out to germinate, embryo-side down, on moist filter paper within closed Petri dishes at 25°C. The ability of a seed to germinate was assessed after 36 h and again after 90 h, the final criterion for successful germination being the production of a radicle 5 mm or longer. Once the radicle had reached this length, the seeds were planted in 'Jiffy Pots', and seedling establishment scored on the production of the first leaf.

Preparation for electron microscopy

Half-radicle tips were prepared for electron microscopy using a standard glutaraldehyde-osmium method.

Results

Moisture contents, viability and incidence of infection

The moisture contents of the various seed batches were all in the range 10 – 12% at the start of storage, the newly harvested (1984) seeds having a moisture content of 11,00%. At the end of the five-month-storage period (1983 harvest) seed moisture was 11,37%, and seeds stored for eight years (1975 harvest) had reached the very similar equilibrium moisture content of 11,10%. The moisture contents of the 12-year-old seeds (1971 harvest) and those which had been stored for 15 years (1968 harvest) were substantially lower, being 8,63% and 9,17%, respectively. Nevertheless, these two older seed samples harboured storage fungi at levels of 24% and 92%, respectively. Only 4% of those seeds stored for five months or for eight years harboured any storage fungi (Figure 1). The 1984 harvest, however, was heavily infected by *Fusarium* spp.

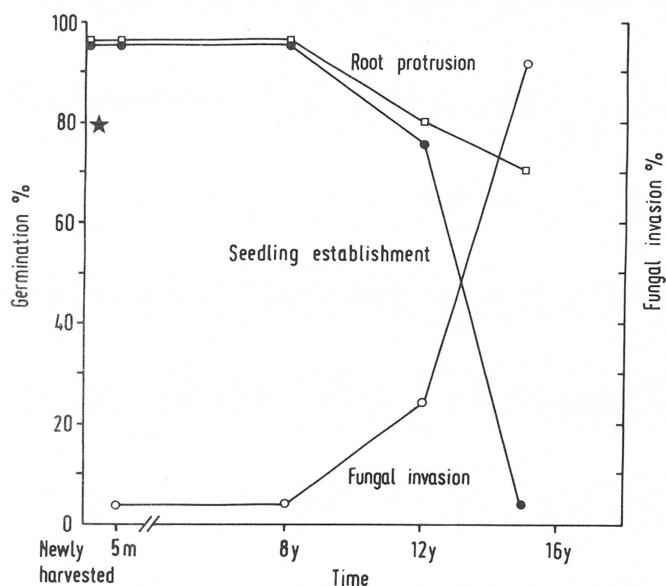


Figure 1 Apparent seed viability (indicated by root protrusion, —□—) and actual viability (seedling establishment, —●—) are shown as a function of storage time, as is the degree of fungal invasion (—○—). The extremely high incidence of infection in the 1984 harvest is indicated by ★.

Figure 1 shows that a final germination level of 96% was attained by fresh seeds, as well as by those which had been stored for five months and for eight years. However, there was a marked lag in germination of the eight-year-stored material, compared with that which had been stored for only five months. Whereas the five-month-stored material achieved 96% germination within 36 h, after eight years of storage, this level of germination took 90 h to achieve, only 24% germination being recorded after 36 h imbibition. Viability of material was depressed to 76% after 12 years storage, declining to only 4% in the 15-year-stored seeds. However, the low germination level in the latter may well have been caused by the extremely high incidence of infected seeds (92%). It is interesting that the ability for seedling

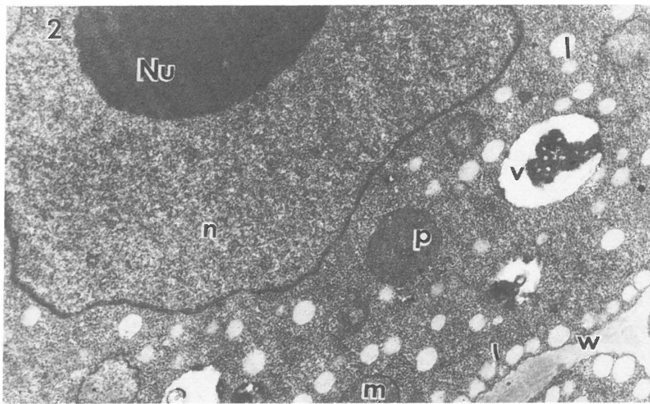


Figure 2 General view of a typical root meristem cell from the newly harvested material. There is little differentiation of organelles and no intracellular damage is apparent. n—nucleus; nu—nucleolus; m — mitochondrion; p — plastid; v — vacuole; l — lipid droplet; w — wall, ×7000.

