UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

VIGOUR OF FUNGICIDE-TREATED AND UNTREATED MAIZE SEED FOLLOWING STORAGE

BY

VELOSHINIE GOVENDER

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

PHILOSOPHIAE DOCTOR

IN THE FACULTY OF NATURAL AND AGRICULTURAL SCIENCES

DEPARTMENT OF MICROBIOLOGY AND PLANT PATHOLOGY

UNIVERSITY OF PRETORIA
PRETORIA

JULY 2008

I, the undersigned, declare that these studies, except where acknowledged in the text, is my own work and has not been previously submitted in any other form to this or any other tertiary institution.

_____

Veloshinie Govender

Vigour of fungicide- treated and untreated maize seed following storage

by

Veloshinie Govender

Supervisor: Prof. T.A.S. Aveling
Co-supervisor: Dr. Q. Kritzinger
Department: Microbiology and Plant Pathology
Degree: Philosophiae Doctor

ABSTRACT

An assessment of the effect that conventional storage structures, used by small-scale farmers in northern Kwa-Zulu Natal and southern Mozambique, had on germination and vigour of maize seeds was conducted. The survey confirmed that the methods of storing the seed decreased the quality of the maize seeds. Storing maize in the field was good as a short-term solution as initial germination was 100%. Following storage at sub-optimum conditions, germination dropped to 25.3%. Commercially treated maize seeds were compared to the test samples collected. After storage, the commercially treated seeds maintained a germination percentage above 75.

Untreated maize seeds were treated with fungicides at the recommended dosages. Thereafter the seeds were subjected to germination and vigour tests according to methods outlined by the International Seed Testing Association. All treatments maintained percentage germination above 75. Apron® XL had the highest percentage germination of 83. This trend was also found following the cold test and greenhouse emergence. None of the treatments differed significantly from the control. In this study none of the treatments caused major imbibition damage as indicated by the percentage weight increase and the low leachate conductivity (1012-1271 $\mu Scm^{-1}g^{-1}$).

The effect of accelerated ageing (AA, 2 and 4 days) and long-term storage (3 and 6 months) on germination and vigour of treated maize seeds was investigated. In the untreated control and treatments there was a gradual decrease in germination following ageing and storage of the seeds. Apron® XL failed to germinate after 3 months. The

decrease in germination was mirrored by the leachate conductivity readings. Thiram was the only treatment to maintain germination after 6 months storage. The seeds were planted in two greenhouse trials to assess the performance of the treatments *in vivo*. The first trial evaluated the emergence and second the emergence and control of *Fusarium graminearum*. Results from the first trial showed that following 2 d AA, seeds treated with Thiram had the highest percentage emergence (70.7) followed by Celest® XL (68) and the untreated control (62.7). Following inoculation, a similar trend was seen for the treatments and the untreated control. In relation to the percentage seedlings emerged, the control had the highest percentage diseased seedlings. Celest® XL had the lowest percentage diseased seedlings (10, 2 and 1) but failed to germinate after 6 months storage. Thiram was the only treatment to emerge after 6 months storage.

The ultrastructural changes in embryonic roots of the untreated control, Celest® XL and Apron® XL were investigated using transmission electron microscopy. These seeds were subjected to 48 hr rapid imbibition and 2 d AA. The most obvious difference between the untreated control, Apron® XL and Celest® XL was the number and position of the vacuoles. In contrast the lipid layer was still attached to the cell wall in the Apron® XL and Celest® XL treatments but in the untreated control they appeared more concentrated in the cytoplasm.

This study proved that Thiram was the best treatment among the fungicides tested. However, these results need to be confirmed using a larger range of maize seed lots.

Keywords: germination, emergence, fungicides, *Fusarium graminearum*, maize, storage, ultrastructure, vigour, *Zea mays*

ACKNOWLEDGEMENTS

I wish to put on record my gratitude to the following

* Prof Terry Aveling, as primary supervisor, for guidance, support, motivation and enthusiasm throughout the course of this study.

* Dr Quenton Kritzinger for invaluable input and support.

* The National Research Fund for financial assistance towards this study.

* The University of Pretoria for funding.

* Syngenta® Ltd (Pty) for the maize seeds and the chemicals used in this study.

* Dr Kloppers from Pannar for providing additional maize seeds.

* Dr Gert Marais and Pranita Dawlal (FABI) for providing the *Fusarium* culture.

* Pam Strauss, Ansie de Vries and the rest of the staff at the Seed Testing unit of Department of Agriculture, Directorate: Plant Production systems – Roodeplaat (Pretoria) for use of the facilities.

* A very big thank you goes to Chris van der Merwe and Alan Hall at the Laboratory for Microscopy and Micro-analysis for your enthusiasm and assistance.

* Marie Theron (Academic Information Service, University of Pretoria), Cathy Barnard (Administration, Faculty of Natural and Agricultural Sciences),

* Thank you to everyone at Plant Pathology Laboratories. Special mention goes to Daleen Muller, thank you for your friendliness and willingness to help.

* To my two best friends Leylani Grobler and Stacey Collignon, thank you for lending me your ears, for the support and most importantly for the laughs!!

* To my family, thank you for your unconditional love and support throughout my studies. To my two special angels: My parents, words cannot express my love and gratitude to you for all that you have done for me. Thank you for molding me into the person I am today and most importantly Thank you for believing in me.

Most importantly, Thank you God for the direction you have given me in my life and the strength to achieve my goals.

TABLE OF CONTENTS

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background and motivation of the study

Maize (*Zea mays* L.) is one of the main staple crops that is grown worldwide. Following rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.), it is the third most important cereal crop in Sub-Saharan Africa (Rehman *et al*., 2002; Dredge, 2004). Maize is primarily a cross-pollinating species, a feature that has contributed to its broad morphological variability and geographic adaptability. Kernels may be colourless (white) or yellow, red, blue or variegated with these colours in mottled or striated patterns (Saunders, 1930; Salvador, 1997).

Traditionally maize is used for human consumption (white maize) (Rehman *et al*., 2002) and for animal feed (yellow maize) (Dredge, 2004). This crop is also versatile and is used in many inedible products, including rubber, plastics, biofuel (Bant, 2007), alcohol fermentation, clothing, food additives and adjuncts and literally thousands of other forms (Abbas *et al*., 2006). In South Africa, figures have revealed, that for 2006-2007 production season, there was 7 125 000 tons produced in this country (www.nda.agric.za) (Table 1.1).

Table 1.1: Calculated final crop of maize- 2006/2007 production season*

CROP	Total Tons	Producer deliveries reported by SAGIS[1)] (Mar-Oct 2007) Tons	Future deliveries (Nov 2007 - Feb 2008) Tons	Retentions on farm for own use/ Seed Tons
White Maize	4 315 000	4 132 107	57 893	125 000
Yellow Maize	2 810 000	2 357 221	42 779	410 000
Total Maize	7 125 000	6 489 328	100 672	535 000

* Table modified from http://www.nda.agric.za/foodsecurity issues

In South Africa, climatic stress makes it imperative to ensure that the amount of seed (Table 1.1) that is retained on farms for own use and seed (mostly by subsistance farmers)

is disease free and of good quality. This is a global problem (Ajayi and Fakorede, 2000, Rehman, 2006) and research into the proper storage of seeds has presented the abiotic stresses that affect seeds (Jayas and White, 2003) as well as problems with the inherent properties of seed (Thamaga-Chitja *et al*., 2004). In many areas of the world, storage facilities for seeds are inappropriate for long-term storage and can lead to a decrease in germinability of the crop (Thamaga-Chitja *et al*., 2004), discolouration of part (usually the embryo) or all of the seed or kernel, heating and mustiness, various biochemical changes that may be a result of the production of mycotoxins (Munkvold, 2003; Presello *et al*., 2007) that if consumed may be injurious to man and to domestic animals and loss in seed weight (Nansen *et al*., 2004).

