CHAPTER SEVEN

ULTRASTRUCTURE OF UNTREATED AND FUNGICIDE TREATED MAIZE SEED

Abstract

Ultrastructural changes within cells are influenced by stress such as fluctuations in temperature due to improper storage, lack of oxygen and blockage in pathways responsible for water uptake. In maize embryos most of the endoplasmic reticulum is formed during the first 48 hrs of germination following imbibition. Any factor that prevents water uptake will essentially prevent normal development of cell structures. The objective of the current study was to assess the effect of the fungicide treatments on the ultrastructure of the embryonic tissue of the maize seeds following 2 d accelerated ageing (AA) and rapid imbibition. Maize seeds were treated with Celest® XL [fludioxonil + mefenoxam] and Apron® XL [metalaxyl –M]. The control consisted of untreated seeds. Following treatment, seeds were subjected to 2 d AA. All seeds were imbibed for 48 hrs. Results showed intact cells with clearly defined nuclei and other organelles in both the treatments and the untreated control. The most obvious difference between the untreated control, Apron® XL and Celest® XL was the number and position of the vesicles. The lipid bodies formed a layer which was still attached to the cell wall in seeds treated with Apron® XL and Celest® XL but in the untreated control they appeared to be more concentrated in the cytoplasm. The mitochondrial structure in the untreated control and the two treatments did not show any major differences, however, one of the mitochondria from a seed from the Apron® XL treatment was not fully developed. Results from this study showed that there was no damage after rapid imbibition and the maize seed tested developed normally not being affected by the stress of accelerated ageing for two days. Maize seeds may be treated with the fungicides tested without adverse effects on imbibition and germination.

7.1 Introduction

The mechanism of seed ageing is an enigma, but it is important to investigate this process especially at a subcellular level (De Castro and Martinez-Hounduvilla, 1984). The earliest stage of imbibition of water by a dry seed involves rapid hydration of the desiccated tissues of the embryo. This is a prequisite for the resumption of processes of growth and development at an ultrastructural level (Baird *et al*., 1979). The seed coat is the first barrier against adverse environmental conditions and the membranes and apoplast of all the living tissue is the major barrier to the uptake of water (Peterson *et al*., 1993). Water entrance at the beginning of imbibition takes place through the thinnest areas of the seed coat (Perissé and Planchuelo, 2004).

Seed treatments are used to protect the seed and have been used with much success (Bradley *et al*., 2001; Galli *et al*., 2005). The success of such treatments are measured by the seed still germinating and developing normally (Bradley *et al*., 2001). In a study by De Castro and Martinez-Hounduvilla (1984), noticeable changes occurred in the ultrastructure of endosperm and embryo cells of maize with loss in the ability to germinate, even in the un-imbibed state. Results of their study showed that the embryo cells were packed with protein bodies and spherosomes or lipid bodies (De Castro and Martinez-Hounduvilla, 1984).

In a study on pines (*Pinus* sp.), De Castro and Martinez-Hounduvilla, (1984) found that after imbibition, vacuoles were present in metabolically active cells as this replaced protein bodies. During imbibition proteolysis takes place more rapidly than lipolysis (De Castro and Martinez-Hounduvilla, 1984). Vacuoles accompanying protein body degradation were frequent and numerous spherosomes were still present. Other components present were mitochondria with well-defined cristae. Signs of membrane damage in the cells of the embryo were more clearly evident in the dry state (dehydrated state) (De Castro and Martinez-Hounduvilla, 1984).

In the maize embryo most of the endoplasmic reticulum is formed during the first 48 hr of germination (Mollenhauer *et al*., 1968, Felker, 1987). The endoplasmic reticulum and the plastids are proposed to be important in graviperception of roots in maize (Moore and

McClelen, 1985). Plastids differentiate into large amyloplasts, amyloplasts act in conjunction with plasmodesmata to form a multiple valve system that controls the movement of growth regulators (eg. abscisic acid) (Moore and McClelen, 1983).