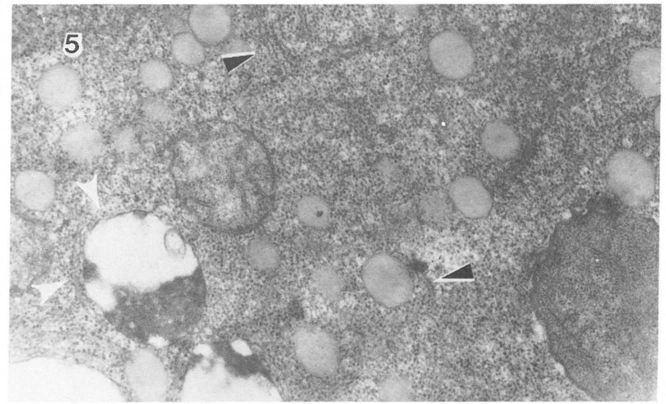
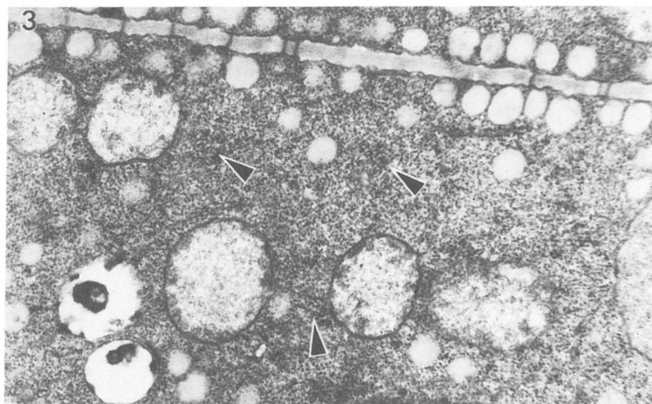
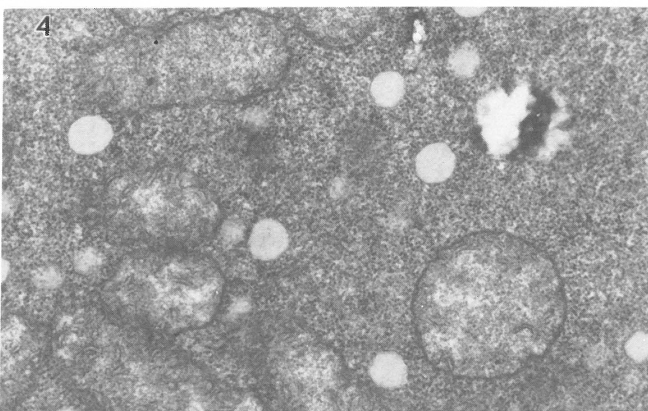


Figure 5 Uniformly dense plastids with a little inner membrane development characterized newly harvested material, as did short ER profiles (arrows) which occurred in intimate association with the small vacuoles and scattered in the cytoplasm. Some polysome formation is evident, ×11000.





Figures 3 & 4 Most of the mitochondria from newly harvested material were essentially spherical and showed a little internal development (Figure 3). However, some of these organelles had elongated and become considerably more differentiated (Figure 4). Some measure of polysome formation (arrows, Figure 3) had also occurred in this material 12 h after the start of imbibition, 3×15600; 4×14100.

establishment of the seed samples declined very much in parallel with the increase in the incidence of infection. It should be noted that a higher proportion of the 12- and 15-year-stored seeds were capable of initial root protrusion than of seedling establishment.