Even if proper storage conditions are met the inherent property of the seed must be taken into account, if maize seed is harvested when the moisture content is high (13-14%) (Thamaga-Chitja *et al*., 2004), storing that seed would not eliminate the possibility of storage fungi (Asiedu and Powell, 1998). Fungicides have been used to control the pathogens of many economically important crops (Agrios, 2005). There are preventative and curative fungicides (Agrios, 2005). Seed treatment fungicides are a viable option to protect the seed from fungal infection after harvesting (Falloon, 1982; Munkvold and O'Mara, 2002).

Protection of valuable seed such as maize seed should not come at the expense of seed quality and viability (Abba and Lovato, 1999). Viability is measured by germination tests, which determines the maximum germination potential of a seed lot as well as the evaluation of a particular seed lot under an ideal set of conditions (ISTA, 2006). Seed vigour assesses the ability to germinate under a wide range of environmental conditions (Shah *et al*., 2002). It remains not a single measurable property of physiological and physical quality like standard germination but a concept describing several characteristics associated with seed lot performance (Hampton, 1995; Copeland and McDonald, 2001). It is therefore important that the fungicides that are used to protect seeds do not interfere with the viability or vigour of the seeds, no matter what stress condition the seeds are grown under. The way in which seeds react to stress or changes in there environment is best shown through ultrastructural changes.

1.2 Objectives of the study

The primary aims of this study were to 1) investigate the effect fungicide seed treatments have on the germination and vigour of maize seeds and 2) to evaluate the effectiveness of those fungicides when treated maize are aged artificially and subjected to long-term storage.

The specific objectives of this study were to:

a) Conduct a survey amongst small-scale subsistence farmers to assess the effect their storage structures have on germination and vigour of their maize seeds.
b) Evaluate germination and vigour of commercially treated maize seed under sub-optimum conditions.
c) Assess the effect fungicide seed treatments have on germination and vigour of maize seed subjected to 2 and 4 day accelerated ageing and 3 and 6 months storage.
d) Identify which of the fungicide seed treatments maintain/improve emergence of maize seeds that were subjected to ageing and long-term storage, under greenhouse conditions.
e) Evaluate which fungicide is effective in the control of *Fusarium graminearum* (Schwabe) under greenhouse conditions.
f) Compare the ultrastructural changes between treated maize seeds and untreated control subjected to 48 hr rapid imbibition.

1.3 Structure of the thesis

One of the chapters presented in this thesis has been published and is presented in the format as it appears in South African Journal of Botany.

Chapter Two: This chapter provides a concise review of the importance of maize seed globally. Information has been provided on control of diseases affecting maize. The reader is introduced to the concepts of germination and vigour and the importance of proper storage when it comes to maintaining viability of seed for the next planting season.

Chapter Three: Conventional storage structures were investigated as to the effect of sub-optimum storage conditions on germination and vigour. This was compared to the effect sub-optimum storage conditions have on commercially treated maize seeds.
Published as: **V. Govender, T.A.S. Aveling, Q. Kritzinger**.2008. The effect of traditional storage methods on germination and vigour of maize (*Zea mays* L.) from northern KwaZulu-Natal and southern Mozambique. *South African Journal of Botany*. 74: 190-196.

Chapter Four: Maize seeds were treated with fungicides and the effect of these treatments on germination and vigour were investigated. This was compared to untreated seeds. Greenhouse emergence was compared to the germination test conducted *in vitro*.

Chapter Five: Treated maize seeds were subjected to 2 and 4 day accelerated ageing and 3 and 6 months storage. Following the incubation periods the treated seeds were subjected to germination and vigour tests. These results were compared to the untreated control that was also aged and subjected to long-term storage.

Chapter Six: This chapter focuses specifically on greenhouse emergence of fungicide treated seeds. There were two trials that were conducted 1) un-inoculated – aged and stored treated maize seed were grown under greenhouse conditions to assess emergence and 2) inoculated – aged and stored treated maize seed were inoculated with *Fusarium graminearum* (Schwabe) and the emergence and disease control were evaluated.

Chapter Seven: Based of results from the previous chapters, the ultrastructural changes of maize seeds treated with two of the fungicide treatments were investigated. This was compared to the untreated control. This

investigation was conducted following rapid imbibition (48 hr) of the seeds.

Chapter Eight: In this chapter, the findings from the study have been summarized.

1.4 Literature cited

Abba, E.J. and A. Lovato. 1999. Effect of seed storage temperature and relative humidity on maize (*Zea mays* L.) seed viability and vigour. *Seed Science and Technology*. 27: 101-114.

Abbas, H.K., Cartwright, R.D., Xie, W. and W.T. Shier. 2006. Aflatoxin and fumonisin contamination of maize (*Zea mays* L.) hybrids in Arkansas. *Crop Protection*. 25: 1-9.

Agrios, G.N. 2005. Plant Pathology – 4th Edition. Academic Press, San Diego. Pages: 209-211.

Ajayi, S.A. and M.A.B. Fakorede. 2000. Physiological maturity effects on seed quality, seedling vigour and mature plant characteristic of maize in a tropical environment. *Seed Science and Technology*. 28: 301-319.

Asiedu, E.A. and A.A. Powell. 1998. Comparisons of the storage potential of cultivars of cowpea (*Vigna unguiculata*) differing in seed coat pigmentation. *Seed Science and Technology*. 26: 211-221.

Bant, M.U. 2007. Tropical maize for biofuels. http://www.aces.uiuc.edu/news/stories/news4169.html. Accessed: 19/09/2007.

Copeland, L.O. and M.B. McDonald. (Eds.) 2001. Pages: 124-128 *In*: Principles of Seed Science and Technology (4th Edition). Kluwer Academic Publishers, Massachusetts.

Dredge, R.D. 2004. Assessment of current economic and physical environment of agriculture. Executive Summary. http://www.nda.agric.za. Accessed: 24/07/2005.

Falloon, R.E. 1982. Fungicide seed treatment of maize to improve establishment and control of pathogens. *New Zealand Journal of Agriculture*. 10: 197-202.

Hampton, J.G. 1995. Methods of viability and vigour testing – a critical appraisal. Pages: 81-117 *In*: Basra, A.S. (Ed.). Seed Quality – Basic mechanisms and agricultural implications. The Haworth Press, New York

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

International Seed Testing Association (ISTA). 2006. International Rules for Seed Testing. Seed Science and Technology. Seed Vigour Testing. ISTA Handbook, 2006.

Jayas, D.S. and N.D.G. White. 2003. Storage and drying of grain in Canada: Low cost approaches. *Food Control.* 14: 255-261.

Munkvold, G.P. 2003. Epidemiology of *Fusarium* diseases and their Mycotoxins in maize ears. *European Journal of Plant Pathology*. 109: 705-713.

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.

Nansen, C., Phillips, T.W., Parajulee, M.N. and R.A. Franqui. 2004. Comparison of direct and indirect sampling procedures for *Plodia interpunctella* in a maize storage facility. *Journal of Stored Products Research.* 40: 151-168.

Presello, D.A., Iglesias, J., Botta, G. and G.H. Eyhérabide. 2007. Severity of *Fusarium* ear rot and concentration of fumonisin in grain of Argentinian maize hybrids. *Crop Protection*. 26: 852-855.