Ultrastructural examination of the root tips of a number of monocotyledons (Berjak and Villiers, 1972) gymnosperms and dicotyledons confirm that membrane systems of aged seeds suffer deteriorative changes during imbibition, including abnormalities in mitochondrial and plastid membrane, fusion of lipid droplets to form larger bodies or irregular pools in the cytoplasm (Smith, 1991). Mitochondria in dry seeds are only partially functional and functionality developed during germination (Hodson *et al*., 1987). Even under strict anoxia functionally adequate mitochondrial membranes (cristae) in maize, shows the stabilization of the inner mitochondrial membrane. The mitochondrial reassemblage may occur under conditions of the complete blocking of oxidative phosphorylation, apparently through the utilization of the energy produced by glycolysis (Vartapetian *et al*., 1987). Treating seeds may interfere with the pathways needed for germination to begin. In one study treating maize seeds with cadmium was evident in the alterations in chloroplast structure which had a direct effect on phytosynthesis (Rascio *et al*., 1993). Investigation on the mitochondrial activity in maize seeds showed that impaired mitochondrial activity, were detected in moderately aged kernels (Dreyer and Van de Venter, 1992).

Exposing maize seeds to heat shock shows that on an ultrastructural level, it is the nucleolus that undergoes the most dramatic change as there is loss of the granular component (Fransolet *et al*., 1979). Investigating the changes in plasmalemma organization, Bliss *et al*. (1984), found that there was a decrease in plasmalemma particle density during imbibition of cowpea (*Vigna unguiculata*) seeds. This change was not linked by any changes in the membranes permeability properties (Bliss *et al*., 1984).

The objective of the current study was to assess the effect of the fungicide treatments on the ultrastructure of the embryonic tissue of the maize seeds following 2 d accelerated ageing and rapid imbibition.

7.2 Materials and methods

7.2.1 Treatment of seeds`

Untreated seed and the chemicals, Celest® XL [*fludioxonil (25 g ai/L) + mefenoxam (10 g ai/L*] and Apron®XL [*metalaxyl –M (350 g ai/L*] were supplied by Syngenta Pty. Ltd (Midrand, South Africa). Seeds were treated as discussed previously in Chapter Four (4.2.1). Representaive seeds from each treatment were subjected to 2 d AA at 45ºC and at 90-100% relative humidity.

7.2.2 Preparation of the seeds

The seeds that were chosen for transmission electron microscopy were those that were treated with Celest® XL, Apron® XL and the untreated control, which consisted of untreated seeds. Representaive seeds from each treatment were subjected to rapid imbibition as outlined in the ISTA (2008) rules. Seeds were individually placed in 4 ml water in a 24 well ice-cube tray. Seeds were incubated for 48 hr. Following the incubation, seeds were removed from the water and the seed coats were removed. Following removal of the seed coat, the seeds were dissected and the embryos were separated from the rest of the seed with the aid of a stereo-microscope (Nikon/SMZ-1, Japan). Small sections of the radicle area of the embryo were removed.

7.2.3 Transmission electron microscopy (TEM)

The samples were fixed overnight in 2.5% glutaraldehyde in 0.075 M phosphate buffer (pH 7.4). The samples were rinsed three times (15 min each) in 0.075 M phosphate buffer and post-fixed in 1% aqueous osmium tetroxide. Thereafter the samples were rinsed three times (15 min each) and dehydrated in an ethanol series (30, 50, 70, 90 and 100%) and embedded in Quetol 651 resin (Van der Merwe and Coetzee, 1992) at 60ºC for 48 hr. Ultra-thin sections were prepared using a Reichert Ultracut E ultramicrotome (Vienna, Austria) and stained with 4% aqueous uranyl acetate and lead citrate (Reynolds, 1963) for viewing with a Philips EM301 transmission electron microscope (Eindhoven, Netherlands).

7.3 Results

Initial view of the overall cell structure showed that there were intact cells with clearly defined nuclei, vacuoles and other organelles (Fig 7.1 a, b, c). There were, however,

differences between the untreated control and the Apron® XL and Celest® XL treated samples. The most noticeable difference was in the structure and position of the vacuoles.