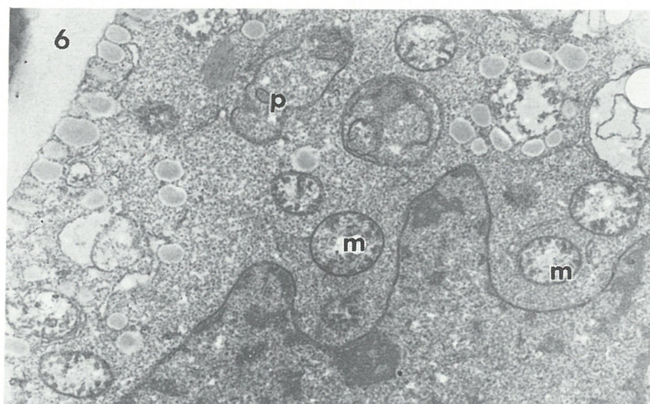
The ultrastructural situation

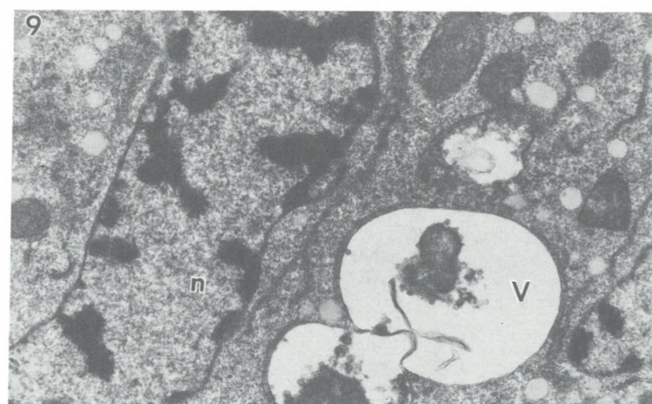
Newly harvested material

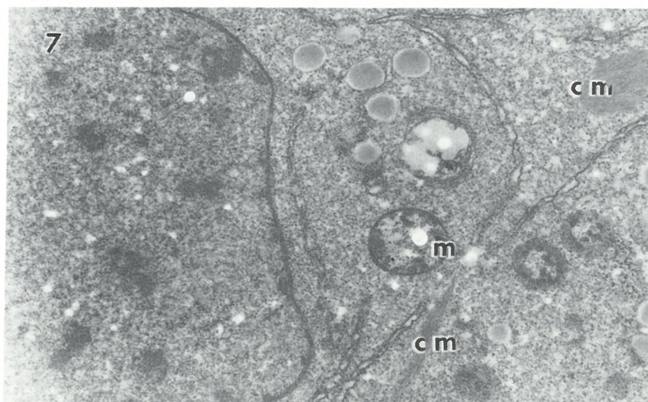
The nuclear profile was essentially spherical, and the matrix homogeneous, with no occurrence of clumped chromatin. The nucleolus was dense and compact and closely associated with the nucleolar organizer. Lipid droplets were disposed both along the cell periphery and within the cytoplasm, and the plasmalemma was in close association with the cell wall (Figure 2). Mitochondria presented generally circular profiles with only a small measure of internal membrane development evident, indicating that most of these organelles were spherical and relatively undifferentiated (Figure 3). However, there was also evidence of development of some of these organelles into elongated structures, showing a considerably greater differentiation of the cristae (Figure 4). Plastids had a relatively dense matrix and small plastoglobuli. Some internal membrane was evident but no starch was present (Figure 5). The endoplasmic reticulum (ER) occurred as short, regular profiles, scattered in the cytoplasm and in close association with the typically unexpanded vacuoles (Figure 5), and some polysome formation had taken place (Figure 3 & 5).

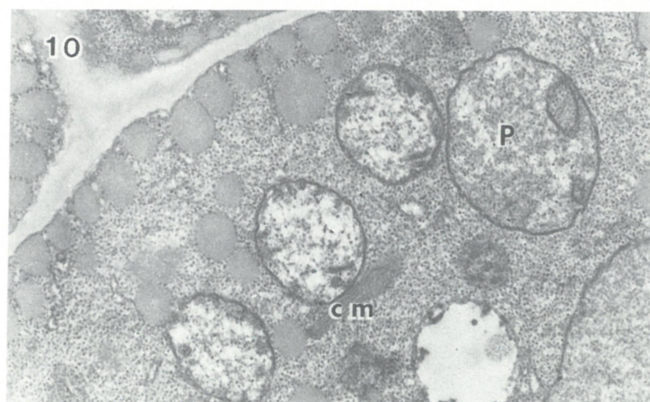
Five-month-stored material

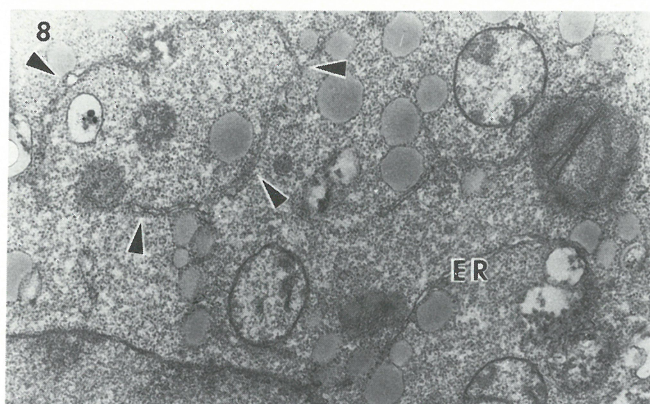
Figure 6 shows that some degree of nuclear lobing had occurred and distinct patches of clumped chromatin were visible after only five months of storage, the nucleolus presenting much the same appearance as in the newly harvested material. The disposition of the storage lipid remained largely peripheral, with some droplets occurring in the bulk cytoplasm. The plasmalemma showed no signs of withdrawal from the cell wall (Figure 6). Mitochondria showed signs of deterioration, even after only this short storage period: the matrix in some of these organelles was markedly non-homogeneous (Figure 7) and there was no evidence of development into the elongate form, nor had cristae differentiated. Generally the plastids appeared much the same as in the fresh material, but although internal membranes were evident, there frequently was irregularity of the outer membrane (Figure 6). Some measure of proliferation of the ER had occurred and the longer cisternae which were scattered in the cytoplasm, showed consistent profile irregularity (Figures 7 & 8). There was also evidence of the early stages of cytolysome formation (Figure 8). Shorter ER profiles were situated in close association with the vacuoles which were somewhat more expanded than in the younger material (Figure 7). Some measure of polysome formation had occurred (Figures 7 & 8) and occasional clumps of cytoskeletal material were observed (Figure 7).