Rehman, Z., Habib, F. and S.I. Zafar. 2002. Nutritional changes in maize (*Zea mays* L.) during storage at three temperatures. *Food Chemistry*. 77: 197-201.

Rehman, Z. 2006. Storage effects on nutritional quality of commonly consumed cereals. *Food Chemistry*. 95: 53-57.

Salvador, R.J. 1997. History of maize. http://maize.agron.iastate.edu/maizearticle.html. Accessed: 25/02/2005.

Saunders, A.R. 1930. Maize in South Africa. Central News Agency Limited, South Africa. Pages: 7-34.

Shah, F.S., Watson, C.E. and E.R. Cabrera. 2002. Seed Vigour testing of subtropical maize hybrids. Research report – Mississippi Agricultural and Forestry Experiment Station. 23. Pages: 1-5.

Thamaga-Chitja, J.M., Hendriks, S.L., Ortmann, G.F. and M. Green. 2004. Impact of maize storage on rural household food security in Northern Kwazulu-Natal. *Journal of Family Ecology and Consumer Science*. 32: 8-15.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction to Maize (*Zea mays* L.)

2.1.1 Origin and biology of *Zea mays* L.

Maize (*Zea mays* L.) is a gigantic domesticated grass of tropical Mexican origin (Saunders, 1930; Salvador, 1997). The crop, which is produced from 50°C latitude N to 40°C S, is adapted to desertic and high rainfall environments and to elevations ranging from 0 to 400 m above sea level (Saunders, 1930). Currently, major maize production areas are located in temperate regions of the globe (Salvador, 1997). Following rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.), it is the third most important cereal crop in sub-Saharan Africa (Rehman *et al*., 2002; Dredge, 2004). Maize is grown throughout the tropics and subtropics and mostly by small-scale farmers, generally for subsistence as part of agricultural systems that feature several crops and sometimes livestock production. These systems often lack essential inputs such as fertilizer, improved seed, proper irrigation and labour (Kidane, 2001; Dredge, 2004).

Maize is a tall, determinate annual plant producing large, narrow, opposing leaves, borne alternatively along the length of a solid stem. Its other distinguishing feature is separation of the sexes among its flowering structures (Sauer, 1993). The maize plant produces male inflorescences (tassels) which crown the plant at the stem apex, and female inflorescences (ears) which are borne at the apex of condensed lateral branches protruding from leaf axils (Sauer, 1993). The male (staminate) inflorescence, a loose panicle, produces pairs of free spikelets each enclosing a fertile and a sterile floret. The female (pistillate) inflorescence, a spike, produces pairs of spikelets on the surface of a highly condensed rachis (central axis or “cob”). The individual maize grain is botanically a caryopsis, a dry fruit containing a single seed fused to the inner tissues of the fruit case (Salvador, 1997).

Maize production is measured in bushels, a term that equates to a quantity equal to 56 pounds of shelled grain (removed from the cob). A single bushel of maize contains roughly 73 000 kernels, each of which can produce a plant bearing one or more ears, each of which in turn can produce roughly 800 new kernels (Salvador, 1997; Rindels, 1995). The high productivity of maize is due to its large leaf area and to modification of its photosynthetic pathway. This modification (shared by other tropical species adapted to survive periods of drought stress) is known as "C4 syndrome" and consists of an efficient mechanism for the exchange of water vapour for atmospheric carbon dioxide (Salvador, 1997). Maize is primarily a cross-pollinating species, a feature that has contributed to its broad morphological variability and geographic adaptability. Kernels may be colourless (white) or yellow, red, blue or variegated with these colours in mottled or striated patterns (Saunders, 1930; Salvador, 1997).

2.1.1.1 Africa

Maize was introduced in Africa by Portuguese explorers in the beginning of the 16th century. It has since become Africa's second most important food crop (Saunders, 1930; Modi, 2004). Per capita consumption of maize in Africa is highest in Eastern and Southern Africa. Production in Eastern and Southern Africa consists almost exclusively of white maize, with small pockets of yellow land races in coastal regions and southern Sudan. West and Central African households use both white and yellow maize (Dredge, 2004). The challenge of improving maize varieties for small-scale farmers in Africa appears to be centred to making the crop more resistant to foliar diseases and more tolerant to drought. Longer-term, there are important opportunities in adding *Striga* resistance, resistance to stem borers and increased protein content. Maize consumption in Kenya, Tanzania, Malawi, Zimbabwe, Zambia and Swaziland averages over 100 kg per year per family giving maize a similar position in terms of dietary importance as rice is in Asia (Saunders, 1930; Rehman *et al*., 2002; http://www.africancrops.net/crops1/maize/index).

In Africa, the factors that contribute to affect declining maize production include excessive rains and floods or excessive dry spells, reduced and late delivery of agricultural implements as a result of transport bottlenecks (http://www.africancrops.net). For these reasons Malawi, Zimbabwe and Zambia have declared a state of emergency and have at

some point appealed for international assistance (http://www.fao.org). Other areas with problems include southern provinces of Mozambique, Lesotho, Swaziland and Namibia. In Eastern Africa (Somalia, Kenya and Ethiopia) there remain acute food shortages due to continuing drought conditions. Alternatively the introduction of plant material into Ethiopia can serve as sources of serious pest introductions (Kidane, 2001). In the Democratic Republic of Congo and Liberia access to the populations remains problematic due to civil strife so food aid is not always received by those that need it (Dredge, 2004; http://www.fao.org). Much of Southern Africa is subject to climatic extremes that often result in poor crop yields.

2.1.1.2 South Africa

Maize was brought by the Portuguese from the West Indies to the island of St Thome and from there taken to the Gold Coast. Apparently it reached South Africa in 1655, shortly after the arrival of the first Dutch colonists (Saunders, 1930). Maize is the most important crop grown in southern Africa, accounting for up to 70% of total human caloric intake (Martin *et al*., 2000). Most maize production is fed by rain. Even in South Africa, irrigated land is less than 1% of the cultivated area. A strong dependence upon agriculture, high population growth rates, and unstable economic conditions compound the sensitivity to climatic extremes (Martin *et al*., 2000). The importance of breeding for local adaptation to South African conditions is extremely important since South Africa has its own stress conditions that need to be bred for (http://www.nda.agric.za). Key traits that are focused on in the breeding programs are yield, dry-down, stand ability, grain quality, and resistance to diseases such as northern leaf blight, rust and grey leaf spot (Hols, 2006).

In 2004 the situation in South Africa was such that following a late start to the rainfall season, farmers planted later than normal but still managed to plant within a “safe planting” window (Dredge, 2004; Reynolds, 2004). Food security for the country as a whole is, in spite of the drought of the years prior to 2004, in a very healthy state mainly due to improved production techniques and overhead irrigation (http://www.nda.agric.za). The recent low prices are the result of a number of factors, including large carry-over stocks from the 2004 crop, relatively low international prices, the rand strength against the dollar and the better than expected rainfall, which has boosted the prospect of a maize crop in

excess of 9 million tons (Dredge, 2004). White maize, used for human food, is regarded as a more important indicator than yellow maize, typically used in animal feed (http://www.nda.agric.za). The main irrigated production areas used by Pannar are situated in the north-eastern South Africa, which has a healthy dry climate and fertile well-drained soils (http://www.pannarseed.co.za).