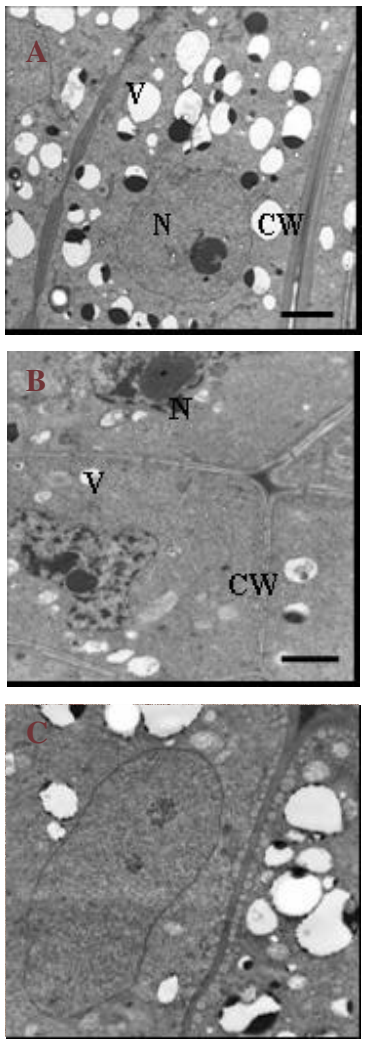

Figure 7.1: TEM micrographs of embryonic tissue of untreated and treated maize seeds following 2 d accelerated ageing and 48 hr rapid imbibition, (Bar = 1 µm) a) Untreated control, b) Apron® XL and c) Celest® XL (CW = cell wall, N = nucleus, V = vacuole).

The black structures within the vacuoles in the untreated control (Fig 7.1 a) and Celest® XL (Fig 7.1 c) treatment were electron opaque remnants of protein bodies. The lipid body layer in the untreated control seemed to be present as lipid droplets in the cytoplasm (Fig 7.2 a),

whilst, in contrast, the lipid layer was still associated with the cell wall in the Apron®XL and Celest® XL treatments (Fig 7.2 b and c).

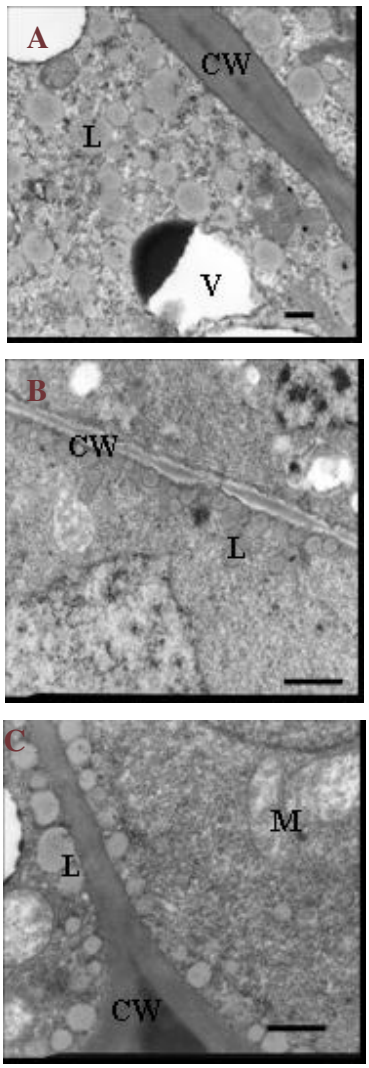

Figure 7.2: TEM micrographs of embryonic tissue of untreated and treated maize seeds following 2 d accelerated ageing and 48 hr rapid imbibition showing differences in the lipid body arrangement, (Bar = 1 µm) a) Untreated control, b) Apron® XL and c) Celest® XL (CW = cell wall, L = lipid, V = vacuole, M = Mitochondrium).

The mitochondrial structure of embryos from the untreated control and the two treatments did not show any major differences. In the Apron® XL sample there was one mitochondrium that had an unconventional shape (Fig 7.3 b).