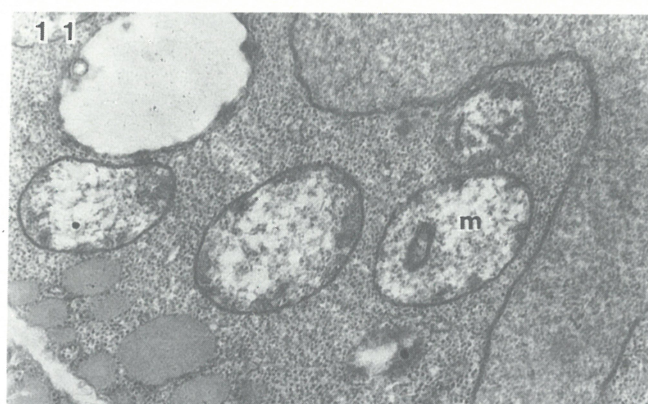










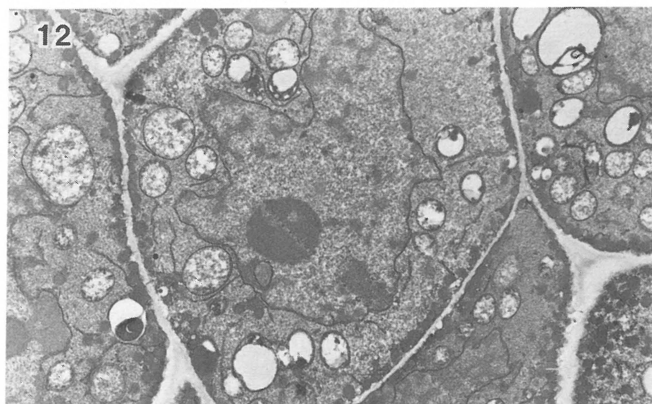


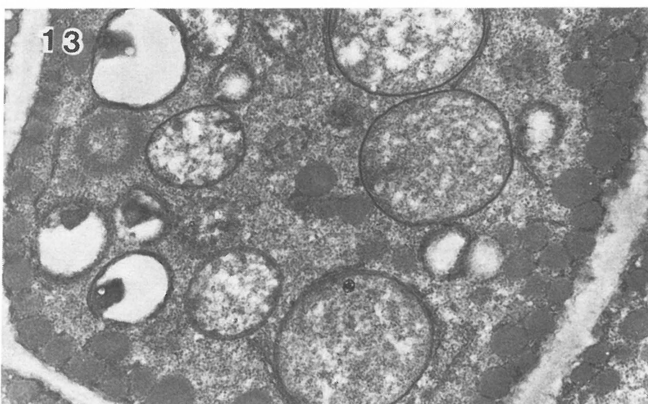
Figures 6, 7 & 8 The ultrastructural situation 12 h after the start of imbibition in root meristem cells of high viability material which had been stored for five months after harvest. The nuclear profile had become irregular, and clumped chromatin was evident. Mitochondria (m) characterized by a lack of internal homogeneity and little development of cristae, were frequent in this material and plastids (p) were often irregular in profile. A measure of ER proliferation (Figures 7 & 8), and the involvement of longish profiles in cytolysome formation (arrows, Figure 8) were evident. Some polysome formation had occurred and occasional clumps of cytoskeletal material (cm) were seen (Figure 7), 6 × 5500; 7 & 8 × 7800.

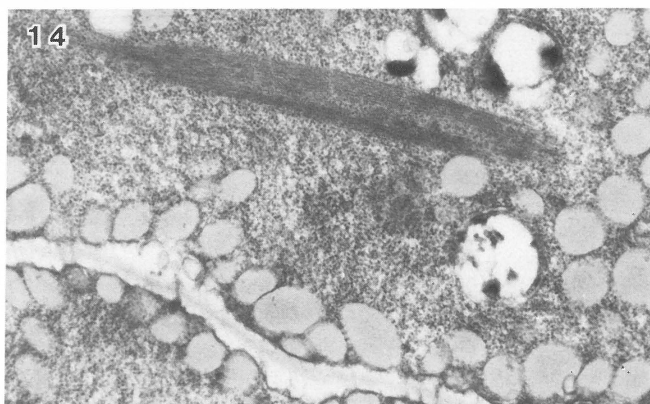
Figures 9, 10 & 11 Althought the eight-year-stored material was still of high viability, a distinct lag in germination occurred. Twelve hours after the start of imbibition considerable subcellular damage was present, including: marked lobing of the nucleus, with irregularly dispersed, dense clumps of chromatin (n, Figure 9); deteriorated mitochondria, often showing derangement of the internal membrane system (m, Figure 11), and similarly damaged plastids (p, Figure 10); somewhat expanded vacuoles with which profiles of the ER were in intimate association (v, Figure 9); and clumps of cytoskeletal material (cm, Figure 10), 9 × 5500; 10 × 7800; 11 × 9400.

Eight-year-stored material

After 8 years of hermetic cold storage the material still maintained a 96% viability, although there was a distinct delay in the onset of germination. This implies the necessity for a repair period, preceding the events of germination proper. The nucleus presented a more deteriorated ultrastructure than in the five-month-stored material, with extensive chromatin clumping and a very lobed profile (Figure 9). The general disposition of the lipid droplets was peripheral and the plasmalemma remained in close association with the cell wall (Figure 10). Many of the mitochondria, however, were considerably more deteriorated than those in the short-term stored material: marked dilution of the matrix had occurred, and the little development of cristae seen earlier had given way to aberrant inner membrane forms (Figure 11). The plastids too, showed signs of abnormal inner membrane formations (Figure 10). While the ER was not as extensive as in embryonic cells from those seeds which had been stored for five months, it had developed into longish profiles, particularly in the perinuclear area, and also occurred in close association with the vacuoles (Figure 9). The vacuoles themselves had expanded somewhat, compared with the fresh material, but there was no evidence of cytoplasmic polysome formation (Figure 9). Infrequent clumps of cytoskeletal material were encountered (Figure 10).







Figures 12, 13 & 14 Viable seeds from material which had been stored for 12 years showed a great deal of subcellular damage when examined 12 h after the start of imbibition. The organelles tended to be clustered round the lobed nucleus which contained irregularly dispersed, clumped chromatin (Figure 12). Mitochondria and plastids were essentially featureless and 'diluted' internally, and appeared somewhat swollen (Figures 12 & 13), and ER profiles occurred not only in association with the vacuoles, but also with plastids (Figure 13). There were substantial clumps of cytoskeletal material evident (Figure 14), 12 × 3100; 13 & 14 × 7800.

Twelve-year-stored material

This material in which germination had declined to 76%, could be divided into viable and non-viable on the basis of the ultrastructural situation after 12 h imbibition.