2.1.2 Uses of maize

Traditionally maize is used for human consumption (white maize) (Rehman *et al*., 2002) and for animal feed (yellow maize) (Dredge, 2004). It is one of the major sources of protein and energy in the preparation of different types of human foods in many parts of the world (Rehman *et al*., 2002). The humble kernel of maize finds its way into your life in more ways than those mentioned previously as inedible products, including rubber, plastics, fuel, alcohol fermentation, clothing, food additives and adjuncts and literally thousands of other forms (Abbas *et al*., 2006). The maize starch is used in the textile industry for fabric and is also suitable for pharmaceutical uses such as disintegrating agents in tablets (Abbas *et al*., 2006). Maize is mainly consumed in African households as a thick porridge, produced by prior soaking followed by hand pounding or grinding in a hammer mill followed by boiling (Saunders, 1930; http://www.africancrops.net/crops1/maize.htm). Recently, research results in the US show that tropical maize, when grown in the Midwest, requires few crop inputs such as nitrogen fertilizer, chiefly because it does not produce any ears, this is ideal for biofuel production (Bant, 2007). The tropical maize is referred to as the sugarcane of the Midwest as it produces, straight from the field with no processing, 25 percent or more sugar in the forms of sucrose, fructose and glucose (Bant, 2007).

The Council for Scientific and Industrial Research (CSIR) has completed a study for South Africa's Maize Trust aimed at developing maize speciality foods for Small, Medium and Mini Enterprises (SMMEs), based on indigenous food concepts. These include biscuits, chocolate bars, and numerous variants of traditional maize bread, using morogo [spinach (*Spinacia oleracea* L.)], sun-dried tomatoes (*Lycopersicon esculentum* L.) and herbs (Anon, 2004). Other speciality products developed included a gluten-free maize bread premix, a maize salsa, and a non-dairy fermented maize dip (Anon, 2004). Some storage structures

for the grains are also weaved using maize grass and depending on the availability, the stalks of maize are used (http://www.idrc.ca).

2.2 Diseases of maize

There are many biotic constraints to maize production including bacteria, fungi, insects and viruses. Insects and viruses (Barrow, 1992) are an important threat to maize production in Africa (Ngoko *et al*., 2002). Major pests include stem and ear borers, armyworms (Burkhardt, 1952), cutworms, grain moths, beetles (weevils, grain borers, rootworms and white grubs) and virus vectors (aphids and leafhoppers) (Annecke and Moran, 1982). False wireworms, *Somaticus* spp. (Coleoptera: *Tenebrionidae*), feed primarily on the subterranean stems of maize seedlings (Drinkwater, 1994). Together with this, there is also attack from nematodes that generally originate from soil to feed on seedling roots (Kommedahl and Windels, 1986).

The extent and severity of infectious maize diseases depend on the presence of a virulent pathogen, proper air and soil and most importantly the susceptibility of the maize host (Shurteleff, 1980). Cool or wet conditions that reduce or delay maize germination or seedling development can also lead to early-season seed rots, seedling blights and/or root rots (Wyckhuys and O'Neil, 2006). In addition, witch-weed, *Striga hermonthica* (L.) Benth. is a parasitic weed which attacks maize, sorghum (*Sorghum bicolor* L.) and other staple cereal crops. It has become an increasing problem to small-scale subsistence farmers in sub-Saharan Africa and represents today the largest single biological barrier to food production in that region (Oswald and Ransom, 2004).

Fungal maize diseases in Southern Africa (SA) include downy mildew, rust, leaf blight, stalk and ear rots, leaf spot (Annecke and Moran, 1982) and some of the more important ones are discussed. A summary of the various diseases affecting maize is outlined in Table 2.1.

Fungal species that are harmful to the maize crop include *Pythium*, *Fusarium*, *Gibberella*, *Trichoderma* and *Penicillium*, but other fungi such as *Diplodia* and *Rhizoctonia* could also be detrimental to maize (Mc Gee, 1988; Ngoko *et al*., 2002; http://www.omafra.gov.on.ca).

Seed rots and seedling blights are more severe in no-till or reduced tillage fields since heavy residue will keep soil temperatures cooler and wetter longer than conventional fields (Ngoko *et al*., 2002). Damping-off will occur in conventional fields when the crop is planted early in conditions that favour disease development or when environmental conditions cause the maize seed to sit in the ground for a prolonged period of time (Suryanarayana, 1978; http://www.omafra.gov.on.ca).

Seed rots are diseases that affect seeds prior to or shortly after germination. Seed rot is caused by various fungi including those belong to the following genera *Pythium*, *Fusarium*, *Diplodia*, *Rhizoctonia* and *Penicillium*. In the case of seed rot the embryo is killed before germination. The onset of seed rot is favoured by prolonged wet and cold soil conditions in the spring and soil temperatures of 10 -13ºC or lower favour seed rot (Rane and Ruhl, 2002). Seeds that have been damaged or have poor seedling vigour are the most susceptible to seed rot, particularly when soil conditions are cooler (10-13°C) and wet for an extended period of time after planting (Mc Gee, 1988). Seedlings that take a long time to emerge are most susceptible to fungal infection (Rane and Ruhl, 2002).

Seedling blight or "damping-off" are characterized into two groups, pre-emergence and post-emergence seedling blight. Pre-emergence seedling blight affect young seedlings prior to emergence. Affected seedlings may die or grow slower than healthy unaffected seedlings. Post-emergence seedling blight (damping-off) affect the roots or lower stems of young seedlings from emergence to second- or third-leaf stage (Suryanarayana, 1978). The most common casual agents are *Pythium*, *Fusarium*, *Gibberella*, *Diplodia*, *Rhizoctonia*, *Penicillium* and *Trichoderma* all live and thrive in the soil. *Fusarium* pathogens may enter maize ears through the silks or through wounds in the ear caused by birds or insects. Many toxigenic species of *Fusarium* are also common pathogens of cereal plants, causing diseases such as head blight of wheat and barley (*Hordeum vulgare* L.) and ear rot of maize. Consequently, when cereal plants are infected with these fungi, there is a risk that grain may become contaminated with *Fusarium* mycotoxins and that these may subsequently be transferred to compound feeds (Placinta *et al*., 1999). *Fusarium* species cause two distinct diseases on ears of maize, *Fusarium* ear-rot (or pink ear-rot) and *Gibberella* ear-rot (or red ear-rot), both of which can result in mycotoxin contamination of

maize grain (Munkvold, 2003). Damping-off of maize caused by *Pythium* spp. and *Fusarium* spp. is one of the most destructive diseases affecting seedling stands and yield (Mao *et al*., 1998). With *Fusarium* spp., *F. verticillioides* Sheld, *F. subglutinans* Toussoun and Marasas and *F. graminearum* Schwabe are the species most frequently isolated from maize kernels and are the most frequently involved in seedling blight. *Fusarium verticillioides* occurs on and within the pericarp in the cavity between the pedicel and the black layer in the embryo and in the floury and horny endosperm (Kommedahl and Windels, 1986).

With *Pythium*, these soil borne fungi infect root tips or mesocotyls of germinating kernels wherever maize is grown. Examples are: *Pythium irregulare* Buis, *P. debaryanum* Hesse and *P. ultimum* Trow. *Diplodia maydis* (Berk.) Sacc is seed but not soilborne and when infected kernels are planted, weak plants or poor stands occur (Mc Gee, 1988; Mao *et al.*, 1998). Although this fungus may be present in either embryo or endosperm, and occasionally the embryo may be destroyed, the hyphae usually do not penetrate deeply enough to affect germination. Consequently the fungus grows when kernels germinate, attacking the plumule and causing death of the seedling just before it emerges from the soil (Kommedahl and Windels, 1986).