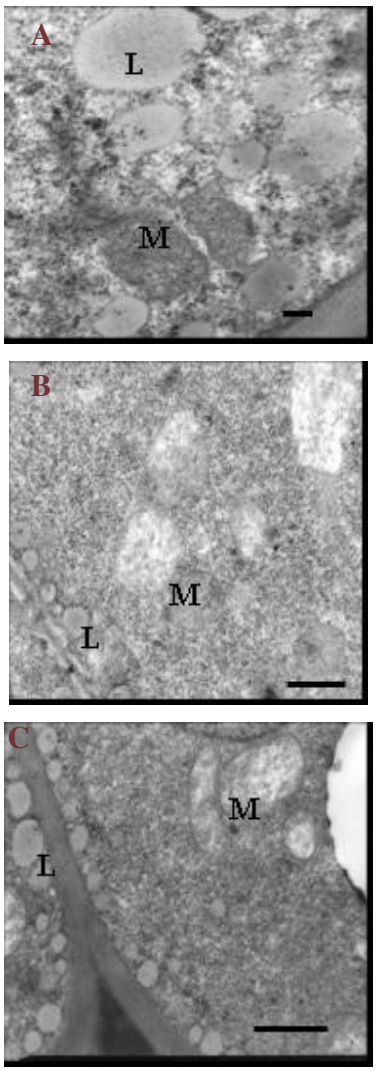

Figure 7.3: TEM micrographs of embryonic tissue of untreated and treated maize seeds following 2d accelerated ageing and 48 hr rapid imbibition showing differences in the mitochondrial structure, (Bar = 1 µm) a) Untreated control, b) Apron® XL and c) Celest® XL. (L = lipid, M = mitochondrium).

7.4 Discussion

In this study there were visible differences in the position of the lipid bodies between the untreated control and the two treatments (Apron® XL and Celest® XL). The characteristic changes in the ultrastucture of the embryonic radicle tip of seeds that have imbibed water are a gradual breakdown of numerous protein bodies, with formation of vacuoles, appearance of the endoplasmic reticulum, the appearance of better defined elongated mitochondria, with more cristae and increase in the number of golgi bodies (Crèvecoeur *et al*., 1976; Bliss *et al*., 1984). Position and occurance of the vacuoles and lipid droplets differed between the untreated control and treatments tested. None of the fungicides tested had a negative effect on cell development.

In plant cells, the vacuoles are visible and are densely present. The mitochondria are cylindrical to ellipsoid or ovoid, sometimes elongated and even branched. In normal functioning cells they are observed to change shape (Gunning and Steer, 1996). As the seeds in this current study were imbibed for 48 hr, this was the average time for the mitochondria to develop normally (Hodson *et al.,* 1987). This was confirmed in a study conducted by Hodson *et al.* (1987), where the difference in mitochondrial ultrastructure is seen in normal imbibition of seeds compared to imbibition under anoxic conditions. In their study they subjected the seeds to stress conditions such as limited oxygen, in this current study seeds were treated with fungicides and subjected to 2 d AA. However in this current study, following this stress that the seeds were subjected to, mitochondria developed normally. In a study by Vartapetian *et al*. (1987), it was found that by 48 hr, soaking of maize seeds nearly all mitochondria were fully formed. They had an oval shape, electron-dense matrix and elongated cristae randomly distributed inside the microchondria (Crèvecoeur *et al*., 1976, Vartapetian *et al*., 1987). This was confirmed with the untreated control and Celest® XL. In contrast this was not the case with the Apron® XL where there was a mitochondrium which seemed to be developing at a slower rate. The shape of the mitochondria differed from those that were found in the seeds that were untreated and the seeds that were treated with Celest® XL. As all the samples were imbibed for the same amount of time, the mitochondrial development would be expected to be the same in all the treatments.

Lipid reserves are broken down during germination and seedling growth (Gunnning and Steer, 1996). As imbibition is the first stage of germination, there were also visible differences in the position of the lipid layer between the untreated control and the treatments. In the untreated control the lipid bodies had already moved away from the cell wall and appeared to be present in the cytoplasm as lipid droplets as the seed had imbibed water normally and the processes for germination could start. This was confirmed by work done on maize seeds by Hodson and co-workers where their findings showed that the layer of lipid bodies near the plasma membrane gradually disappeared, the lipids being incorporated into the plasma membrane (Hodson *et al*., 1987). In contrast the lipid body layer was still attached to the cell wall in both the treatments. The disappearance of lipid bodies is of considerable interest and might be directly related to the restoration of the selective permeability of the membranes, which is a feature of germination (Simon and Raja Harun, 1972). This was further confirmed by Mollenhauer and co-workers who found that in the ultrastructure of germinating maize seeds, many of the lipid droplets were converted to a smooth membrane of the cell (Mollenhauer *et al*., 1968). The present study has shown fusion of lipid bodies to be a feature in aged seeds which confirmed observations by Smith *et al*. (1991).