Embryos from viable seeds

Figure 12 shows the typical appearance of embryonic cells from the viable material. The nuclei were as lobed as formerly and the nuclear envelope appeared damaged in places. Internally the clumped chromatin which is typical of deteriorated embryos was evident, the nucleolus remaining in its characteristic compact form. The lipid droplets were mainly orientated at the periphery of the cells and had a denser appearance than formerly while generally the plasmalemma remained in close contact with the cell wall (Figure 12). However, in some of the cells it had drawn a little inwards, away from the wall (Figure 14). Both mitochondria and plastids were considerably deteriorated, being virtually devoid of any inner membrane formations (Figure 13). The ER was generally in close association, not only with the vacuoles in this case, but also with the deteriorated plastids and the situation pertaining to vacuolar ultrastructure was similar to that encountered in the eight-year-stored material (Figure 13). Substantial clumps of cytoskeletal elements were observed (Figure 14). Organelles showed the tendency to be more aggregated in the vicinity of the lobed nucleus leaving areas of the peripheral ground cytoplasm free of obvious membranous structures. This effect was widespread, although it had not reached extreme proportions (Figure 12). Again no polysomes had been formed (Figures 12 and 13).

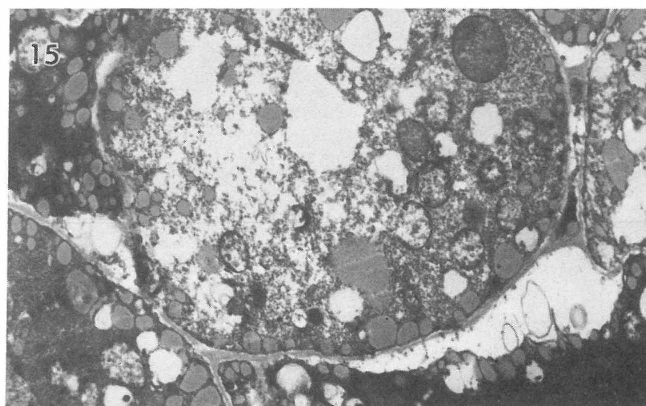


Figure 15 Non-viable, 12-year-stored material was characterized by cytoplasmic lysis and withdrawal of the plasmalemma from the cell wall., × 9400.

Embryos from non-viable seeds

This material presented a mosaic of more and less deteriorated cells (Figure 15). In the latter cytoplasmic lysis was underway, coincident with vacuolar dissolution and the other organelles could be seen in various stages of degeneration. Those cells in the later stages of deterioration presented a totally disorganized appearance, in which the nucleus was visible only by virtue of its being an area in which the clumped chromatin and nucleolus remained. The plasmalemma had pulled away from the wall in the grossly deteriorated cells (Figure 15).

Fifteen-year-stored material

As the incidence of seeds infected by storage fungi was 92%, it proved impossible to obtain samples of uninfected material to allow for the necessary replication.

Discussion

The stored seeds

The moisture content of an individual seed batch upon removal from hermetic storage, represents the equilibrium reached between the seeds and the storage atmosphere. The exact original moisture contents of the seeds used in the present investigation are not known, but they all were in the range 10 – 12%, and the relative humidity at the start of storage was 45 – 50%. It must be assumed that the two oldest

samples were at the lower end of the 10 – 12% moisture content range, and the relative humidity nearer 45% than 50%, as these seeds were at the lowest equilibrium moisture contents upon removal from storage.

It is interesting that these two lower moisture content seed batches harboured storage fungi at levels far exceeding those of the five-month- or eight-year-stored material, the 15-year-old material being 92% infected. As the moisture content of these seeds precluded any active fungal growth or consequent spread during the storage period (Christensen & Kaufmann 1974), their status must reflect the incidence of infection at the time they were put into storage.

The viability curve and that showing the extent of fungal invasion are practically mirror images of each other, suggesting that in the long-stored seeds the presence of fungal infection at a certain level had a marked effect on the ability for seedling establishment. However, even in the uninfected seeds from the 12-year-stored batch sampled for electron microscopy, it was obvious that some were not viable.

The ultrastructural situation

The five-month-stored material showed a surprisingly high incidence of deleterious change compared with that which had been stored for eight years. This was probably partly occasioned by the ambient temperature — in the region of 20°C — as these seed batches were at similar moisture contents on removal from storage. Effects of oxygen in open storage are not thought to cause any significant deterioration of air-dry seeds (Roberts 1983).

Although there was no decline in viability of the five-month-stored material compared with that which had been newly harvested, ultrastructural damage was evident in the nuclei, mitochondria and plastids of the embryonic cells. Chromatin clumping and nuclear lobing which occurred in this material and became more extreme in the seeds stored for eight and 12 years, have been found to progress in parallel with viability loss in maize seed subjected to an accelerated ageing regime (Berjak & Villiers 1972a; 1972b; 1972c).

Osborne and her co-workers (Cheah & Osborne 1978; Osborne 1980; 1983) have shown that activation of nuclear DNAases and fragmentation, but not loss, of DNA occur in the cells of air-dry, stored rye and have suggested that the loss of DNA integrity results from the action of the activated endonucleases. Banerjee *et al.* (1981) have reported an actual loss of DNA and nucleoprotein as well as a decreased ability for ^{32}P incorporation into histones, in rice seeds aged in hermetic storage at 10°C, as well as in seeds of the same cultivar which had been subjected to accelerated ageing. It is well known that gross damage visible as chromosome aberration, occurs in the embryos of aged seeds (Roberts 1983) and Piech & Supryn (1979) working with wheat, have indicated that certain chromosomes are more prone to damage than others.