With maize that is infected with *Drechslera maydis* (Nisik.) Subram. and Jain, the planted kernels may rot or if they germinate, the resultant seedlings are blighted or unthrifty in growth. The fungus grows and sporulates mainly on the pericarp but does not penetrate it and therefore chemical treatment is especially effective (Adenle and Cardwell, 2000). *Colletotrichum graminicola* (Ces.) G.W.Wils, a leaf and stalk rot pathogen of maize also occurs also on the kernels. Planting diseased kernels can result in stunted seedlings and seedling blight. In addition there are eight species of downy mildew that occur on maize, mainly in tropical countries and all of them survive from season to season in crop refuse or in kernels, either as oospores or mycelium (Adenle and Cardwell, 2000). Oospores in soil or in crop refuse germinate to produce zoospores that infect germinating kernels. It is therefore essential to treat kernels with fungicides, especially systemics to protect them from both seed and soil and soil borne propagules (Adenle and Cardwell, 2000).

Root rot-causing organisms infect the seedlings' root system, including lateral roots and root hairs (Mathre *et al*., 1994). Affected plants may be stunted, off-colour or lack vigour. Infection can result in seedling death when disease infection is severe, and infected plants may be more susceptible to stalk rots later in the season. Seed, seedling and roots infected by *Pythium* are most often soft (wet) and dark coloured (Mathre *et al*., 1994) as opposed to roots infected with *Fusarium*, *Gibberella*, *Diplodia* and *Rhizoctonia*, which are firm or leathery. The colour of the roots most often provides a good indication of which organism(s) are present: greyish-white indicates *Diplodia*, tan to pink indicates *Fusarium* or *Gibberella*, reddish to brown indicates *Rhizoctonia* and blue-green indicates *Penicillium* or *Trichoderma* (Mathre *et al*., 1994).

Seed treatments will provide additional protection to young vulnerable seedlings. It is recommended that all maize seed be treated with a fungicide seed treatment to prevent early season pre-emergence and post-emergence disease problems (Rane and Ruhl, 2002). Seeds that are cracked or have been damaged through harvest or handling are most prone to these organisms and should be removed. The major strategies for disease management involve the use of pesticides and agricultural practices such as crop rotation and irrigation (Mao *et al*., 1998). For field crops such as maize, soybean (*Glycine max* L.), wheat and rice (*Oryza sativa* L.), seed treatments remain one of the most suitable methods.

Table 2.1: Summary of some of the diseases infecting maize (*Zea mays* L.)

**The diseases that are indicated in bold indicate the diseases that are prevalent in Southern Africa.

Disease**	Pathogen	Parts of maize plant affected	Reference
Bacterial Diseases			
Stewarts Bacterial	*Erwinia stewartii* (Smith) Dye 1963	Leaves, stalk, vascular system	Buchanan and Gibbons, 1974
Goss' Bacterial Wilt and Blight	*Corynebacterium nebraskense* (Vivader and Mandel, 1973)	Leaves, vascular bundles, roots	Calub *et al*., 1974
Holcus Spot	*Pseudomonas syringae* (Kendrick) 1926	Lower leaves	Kendrick, 1926
Bacterial Stripe and Leaf Spot	*Pseudomonas andropogonis* (Smith) Stapp 1928	Leaves	Vidaver and Carlsen, 1978
Bacterial Leaf Blight	*P. avenae* (Rosen 1922)	Leaves	Sumner and Schaad, 1977
Bacterial leaf streak	*Xanthomonas campestris* pv *zeae*	Leaves	Kloppers, 2005
Chocolate Spot	*P. atrofaciens* pathovar *zeae*	Leaves edges and tips	Ribeiro *et al*., 1977
Bacterial Stalk Rot	*E. chrysanthemi pathovar zeae* (Sabet, 1954)	Stalks, uppermost leaves	Christensen and Wilcoxson, 1966
Mycoplasma Diseases			
Maize Stunt	Motile, cell wall-free prokaryote: *Spiroplasma*	Leaves	Nault and Bradfute, 1979
Maize Bushy Stunt	A mycoplasma like	Leaves, ears	Nault and

	organism (MLO)		Bradfute, 1979
Fungal Diseases			
Seed Rots and Seedling Blights	*Fusarium verticillioides* Sheld, *Penicillium* spp.	Leaves, seeds, stem tissues	Furtell and Kilgore, 1969
Root Rots	*Pythium debaryanum* Hesse, *Fusarium roseum* Link	Primary roots	Furtell and Kilgore, 1969
Helminthosporium Leaf Spots and Blights	*Helminthosporium maydis* (Y. Nisik. & T. Miyake)	Leaves, ear, stalks	Shurtleff, 1980
Physoderma Brown Spot	*Physoderma maydis* Miyabe	Leaf blade, Leaf sheath, stalk	Sparrow, 1974
Phyllosticta Leaf Spot	*Aureobasidium zeae* Dingley	Leaves, outer husks, kernels	Arny and Nelson, 1971
***Phaeosphaeria* Leaf Spot**	*Phaeosphaeria maydis* (Berk.)	Leaves	Kloppers, 2005
Anthracnose	*Colletotrichum graminicola* (Wils)	Leaf, stalk	Dale, 1963
Eyespot	*Kabatiella zeae* (Narita & Hirats.) Dingley		Kloppers, 2005
Grey Leaf Spot	*Cercospora zeae-maydis* (Tehon and Daniels)	Leaves, stalk	Latterell and Rossi, 1977
Diplodia Leaf Streak	*Diplodia macrospora* (Earle) Petr. & Syd.	Leaves	Eddins, 1930
Northern Corn Leaf Blight	*Exserohilum turcicum* (Pass.)	Leaves	
Alternaria Leaf Blight	*Alternaria alternata* (Nees.) (Fr) Keissler	Leaves	Shurtleff, 1980

Sorghum Downy Mildew	*Peronosclerospora sorghi* (W. Weston & Uppal) C. G. Shaw		
Sugarcane Downy Mildew	*Peronosclerospora sacchari* Schw	Systemic infection, leaves, ears	Shaw, 1978
Ergot	*Claviceps digitariae* Hansf.	Kernels	Shurtleff, 1980
Common Smut	*Ustilago maydis* (DC.) Corda	Leaves, stalks, ears	Shurtleff, 1980, Kloppers, 2005
Common Maize Rusts	*Puccinia sorghi* Schw	Leaves (upper and lower)	Shurtleff, 1980
***Fusarium* Stalk Rot**	*Fusarium verticilliodes* Sheld	Roots, plant base and lower internodes	Shurtleff, 1980
***Diplodia* Cob Rot and Stem Rot**	*Stenocarpella maydis* (Berk.) B. Sutton	Stems and cobs	Kloppers, 2005
***Gibberella* Ear and Stem Rot**	*Fusarium graminearum* (Schwabe)	Stalks and ears	Kloppers, 2005
Cob and Tassel Smut	*Sphacelotheca reiliana* (Kühn) Clinton	Maize cobs and tassels	Kloppers, 2005
Viral Diseases			
Maize streak disease	Maize streak virus		Kloppers, 2005

Adapted from Shurtleff, 1980 and Kloppers, 2005.

2.3 Chemical seed treatment to control maize diseases

Seed treatments promote seedling establishment, help ensure yield and reduce quality losses due to many diseases and insects. The ability of seed treatments to control many fungal diseases has made them one of the biggest success stories of plant disease prevention (Mikkelson *et al*., 2003). Seed treatments control fungi residing on the seed

surface or inside the seed and are effective against pathogens that reside in the soil and cause seed rots, damping off and root rots (Anaso *et al*., 1989; Mikkelson *et al.*, 2003).