The ultrastructure of the untreated control and the treated maize seed samples showed cell structures with clearly defined organelles. The vacuoles in the untreated control sample were more numerous compared to the two treatments. According to the literature the black segment of the vacuoles could be electron opaque remnants of protein bodies (Hodson *et al*., 1987). De Castro and Martinez-Hounduvilla (1984) provided proof that the breakdown of proteins takes place at a faster rate than the breakdown of lipids during imbibition, which could explain the numerous lipid bodies compared to protein bodies in the current study.

Results from this study showed the subtle differences between the untreated control and the two treatments following rapid imbibition 2 d accelerated ageing. However, all the samples tested showed normal development of the cell with intact organelles.

7.5 Literature cited

Baird, L.A.M., Leopold, A.C., Bramlage, W.J. and B.D. Webster. 1979. Ultrastructural modifications associated with imbibition of the soybean radicle. *Botanical Gazzete*. 140: 371-377.

Berjak, P. and T.A. Villiers. 1991. Ageing in plant embryos. II. Age –induced damage and its repair during early germination. *New Phytologist*. 71: 135-144.

Bliss, R.D., Platt-Aloia, K.A. and W.W. Thomson. 1984. Changes in plasmalemma organization in cowpea radicle during imbibition in water and NaCl solutions. *Plant, Cell and Environment*. 7: 601-606.

Bradley, C.A, Wax, L.M, Ebelhar, S.A., Bollero, G.A and W.L. Pedersen. 2001. The effect of fungicide seed protectants, seeding rates, and reduced rates of herbicides on no-till soybean. Crop Protection. 20: 615-622.

Crèvecoeur, M., Deltour, R. and R. Bronchart. 1976. Cytological study on water stress during germination of *Zea mays. Planta*. 132: 31-41.

Crèvecoeur, M., Deltour, R. and R. Bronchart. 1983. Effects of subminimal temperature on physiology and ultrastructure of *Zea mays* embryo during germination. *Canadian Journal of Botany*. 61: 1117-1125.

De Castro, M.F.G. and C.J. Martinez-Honduvilla. 1984. Ultrastructural changes in naturally aged *Pinus pinea* seeds. *Plant Physiology*. 62: 581-588.

Dreyer, M. and H.A. Van de Venter. 1992. Differential effect of temperature on mitochondrial activity in shoots from freshly harvested and moderately aged kernels of maize (*Zea mays* L.). *Plant Growth Regulation*. 11: 267-271.

Felker, F.C. 1987. Ultrastructure of maize endosperm suspension cultures. *American Journal of Botany*. 74: 1912-1920.

Fransolet, S., Deltour, R., Bronchart, R. and C. Van de Welle. 1979. Changes in ultrastructure and transcription induced by elevated temperature in *Zea mays* embryonic root cells. *Planta.* 7-18.

Galli, J.A., fessel, S.A. And R.C. Panizzi. 2005. Effect of *Fusarium graminearum* and infection index on germination and vigour of maize seeds. *Fitopatologia Brasileira* 30:470-474.

Gunning, B.S. and M.W. Steer. 1996. Plant cell biology – structure and function. Jones and Bartlett Publishers, Boston. Pages: 3, 4, 8, 30.

Hodson, M.J., Nola, L.D. and A.M. Mayer. 1987. The effect of changing temperatures during imbibition on ultrastructure in germinating pea embryonic radicles. *Journal of Experimental Botany*. 38: 525-534.

International Seed Testing Association. 2008. International Rules for Seed Testing. Seed Science and Technology. Seed Vigour Testing. ISTA Handbook, 2008.

Moore, R. and C.E. McClelen. 1983. Ultrastructural aspects of cellular differentiation in the root cap of *Zea mays. Canadian Journal of Botany*. 61: 1566-1572.

Moore, R. and C.E. McClelen. 1985. Changes in the distribution of plastids and endoplasmic reticulum during cellular differentiation in root caps of *Zea mays. Annals of Botany*. 56: 73-81.

Mollenhauer, H.H., Kogut, C. and C.F. Kettering. 1968. Ultrastructure of germinating seeds. *The Journal of Cell Biology*. 39: 156-159.

Perissé, P. and A.M. Planchuelo. 2004. Seed coat morphology of *Lupinus albus* L. and *Lupinus angustifolius* L. in relation to water uptake. *Seed Science and Technology*. 32: 69-77.

Peterson, C.A., Murrmann, M. and E. Steudle. 1993. Location of the major barriers to water and ion movement in young roots of *Zea mays* L. *Planta*. 190: 127-136.