It is probable that molecular level, endonuclease-mediated degradation of DNA (Cheah & Osborne 1978; Osborne 1980; 1983) underlies the increasingly clumped nature of the chromatin observed in both the long-stored and rapidly aged, air-dry maize seeds as they deteriorate. This ultrastructurally visible event, in turn, is likely to be the basis for the gross damage manifested as chromosome aberration at the light microscope level.

Degeneration of mitochondria (and plastids) was found to have been initiated in rapidly aged maize seed considerably earlier in the deterioration progression than any loss in viability occurred (Berjak & Villiers 1972a) and a similar situation has presently been encountered in the long-stored material. Additionally, the visible mitochondrial degeneration occurring during long-term storage is of the same type as those authors described for maize seeds subjected to an age-accelerating regime. Hallam (1973) and Hallam *et al.* (1973) also reported mitochondrial damage to be a significant feature of the embryonic cells of non-viable rye seeds which had been stored (apparently for some years) at 15% moisture content. Woodstock & Taylorson (1981) found that embryonic axes of soybean aged both in the long term and subjected to an age acceleration regime, showed decreasing rates of oxygen uptake and high respiratory quotients, as well as increasing levels of acetaldehyde and ethanol, compared with fresh material. Those authors suggest that an imbalance develops between tricarboxylic acid and glycolytic activities, which may reflect a decline in mitochondrial activity associated with peroxidative deterioration of mitochondrial membranes, with increasing age of the seeds. More recently, Woodstock *et al.* (1984) have confirmed that deterioration in soybean axes from rapidly aged seeds, is correlated with impairment of respiratory metabolism.

In both the eight-year-stored maize seed presently investigated, and that which had been stored under age accelerating conditions for around 12 days [of the total of 30 days (Berjak & Villiers 1972a)], a lag occurred in the initiation of the visible events of germination. It was established for the latter, that intracellular repair processes occurred during the lag phase and this was probably presently also the case. Osborne (1983) has indicated that DNA repair is thought to occur in damaged rye embryos also during a lag phase preceding the events of germination proper. Viability of the rapidly aged maize seed which had reached a stage of deterioration resembling that seen in the eight-year-stored material, was similarly unimpaired (Berjak & Villiers 1972a).

The proliferation of the rough ER (compared with the fresh material) which was seen in the five-month-stored seeds and, to a lesser extent in those seeds which had been stored for eight years, 12 h after the start of imbibition, is suggested to be geared towards vacuolation. This probably occurs both by cytolysome formation, as is indicated in the five-month-stored material, and by vesiculation of the ER, which is suggested by the irregularity in cisternal profile. Both modes of vacuole formation have previously been described as normal developmental events during germination of fresh maize seeds (Lamb & Berjak 1981), but their entrainment presently described for five-month- and eight-year-stored material after only 12 h of imbibition, is considered precocious. This developmental event is probably part of the spectrum of subcellular repair phenomena, and early vacuole development (and autophagic activity in the later stages of imbibition) occurs in rapidly aged maize seeds and has been implicated in the general repair process (Berjak & Villiers 1972a).

Aggregations of cytoskeletal elements which became more frequent in the older material, have previously been observed (Berjak 1978) in the embryos of stored maize seeds which showed generally similar degenerative changes to those described for the five-month and eight-year-stored material. In terms of the now-recognized role of the cytoskeleton in the organization of the cytoplasm (Schliwa *et al.* 1982), it is probable that this age related phenomenon is indicative of the breakdown of the framework which appears intrinsic to the ordering of the cytoplasm. Similarly, the nuclear lobing which accompanies both rapid (Berjak & Villiers 1972b) and long-term age related deterioration in maize seeds, may well be indicative of the degeneration of

the nuclear skeletal framework, which has been so elegantly demonstrated (Penman *et al.* 1982). In this regard, Sargent *et al.* (1981) have suggested that the orderly reassembly of both cytoplasmic and nuclear micro-tubules (in imbibed embryos of rye which have been dehydrated and then rehydrated) is a characteristic of only those cells which remain viable. Displacement of organelles towards the centre of the cells is seen to have occurred to some extent in the 12-year-stored, but apparently viable material. This too is a phenomenon which accompanies rapid ageing of maize seeds (Berjak & Villiers 1972b), and can be ascribed to the disorganization of the cytoskeleton.

The pattern of more and less deteriorated cells which is seen in 12-year-stored non-viable material is essentially the same as that described for embryos of maize seeds rendered non-viable after some 20 days of accelerated-ageing treatment (Berjak & Villiers 1972c), and for which similar ultrastructural details of cytoplasmic lysis accompanying vacuolar breakdown were described.

Long-term ageing and age acceleration

Considering the potential for seedling establishment as a measure of viability of the long-aged seeds described here, this declines sharply in the 8 – 15-year-storage period. The shape of the survival curve is similar to that obtained when maize seeds were rapidly aged, where a sharp decline in viability occurred between 15 and 22 days of the 30 day experimental period (Berjak & Villiers 1972a). A lag in the onset of germination also occurred in samples of seeds which had been rapidly aged yet still maintained a high viability, as has been shown here for the 8-year-stored seeds. The ultrastructural situation in seeds aged in the long term shows many striking similarities to those subjected to a short period of accelerated ageing. What is of particular significance, is the occurrence early in the deteriorative progression of internal derangement of the mitochondria and of nuclear abnormality, whether the seeds are rapidly aged or have deteriorated in the long term.