Almost all commercially produced maize sold is treated with a fungicide prior to sale to protect the seed from fungal infection after planting (Falloon, 1982; Munkvold and O'Mara, 2002). Without protection, kernels can rot or succumb to blight just prior to or immediately after emergence of seedlings from soil (Kommedahl and Windels, 1986). The treatment of seeds to protect them from pests and diseases and to ensure a good harvest has a long history (Jeffs, 1986). In 1637 a seed treatment for bunt (*Tilletia* sp) is the earliest record of the use of brine. A consignment of grain soaked with seawater was salvaged from a shipwreck and subsequently sown. The crop produced was said to be free from bunt while the neighbouring stands from untreated seed were heavily infected (Jeffs, 1986). Although fungicides can protect seeds that are damaged at harvesting, the incidence of such damage is greater as the moisture content of the seeds increases above 12% after harvest (Kommedahl and Windels, 1986; Munkvold and O'Mara, 2002).

In order for seed treatments to be successful the biological requirements must be met and are: the pesticides should (1) be consistently effective under the varied conditions met in practice, (2) be safe to operators during handling and sowing, (3) be safe to wildlife, (4) have a wide safety margin between the dose that controls harmful organisms and the dose that harms the plant, (5) be compatible with other materials used on the seed and (6) not produce harmful residues in the plant or soil (Hewett and Griffiths, 1986). Seed treatments with a fungicide, such as captan or thiram, controls some seed rot and seedling blight (Shurtleff, 1980; Agrios, 2005). Fungicides in the strobilurine group are particularly effective against against common rusts (Kloppers, 2005). The control of *Fusarium* infection of maize ears and control of deoxynivalenol (DON) production has been effectively obtained by fungicides such as tebucanozole and metconazole (Magan *et al*., 2002). Sugarcane downy mildew, caused by *Peronosclerospora sacchari* Schw, is a potentially destructive disease of maize in several tropical countries. This disease can be eliminated completely by seed treatment with metalaxyl, which is a systemic fungicide (Singh and Lal, 1985). Alternatively, the fungus, *Cercospora zeae-maydis*, has caused excessive yield losses. This pathogen is the causal agent of Gray leaf spot (GLS) and is in part controlled

by propiconazole. The major limitation in making fungicide application is the inability to predict disease severity (Ward *et al*., 1999; Munkvold *et al*., 2001).

As more pathogens become resistant to most fungicides (McKim, 1994) there is a need to explore other fungicides and their effectiveness in bringing about control. In a study conducted by Drinkwater (1994), the active ingredient of soil-applied and seed dressing systemic carbamate insecticides was shown to be translocated only in sub-lethal quantities through the stems of maize seedlings (Drinkwater, 1994). Rather than a systemic mode of action the insecticides were found to function in a contact or repellent action (Drinkwater *et al*., 1990 as cited in Drinkwater, 1994).

2.3.1 Apron® XL

Reduction in losses to *Phytophthora* and *Pythium* damping-off in several crop species has been achieved by seed treatment with certain systemic, Oomycete-specific fungicides, including metalaxyl [methyl N-(2-methoxyacetyl)-N-(2,6-xylyl)-DLalaninate] (Ciba-Geigy Corp.) and pyroxyfur (6- chloro-4-trichloromethyl-2-pyridyl furfuryl ether) (Dow Chemical Co.) (Lewis, 1988). Metalaxyl is currently registered for use as a seed treatment on forage legumes. Since its registration, metalaxyl has been a valuable aid in controlling damping-off of alfalfa (*Medicago sativa* L.) caused by *P. megasperma* f.sp. *medicaginis* (Rhodes and Myers, 1989). The increase in plant height of seedlings in the metalaxyl treatment over those in the untreated control in *Phytophthora*-infested soil indicates that not only survival, but also vigour of surviving plants, was enhanced by metalaxyl seed treatment (Pommer and Lorenz, 1982; Rhodes and Myers, 1989).

It is possible that without the addition of metalaxyl, *Pythium* may infect the maize seedlings. Because conditions favourable for *Pythium* infection usually occur early in the season (Chen, 1999), *Pythium* most likely damages the roots first with the rootworm larvae feeding later. The rootworm larvae may have been more attracted to these *Pythium* infected maize roots, therefore, causing more damage. This agrees with other reports in which rootworm damage was related to increased root colonization by *Pythium* and *Fusarium* in peanut (*Arachis hypogaea* L.) and maize, respectively (Pedersen *et al*., 2003).

Apron® XL has mefenoxam as the active ingredient. It is sold under various formulations and the trade names include Ridomil gold, Apron® XL, Subdue, and Maxx (Nuninger *et al*., 1996). Maize seeds treated with Apron® XL increases yield and vigour and accordingly fewer seeds are destroyed (www.syngenta.com). In their study, Babadoost and Islam (2003) reported that mefenoxam did not have any effect on either seed germination or seedling vigour when pumpkin (*Cucurbita maxima* L.) seeds were sown on blotter paper or in sterilized soil in the greenhouse. Previous studies have shown that metalaxyl as a seed treatment may protect against stand loss caused by these pathogens (Pedersen *et al.*, 2003). Metalaxyl is used as a seed treatment on most commercially available hybrid maize seed in the United States. It is used to protect seeds and seedlings against plant pathogens in the Oomycete genera *Pythium* and *Phytophthora*, which cause damping-off diseases.

Metalaxyl is a systemic fungicide approved by the Environmental Protection Act (EPA) for treating seeds of at least 30 crops including maize (Anaso *et al*., 1989). Metalaxyl is applied as a water-based slurry as it is compatible with other registered seed treatment fungicides and insecticides (Pedersen *et al.*, 2003). The seed treatment as 25% is designed specifically for seed rot and damping-off caused by species of *Pythium* and *Phytophthora*. In a study by Anaso and co-workers (1989), metalaxyl (Apron 35SD), used as a seed treatment, was highly effective in controlling sorghum downy mildew of maize induced by *P. sorghi* (Anaso *et al*., 1989). In a study by Petch *et al*. (1991) under glasshouse conditions a single coating of metalaxyl (10 g ai kg^{-1} t^{-1} seed) gave plant survival, yield, and control of cavity spot in infested soil equivalent to that from the commercially recommended metalaxyl drench treatment (Petch *et al*., 1991; Clear *et al*., 2002).

2.3.2 Apron® Star

In a study conducted by Ward *et al*. (1997), resistance to benomyl resulted in other combinations of fungicides to be tested for their control of Gray Leaf Spot (GLS). Eria (carbendazim/difenoconazole) and Score (difenoconazole) were found to be effective against GLS and gave a low disease severity compared with the untreated control. Even though carbendazim and benomyl also gave a relatively low disease severity, these fungicides are no longer registered on maize due to a resistance management strategy (Ward *et al*., 1997).

Apron® Star 42 WS is a combination of three active ingredients, namely thiamethoxam, metalaxyl-m and difenoconazole (http://www.syngenta.com). The active ingredient thiamethoxam is a new chemical having a wide spectrum neonicotinoid insecticide (Maienfisch *et al*., 2001; http://www.syngenta.com). In their study, Wu *et al*. (2001), the use of thiamethoxam as a seed treatment on *Brassica oleracea* did not significantly affect seed germination. Difenoconazole is a well-known fungicide that provides protection to plants and reduced resistance risk when compared to the fungicides such as benzimidazoles and dicarboxide (Wu *et al*., 2001).

Difenoconazole has previously been shown to improve seed germination of wheat (Allen *et al*., 2004) and maize (Munkvold and O'Mara, 2002 as cited in Allen *et al*., 2004). In a study by Ronchi *et al*. (1997), it was found that tetraconazole, a triazole fungicide, acted by being a potential activator of plant defence responses to abiotic and biotic stresses. The compound helped the plant become resistant to drought by allowing the plant to remain turgid under water stress while control plants were visibly wilted (Ronchi *et al*., 1997). In a study by Khalil *et al*. (1990) the effect of triazole fungicides were tested in maize seedlings. Due to the systemic action of this fungicide there was accumulation of these fungicides in the roots and shoots of these seedlings, however there were no phytotoxic effects of these fungicides and both these organs functioned normally (Khalil *et al*., 1990).