Rascio, N. Vecchia, F.D., Ferretti, M., Merlo, L. and R. Ghisi. 1993. Some effects of cadmium on maize plants *Archives of Environmental contamination and Toxicology*. 25:244-249.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology.* 17**:** 208–212.

Simon, E.W. and R.M. Raja Harun. 1972. Leakage during seed imbibition. *Journal of Experimental Botany*. 23: 1076-1085.

Smith, M.T. 1991. Ultrastructural changes during imbibition in seeds of lettuce (*Lactuca sativa* L.) after gamma irradiation. *Seed science and Technology.* 19: 385-395.

Van der Merwe, C.F and J. Coetzee. 1992. QUETOL 651 for general use: a revised formulation. Proceedings for Electron Microscopy Society of Southern Africa. Pietermaritzburg. Pages: 31-32.

Vartapetian, B.B., Snkhchian, H.H. and I.P. Generozova. 1987. Mitochondrial fine structure in imbibing seeds and seedlings of *Zea mays* L. under anoxia *In* Crawford, R.M.M. (Ed). Plant life in aquatic and amphibious habitats. Blackwell Scientific Publishing. Oxford. Pages: 205-233.

CHAPTER EIGHT

GENERAL CONCLUSION

Biofuels are becoming a viable alternative to fossil fuels. Utilizing agricultural crops for the production of biofuel has drawn much interest. As one of the main crops, maize (*Zea mays* L.) offers promise in this regard. Compared to other crops with biofuel potential, maize can provide both starch (seed) and cellulose material for production. Due to climatic stress, it becomes increasingly important to make sure we are equipped with quality seed for quality crops. An even more urgent reason for ensuring quality is that maize is the most important crop grown in southern Africa, accounting for up to 70% of total human caloric intake (Martin *et al*., 2000). Of the 7 125 000 tons produced in South Africa in 2006-2007, 535 000 tons are retained on farms for own use and seed for next season planting (http://www.nda.agric.za).

Most of the farmers are subsistance farmers and production of the crop is only to sustain their households. In a survey that was conducted, evaluation of conventinal storage structures showed that the seeds are being stored under sub-optimum conditions. Initial germination results gave percentages over 85% for most of the samples. The result for the sample that had a percentage germination below 20% was expected as those seeds were infested with insects. Maize that was left in the field was used as one of the controls and gave 100% germination. However, when those seeds were stored for a year under simulated sub-optimum conditions, percentage germination dropped to 25.3%. The moisture content of a sample is the loss in weight when it is dried in accordance with the rules outlined by International Seed Testing Association (ISTA) (ISTA, 2008). Control of moisture content of seed is imperative. Water is held in the seed with varying degrees of strength, ranging from free water to chemically bound water (Grabe, 1989). In this study, the drying of the maize in the field was excellent in terms of decreasing of the moisture content. However, with inconsistencies in the climate this process is not controlled.

The decrease in germination of the field sample, proved that even seed in a fairly good condition are susceptible to attack by storage fungi. In a study by Qasem and Christensen (1958), the storage fungi most often involved in deterioration of field- stored maize were typically found after maize had been stored under warm conditions, when the moisture content was between 14 and 18%. The samples that were obtained from the farmers were concurrently compared with commercially treated Celest® XL [*fludioxonil* (25 g ai/L) + *mefenoxam* (10 g ai/L)] maize seed. The treated seed had an initial germination of 94%, following storage with the other samples and the field stored maize, germination decreased to 86%. This was still an acceptable decrease as the acceptable percentage germination of maize is 70% according to the Plant Protection Act (1976). Storing these treated seeds had little effect on the germination. Subsistence farmers may not be equipped to change the moisture content of their seeds to be stored but they could use seed treatments to protect their seeds.

Investigating the effect maize seed treatments has on germination and vigour revealed that when seeds are treated and processed immediately there were no significant differences between the treatments and the untreated control. Seeds treated with Apron® XL had the highest percentage germination (83%) followed by Apron® Star and Thiram (82 and 82.5%, respectively). Both Celest® XL the untreated control also had germination percentages above 80%. This study also showed that and there was a correlation between the percentage germination following the standard germination test of unstored seed, germination following the cold test and the percentage emergence. Unstored seed treated with Apron® XL maintained a higher percentage germination than the other treatments. Seed treatments do not have a negative effect on germination and in most cases, fungicide treatment of maize seed improves emergence and yield compared to non-treated seeds (Munkvold and O' Mara, 2002).