Although age accelerating techniques involving manipulations of storage temperature and relative humidity are widely used to test seed viability and vigour and have been taken as representing a speeding-up of the long-term ageing process, there is presently no certainty that the latter is a valid view (Bass 1984). The uncertainty arises partly because of the controvertial views on the involvement of free radical induced peroxidation in long- and/or short-term ageing processes in air-dry seeds. A consideration of the relevant literature (Harman & Mattick 1976; Priestley & Leopold 1979; 1983; Stewart & Bewley 1980; Pearce & Samed 1980; Powell & Matthews 1981) shows that the free radical status *per se* is seldom assessed, most of the conclusions being based on whether or not changes in macromolecules, particularly lipids, can be detected, and/or accumulation of breakdown products occurs. An additional factor underlying the often conflicting results which have been reported by those authors, may be partly correlated with analyses of whole seeds, or of embryonic axes and storage tissues separately.

However, although the overview is equivocal, certain results indicate that free radical mediated deterioration might underlie viability loss in seeds aged either in the long term, and/or during age accelerating storage regimes (Harman & Mattick 1976; Stewart & Bewley 1980; Powell & Matthews 1981; Priestley & Leopold 1983). It is pertinent in this respect that Buchvarov & Gantcheff (1984), who directly assessed the free radical status of soybeans, found that compared with fresh material, a substantial elevation occurred in axes from long-term aged seeds as well as those subjected to an accelerated ageing treatment, and that no indication of free radical accumulation was evident in the cotyledons of any of their material.

In view of the results presently reported for maize seeds aged in the long term compared with those obtained for material subjected to an age accelerating regime (Berjak & Villiers 1972a; 1972b; 1972c), it appears that the subcellular events underlying viability loss are the same, irrespective of the rate of seed ageing. We suggest that the deteriorative events which underlie the visible ultrastructural degeneration are similar in both cases, and are initiated at particular, key subcellular sites — viz. in the mitochondria and the chromatin.

In view of the evidence of some of the groups of workers cited above, it is feasible that free radical mediated damage might well underlie viability loss in stored seeds. However, we propose that such damage is unlikely to be distributed equally, either within an individual cell, or from tissue to tissue. Our contention is that it could be preferentially concentrated at the particular ultrastructural sites which first show damage — the mitochondria and the chromatin. The degree of damage exhibited by the embryonic axis or the storage tissues respectively, is proposed to be a function of the volume ratios of such sites: cells in the tissues concerned. Viewed in this light it is not surprising that Buchvarov & Gantcheff (1984) showed free radical accumulation in the embryonic axes, but not in the cotyledons of variously aged soybean seeds. It is also possible that in many cases where whole seeds have been analysed for changes in saturated: unsaturated lipid ratios and/or the occurrence of peroxidation products, the negative results obtained may be explicable in terms of a quenching effect from the largely inert storage tissues which often constitute the bulk of the seed preparation. We also suggest that assessments of possible free radical damage to macromolecules other than lipid might prove a worthwhile exercise.

We tentatively propose the following explanation of the differential rate of damage accumulation at particular sites within the embryonic cells of ageing seeds: A mitochondrion is a compact compartment with a high membrane: volume ratio, containing many enzymes, other macromolecules and particles. All these intra-mitochondrial structures have the potential to bind water tenaciously (Clegg 1979), which might result in an elevated water content of these organelles compared with other subcellular sites (Berjak & Smith 1986). The same argument can be applied to the *milieu* of the chromatin, the other major subcellular site within the embryonic axes of stored seeds showing ultrastructurally visible damage long before the viability of the sample has started to decline. The early ultrastructurally visible damage to the mitochondria and chromatin is proposed to be a function of the concentration of macromolecular structures vulnerable to free radical attack, occurring within such confined locations which might have relatively high water contents. The effects of free radical generation at such sites would be greatly exacerbated, compared with subcellular locations having lesser localized concentrations of macromolecular structures.

From the present work it is proposed that the intracellular deteriorative events occurring in air-dry seeds (at least of maize) are similar, irrespective of whether the seeds are stored in the long term, or have been subjected to an age accelerating storage regime. Considered in the light that all air-dry storage of the vast majority of quiescent seeds is a highly artificial process, it is incorrect to distinguish the deteriorative events

occurring during long-term storage as 'natural ageing processes' as opposed to those occurring in the short term under conditions of environmental manipulation, as 'artificial ageing processes'. The hypothesis put forward here is that free radical mediated, deteriorative events might be concentrated at localized intracellular sites, in particular in the mitochondria and in the *milieu* of the chromatin. Therefore our current investigations are aimed at ascertaining whether there is an increasing incidence of specific damage ascribable to free radical generation, occurring particularly in mitochondria isolated from seeds sampled at various times during both long-term and accelerated ageing.

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References

BANERJEE, A., CHOUDHURI, M.M. & GHOSH, B. 1981. Changes in nucleotide content and histone phosphorylation of ageing rice seeds. *Z. Pflanzenphysiol.* 102: 33 – 36.

BASS, L.N. 1984. Report of the Seed Storage Committee 1980 – 1983. *Seed Sci. & Technol.* 12: 227 – 231.

BERJAK, P. 1978. Viability extension and improvement of stored seeds. *S. Afr. J. Sci.* 74:365 – 368.

BERJAK, P. & SMITH, M.T. 1986. The enigma of free radical mediated deterioration in stored, orthodox seeds. (In prep.).