2.3.3 Thiram

Thiram belongs to the ethylene bisdithiocarbamate (EBDC) chemical class. The EBDCs are fungicides used to prevent crop damage in the field and to protect harvested crops from deterioration in storage or transport (Maude, 1977; Frederickson and Leuschner, 1997). The role of this conventional fungicide in the treatment of fruit, vegetable, ornamental and turf crops fungal diseases is mainly a protection role rather than a cure (Maude, 1977). It is also used as an animal repellent to protect fruit trees and ornamentals from damage by rabbits, rodents and deer. Thiram is available as dust, flowable, wettable powder, water dispersible granules, and water suspension formulations and in mixtures with other fungicides (Maude, 1977). Thiram is nearly immobile in clay soils or in soils high in organic matter. Because it is only slightly soluble in water (30 mg L^{-1}) and has a strong tendency to adsorb to soil particles thiram is not expected to contaminate groundwater (http://www.cornell.edu). Due

to the soaking and subsequent drying of seeds necessary in the thiram soak treatment, the method has proved of commercial use mainly against the pathogens of small, high-value seeds (Maude, 1977). Thiram (tetramethylthiuram disulfide) was the most important alternative to captan as a seed treatment fungicide for maize. Thiram is effective against the common seed, seedling blight and root rot fungi prevalent in maize fields (Kommedahl and Windels, 1986).

Improved emergence and high plant stand in the fungicide/insecticide mixture treatments compared to the untreated control could have resulted from control of seed rot and pre-emergence damping off diseases reported earlier (Ahmed *et al*., 2001). Results from several studies showed that Tebuconazole and thiram alone or mixed with imidacloprid delayed emergence when treated wheat seeds were planted in stubble mulch soil (Ahmed *et al*., 2001). In a study where wheat, which is an irrigated and shallow planted crop, it was found that low rates of the fungicides stimulated emergence in all trials and over all seasons compared to the untreated control. The findings coincided with previous findings that fungicides modify emergence characteristics of seeds planted at shallow depth in irrigated fields (Pike *et al*., 1993). In a study by Ahmed and co-workers (2001), findings showed that the projected increase in food demand and the goal of minimizing expansion of cropped area suggested that wheat yield could be increased by improving crop management practices while meeting acceptable environmental standards (Ahmed *et al*., 2001).

2.3.4 Celest® XL

Celest® XL includes two active ingredients, namely fludioxonil and mefenoxam. This is a water-based odourless chemical (http://www.syngenta.com). It is marketed in some countries under the name Maxim. Mc Govern *et al.* (2002) reported that fludioxonil consistently decreased the incidence of *Rhizoctonia* blight but did not increase the effectiveness of solarization or effect populations of phytoparasitic nematodes. It was found in their studies that *P. ultimum* is a fludioxonil-insensitive fungus (Okada *et al*., 2005). These results were confirmed by Martinez *et al*. (2005) during the evaluation of fungicides for the control of carrot (*Daucus carota* L.) cavity.

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 (Munkvold and O'Mara, 2002; Broders *et al*., 2007) and soybean seeds and seedlings. Of the fungicides tested, only fludioxonil that provided sufficient inhibition of mycelial growth *in vitro* (Broders *et al*., 2007). In another study, fludioxonil was found to reduce certain parameters associated with the disease in barley, including incidence, severity, and deoxynivalenol concentration, while increasing the percentage of plump kernels and yield (Jones, 2000).

African subsistence farmers typically cultivate maize with judiciously used, small inputs of fungicide and insecticide, when they can see their value. For example, they use seed dressings of insecticide and fungicide at planting, and then weeks later, put a few granules of insecticide to control stem borers into the funnel formed by the whorl of maize leaves (Uremis *et al*., 2004).

2.4 Storage of grain

Grains are highly perishable but if they are harvested at the correct time and the moisture content is kept low they may retain their original processing quality and even their original germinability (Christensen and Kaufmann, 1969). Quality of seed is determined by many factors, the most important of this is the way in which grain is stored (Ajayi and Fakorede, 2000; Rehman, 2006). In 1969, the Food and Agricultural Organization (FAO) estimated that 5% of all grain foods harvested are lost before consumption (Christensen and Kaufmann, 1969) but in Africa and some South American countries the loss of the annual harvest totals 30% (Christensen and Kaufmann, 1969; Justice and Bass, 1979). This huge percentage in loss is due to the nature of the storage. After harvest, correct storage of the grain is important to prevent mould spoilage, pest infestation and grain germination (Blaney *et al*., 1984; Abbas *et al*., 2006).

Deterioration of stored grain results from interactions among physical, chemical and biological factors namely temperature, moisture, carbon dioxide (CO_2), oxygen (O_2), the grain characteristics, micro-organisms, insects, mites, rodents, birds, geographical location and granary structure (Jayas and White, 2003). Therefore it becomes important to know

exactly how and where to store grain. Storage fungi, which reduce seed quality, become active in seeds when moisture is 13 percent or higher (Thamaga-Chitja *et al*., 2004). Their proliferation causes rapid loss of seed germination and vigour potential (Shurtleff, 1980; Asiedu and Powell, 1998). Insects in grain at worst will cause major financial losses (Modi, 2004; Thamaga-Chitja *et al*., 2004). If dry grains are held for only a few months, minimum nutritional changes will take place, but if the grains are held with a higher amount of moisture, the grain quality can deteriorate because of starch degradation by grain and microbial amylases (enzymes) (Rehman *et al*., 2002; McKevith, 2004).

Mycotoxins are fungal metabolites that are toxic when consumed by animals or man (Abbas *et al*., 2006). They can accumulate in maturing maize in the field and in grain during transportation (Shurtleff, 1980; Blaney *et al*., 1984) and storage under conditions of moisture, humidity and temperatures favourable for growth of the toxin-producing fungus or fungi (Shurtleff, 1980; Mislivec, 2000; Spears, 2002). *Fusarium* species cause two distinct diseases on ears of maize, *Fusarium* ear rot (or pink ear rot) and *Gibberella* ear rot (or red ear rot), both of which can result in mycotoxin contamination of maize grain (Mduduzi *et al*., 2005; Abbas *et al*., 2006). The primary causal agent for *Fusarium* ear rot is *Fusarium verticillioides* (Sacc.), but *F. subglutinans* (Wollenw. & Reinking) and *F. proliferatum* are also important. *Gibberella* ear rot is caused primarily by *F. graminearum*, but *F. culmorum* (Wmmm.G.Sm) Sacc. can also be important (Yates *et al*., 2005). The primary mycotoxins produced by these fungi are fumonisins and deoxynivalenol (Munkvold, 2003). The other main class of toxins are aflatoxins, which are secondary metabolites produced by *Aspergillus flavus* Link and *A. parasiticus* Speare. These fungi are responsible for spoilage of many stored grains including maize (Egal *et al*., 2005; Liu *et al*., 2006). Not only do these aflatoxins and fumonisins have an important economic impact on the grain industry but the fumonisins are a much greater risk to human and animal health (Abbas *et al*., 2006). The maximum levels permitted by the United States Food and Drug Administration (USFDA) for aflatoxin and fumonisins are 20 ppb and 2 ppm respectively (Abbas *et al*., 2006).