Greenhouse emergence of seeds that have been treated gives a good indication of which fungicide is effective protecting the seeds in the presence of pathogens. In order to test the seed treatments under stress conditions, fungicide treated seeds were stressed by subjecting the seeds to 2 and 4 d accelerated ageing (AA) and 3 and 6 months storage. Evaluating germination and vigour of these seeds gives a good indication of which treatment can be

successfully used to protect the viability of the seeds. This study showed that for most of the samples and the untreated control, germination percentages after 2 d AA did not differ significantly from those stored for 3 months under unfavourable conditions. There was a gradual decrease in germination from 2 – 4 d AA and for some treatments from 3 to 6 months storage. The vigour tests conducted confirmed these results. Interestingly, Apron® XL failed to germinate after 3 months storage. The reason for this is unknown as the percentage weight increase following imbibition and conductivity results for this treatment was not excessively high. After 6 months storage, only Thiram had 12.5% germination and none of the other treatments or the untreated control germinated.

The aged and stored fungicide treated maize seed was grown under greenhouse conditions. In the first trial emergence of the different treatments was compared to the untreated control. Comparing after 2 d AA, Thiram had the highest percentage emergence (70.7%), followed by Celest® XL (68%). Apron® XL had the lowest emergence, decreasing to 6.6% after 4 d AA. After 3 months storage, these seeds failed to germinate. This mirrored the results obtained *in vitro*. After 6 months storage, percentage emergence was only recorded in the untreated control and the Thiram treated seed. The untreated control performed well under greenhouse conditions compared to the treatments. One possible explanation could be that storage fungi of the seeds subjected to the chemical treatments and harsh ageing experiments/storage conditions coupled with ultrastructual changes (Anderson *et al.,* 1970) lowered the germination potential of those seeds. Fungi are known to attack seeds during storage and cause deterioration, these losses include decrease in germinability, discolouration of seed and increase in fatty acids (Anderson *et al*., 1970). With the exception of Thiram none of the fungicides used in this study have been proven to be effective in the control of all the common storage fungi associated with seeds subjected to poor storage conditions.

The second trial consisted of inoculating the treated seeds and the untreated control with *Fusarium graminearum* (Schwabe). Apart from being the casual agent of seedling blight, *F. graminearum* is also a serious storage fungus, producing mycotoxins, which have deleterious health implications (Clear *et al.*, 2002). Emergence following inoculation with *F. graminearum* showed differences in the results. After 2 d AA, the Celest® XL treatment

had the highest percentage emergence (64%) but did not differ from the Thiram treatment and the untreated control. Apron® XL treated seed failed to germinate after 3 months storage and only the untreated control and Thiram treated seed emerged after 6 months storage.. In a study by Broders *et al*. (2007) seed treatment fungicides azoxystrobin, trifloxystrobin, fludioxonil and captan were tested for their effectiveness against *Fusarium graminearum* on maize. Of the fungicides tested, only fludioxonil that provided sufficient inhibition of mycelial growth *in vitro* (Broders *et al*., 2007). One of the major problems during storage is storage fungi and fungicides are needed to protect seeds. In this study seeds treated with Thiram still germinated and emerged following harsh storage conditions.

This study showed the effect ageing and storage has on the performance of seeds treated with Apron® XL. One possible explantion may be the ultrastructural changes that occur when a seed is treated. These seeds were treated by soaking the seeds in the fungicide at the recommended time and dosage. The way in which the fungicide gains access to the seed is through imbibition, coupled with the stress the seed was subjected could have resulted in ultrastructural changes which may have affected germination and thus emergence. Ultrastructural changes within cells are influence by stress such as fluctuation in temperature, lack of oxygen, blockage in pathways responsible for water uptake (Baird *et al*., 1979). In this current study the effect of the fungicide treatments on the ultrastructure of the embryonic tissue of the maize seeds following 2 d AA followed by 48 hr fast imbibition was assessed. The most obvious difference between cells of the radicles of seeds of the untreated control, Apron®XL and Celest® XL treatments was the number and position of the vacuoles. Vacuoles in cells of the untreated control had more pronounced electron opaque remnants of protein bodies (Hodson *et al*., 1987). This is an indication of the process of germination as a result of imbibition. The characteristic changes in the ultrastucture of the embryonic radicle tip of seeds that have imbibed water are a gradual breakdown of numerous protein bodies, with formation of vacuoles, appearance of the endoplasmic reticulum, the appearance of better defined elongated mitochondria (Bliss *et al*., 1984). The lipid layer was still attached to the cell wall in cells of the Apron® XL and Celest® XL treatments but appeared more concentrated in the cytoplasm of cells ofthe untreated control. The position of the lipid layer alongside the cell wall resembles those found in dry seed, where the process of germination has not yet began. As the treated seeds