BERJAK, P. & VILLIERS, T.A. 1972a. Ageing in plant embryos II. Age-induced damage and its repair during early germination. *New Phytol.* 71: 135 – 144.

BERJAK, P. & VILLIERS, T.A. 1972b. Ageing in plant embryos IV. Loss of regulatory control in aged embryos. *New Phytol.* 71: 1069 – 1074.

BERJAK, P. & VILLIERS, T.A. 1972c. Ageing in plant embryos V. Lysis of the cytoplasm in non-viable embryos. *New Phytol.* 71: 1075 – 1079.

BUCHVAROV, P. & GANTCHEFF, Ts. 1984. Influence of accelerated and natural aging on free radical levels in soybean seeds. *Physiol. Plant.* 60: 53 – 56.

CHEAH, K. & OSBORNE, D.J. 1978. DNA lesions occur with loss of viability in embryos of ageing rye seed. *Nature* 272: 593 – 599.

CHRISTENSEN, C.M. & KAUFMANN, H.H. 1974. Microflora. In: Storage of cereal grains and their products, ed. Christensen, C.M. University of Minnesota Press. Minnesota.

CLEGG, J.S. 1979. Metabolism and the intracellular environment: The vicinal water-network model. In: Cell associated water, eds Drost-Hansen, W. & Clegg, J.S. Academic Press, New York.

DINI, M., GEVERS, H.O. & BERJAK, P. 1984. Deterioration in long-stored fungal-infected maize seeds. *Proc. Electron Microsc. Soc. South. Afr.* 14: 39 – 40.

HALLAM, N.D. 1973. Fine structure of viable and non-viable rye and other embryos. In: Seed ecology, ed. Heydecker, W. Butterworths, London.

HALLAM, N.D., ROBERTS, B.E. & OSBORNE, D.J. 1973. Embryogenesis and germination in rye. *Planta* 110: 279 – 290.

HARMAN, G.E. & MATTICK, L.R. 1976. Association of lipid oxidation with seed ageing and death. *Nature* 260: 323 – 324.

LAMB, J.M. & BERJAK, P. 1981. A unifying view of vacuolar ontogeny from studies on the root cap of *Zea mays* L. *S. Afr. J. Sci.* 77: 120 – 125.

OSBORNE, D.J. 1980. Senescence in seeds. In: Senescence in plants, ed. Thimann, K.V. CRC Press, Boca Raton, Florida.

OSBORNE, D.J. 1983. Biochemical control systems operating in the early hours of germination. *Can. J. Bot.* 61: 3568 – 3577.

PEARCE, R.S. & SAMED, M.A. 1980. Changes in fatty acid content and polar lipids during ageing of seeds of peanut (*Arachis hypogea* L.). *J. Exp. Bot.* 31: 1283 – 1290.

PENMAN, S., FULTON, A., CAPCO, D., BEN ZE'EV, A., WITTELSBERGER, S. & TSE, C.F. 1982. Cytoplasmic and nuclear architecture in cells and tissue: Form, functions, and mode of assembly. Cold Spring Harbor Symposia on Quantitative Biology Vol. XLVI. Organization of the cytoplasm. pp. 1013 – 1028.

PIECH, J. & SUPRYN, St. 1979. Effect of chromosome deficiencies on seed viability in wheat, *Triticum aestivum* L. *Ann. Bot.* 43: 115 – 118.

POWELL, A.A. & MATTHEWS, S. 1981. Association of phospholipid changes with early stages of seed ageing. *Ann. Bot.* 47: 709 – 712.

PRIESTLEY, D.A. & LEOPOLD, A.C. 1979. Absence of lipid oxidation during accelerated aging of soybean. *Plant Physiol.* 63: 726 – 729.

PRIESTLEY, D.A. & LEOPOLD, A.C. 1983. Lipid changes during natural aging of soybean seeds. *Physiol. Plant.* 59: 467 – 470.

PRIESTLEY, D.A., McBRIDE, M.B. & LEOPOLD, C. 1980. Tocopherol and organic free radical levels in soybean seeds during natural and accelerated aging. *Plant Physiol.* 66: 715 – 719.

ROBERTS, E.H. 1983. Loss of seed viability during storage. In: Advances in research and technology of seeds, ed. Thomson, J.R. Part 8. Purdoc, Wageningen.

SARGENT, J.A., SENMANDI, S. & OSBORNE, D.J. 1981. The loss of desiccation tolerance during germination: An ultrastructural and biochemical approach. *Protoplasma* 105: 225 – 239.

SCHLIWA, M., VAN BLERKOM, J. & PRYZWANSKY, K.B. 1982. Structural organization of the cytoplasm. Cold Spring Harbor Symposia on Quantitative Biology Vol. XLVI. Organization of the cytoplasm. pp. 51 – 67.

STEWART, R.R.C. & BEWLEY, J.D. 1980. Lipid peroxidation associated with accelerated aging in soybean. *Plant Physiol.* 65: 245 – 248.

WOODSTOCK, L.W. & TAYLORSON, R.B. 1981. Ethanol and acetaldehyde in imbibing soybean seeds in relation to deterioration. *Plant Physiol.* 67: 424 – 428.

WOODSTOCK, L.W., FURMAN, K. & SOLOMOS, T. 1984. Changes in respiratory metabolism during ageing in seeds and isolated axes of soybean. *Plant & Cell Physiol.* 25: 15 – 26.