were already imbibed, it is posible that the treatments may interfere with the pathway responsible for water uptake when seeds have been subjected to accelerated ageing and rapid imbibition.

This study reiterated the importance of good storage practices, especially by subsistence farmers. When comparing the fungicide/pesticide treatments on maize seeds it was found that seed treated with Thiram still germinated even after 6 months storage under sub-optimum conditions probably playing an important role in controlling storage fungi. In the inoculated trial this treatment had 0% diseased seedlings among the seedlings that had emerged. As newer technologies are emerging in agriculture, not everyone has access to these technologies. The use of seed treatments is one option that is available to most farmers. Results in this current study are promising but as the fungicide seed treatments were tested on only one maize lot, different seed lots may give different results. The research in this study needs to be repeated using other maize seed lots and results need to be confirmed in field trials.

8.1. Literature cited

Anderson, J.D., Baker, J.E. and E.K. Worthington. 1970. Ultrastructural changes of embyos in wheat infected with storage fungi. *Plant Physiology*. 46: 857-859.

Baird, L.A.M., Leopold, A.C., Bramlage, W.J. and B.D. Webster. 1979. Ultrastructural modifications associated with imbibition of the soybean radicle. *Botanical Gazzete*. 140: 371-377.

Bliss, R.D., Platt-Aloia, K.A. and W.W. Thomson. 1984. Changes in plasmalemma organization in cowpea radicle during imbibition in water and NaCl solutions. *Plant, Cell and Environment*. 7: 601-606.

Broders, K.D., Lipps, P.E., Paul, P.A. and A. E. Dorrance. 2007. Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. *Plant Disease*. 91: 1155-1160.

Clear, R.M., Patrick, S.K., Turkington, T.K. and R. Wallis. 2002. Effect of dry heat treatment on seed-borne *Fusarium graminearum* and other cereal pathogens. *Canadian Journal of Plant Pathology*. 24: 489-498.

Grabe, D.F. 1989. Measurement of moisture content. Pages: 69-92 *In*: Stanwood, P.C. and M.B. McDonald (Eds). Seed Moisture. Crop Science Society of America, Inc, Madison.

Hodson, M.J., Nola, L.D. and A.M. Mayer. 1987. The effect of changing temperatures during imbibition on ultrastructure in germinating pea embryonic radicles. *Journal of Experimental Botany*. 38: 525-534.

http://www.nda.agric.za. Accessed: 30/11/2007.

International Seed Testing Association (ISTA). 2008. International Rules for Seed Testing. Seed Science and Technology.

Martin, R.V., Washington, R. and T.E. Downing. 2000. Seasonal Maize forecasting for South Africa and Zimbabwe derived from an agro-climatological model. *Journal of Applied Meterology*. 39: 1473-1479.

Munkvold, G.P. and J.K. O'Mara. 2002. Laboratory and growth chamber evaluation of fungicidal seed treatments for maize seedling blight caused by Fusarium species. *Plant Disease*. 86: 143-150.

Plant Protection Act, 1976. Provisions Relating to Seed and Seed Samples. Act no. 53 of 1976. http://www.nda.agric.za. Accessed: 30/03/2006.

Qasem, S.A. and C.M. Christensen. 1958. Influence of moisture content, temperature, and time on the deterioration of stored maize by fungi. *Phytopathology*. 48: 544–549.

Vertucci, C.W. 1989. The Kinetics of seed imbibition: Controlling factors and relevance to seedling vigour. Pages: 93-115 *In*: Stanwood, P.C. and M.B. McDonald (Eds). Seed Moisture. Crop Science Society of America, Inc, Madison.