Many storage facilities are inappropriate for long-term storage and can lead to a decrease in germinability of the crop (Thamaga-Chitja *et al*., 2004), discolouration of part (usually the

embryo) or all of the seed or kernel, heating and mustiness, various biochemical changes, production of toxins (Nansen *et al*., 2004).

Grain can be stored in a variety of containers such as clay pots, wooden crib-like structures with woven roof (Fig 2.1 c) grass baskets, granaries (these are hut-like structures that are normally built on stands so that the structures are not directly on the ground) (Fig 2.1 a and b), grain wells, bags and grain huts (Fig. 2.1) and Ferrumbus (these structures are constructed with a circular foundation of stones and cement.

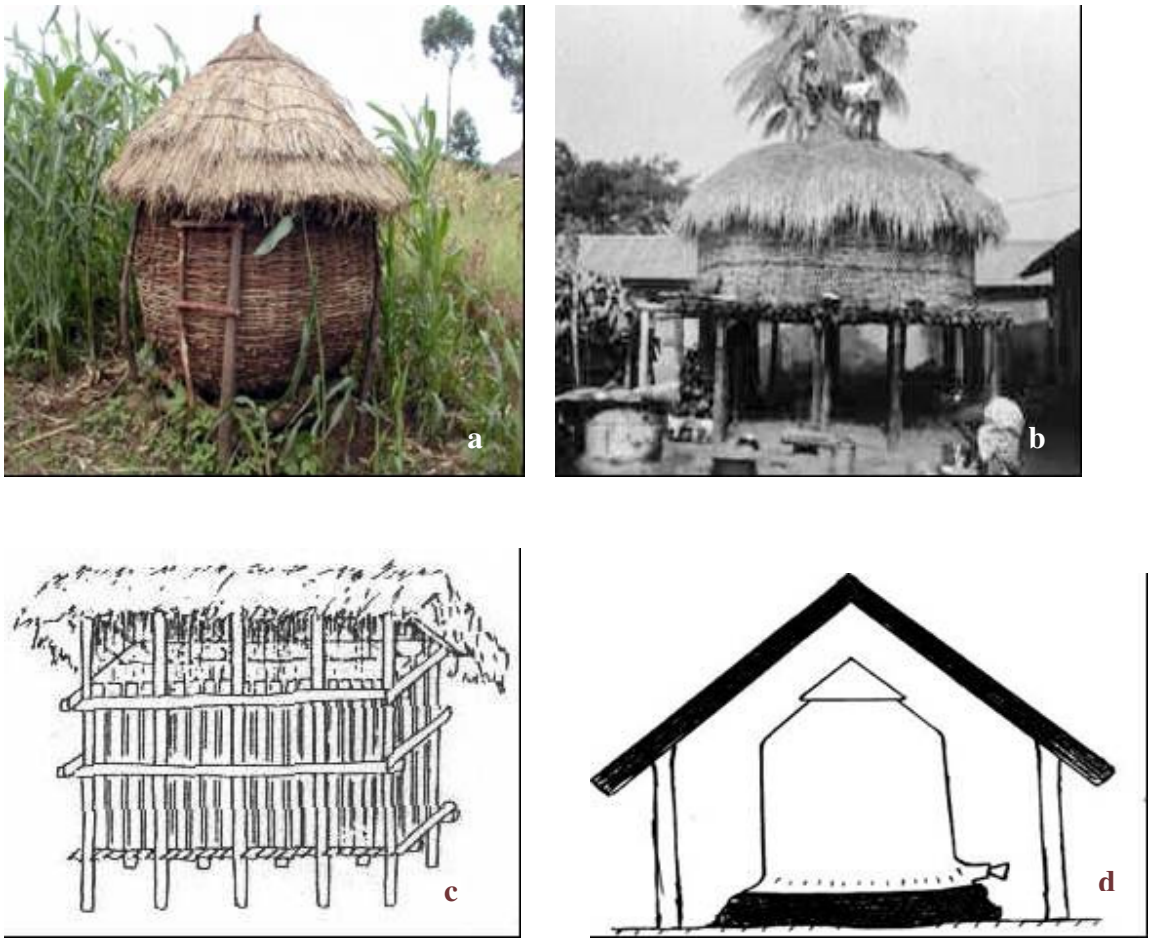


Figure 2.1: Storage structures used in South Africa, a woven wall granary (a), a high granary that is designed to keep moisture out (b), a rectangular crib, with woven roof (c) and a ferrumbu (d). (Pictures obtained from http://www.solutions-site.org and http://www.fao.org)

The foundation is about 60 cm deep. A bin with an outlet is placed on the foundation. This is then housed in a small hut-like construction) (Fig 2.1 d). These are structures that are commonly used in South Africa. Grain huts are used for wheat, sorghum, dry beans and maize and are not built too close to the fields (http://www.nda.agric.za; Thamaga-Chitja et al., 2004). Clay pots are often made from a mixture of ash and cattle manure and the odour of the manure and ash wards off insects. These pots may be kept in an ordinary hut or under the roof of these huts (Thamaga-Chitja *et al*., 2004).

Grass baskets can be weaved from bamboo splits as well as grass in South Africa and come in different sizes (Modi, 2004). They are made water tight to keep the grain dry. Grain wells (izisele) are dug in the kraal (is an enclosure for cattle or other livestock, located within an African homestead or village surrounded by a palisade, mud wall, or other fencing, roughly circular in form) or against a steep incline. The opening to these grain wells are very small, just enough space for a man to enter. The well itself is a big hole which can be plastered with cattle manure or lined with wattle (Thamaga-Chitja *et al*., 2004). A grain well could be dangerous because of the gas build–up from cattle manure. In a study by Modi (2004) it was found that persons climbing down the well have been enveloped by the poisonous gases and have died. The safest alternative for storing grain is within a grain hut or in bags. These methods may allow for easy control of insects and mechanical damage. Some farmers store their maize un-husked over the kitchen fire (Appert, 1987; Modi, 2004). In other crops the application of smoke stimulates seed germination (Sparg *et al*., 2005).

Some farmers are forced to sell their maize at harvest time for a low price and then purchase it back for their own consumption at a higher price (Olakojo and Akinlosotu, 2004; Quezada *et al*., 2006). Alternatives to decrease both the qualitative and quantitative loss of maize without the use of fungicides must be found (Quezada *et al*., 2006), as most of the subsistence farmers cannot afford a fungicide regime. One of the solutions is hermetic (an airtight seal) grain storage. This storage method makes use of the elimination of oxygen and the increase of carbon dioxide. The insects are not able to survive at oxygen levels less than 3% and the fungal development ceases when the oxygen is 1% or less (Quezada *et al*., 2006). These lower oxygen levels caused 100% mortality rate of the

insects (Quezada *et al*., 2006). However, hermetic storage is not practised widely in rural storage as the maize grain moisture content is between 13-15% (Quezada *et al*., 2006). This is considered a high percentage moisture for grain and negatively affects the storage life of the grain (Quezada *et al*., 2006; ISTA, 2008)

Beninese farmers often change their storage structures during the storage period, transferring the maize from a drying or temporary store to a more durable one. Most of the farmers complained about insects damaging stored maize (Hell *et al.*, 2000). The primary pest of stored maize is *Prostephanus truncatus* (Horn) and has been introduced in Africa. The African farmers most at risk are the small-scale producers who grow and store maize for home consumption (Tigar *et al*., 1994).

The association between the physical changes and the changes in the chemical composition of food has made the biochemical and nutritional quality control of the stored products increasingly essential (Rehman *et al*., 2002). In their study, freshly harvested maize was obtained and stored at differing temperature range (10 - 45ºC) for a period of six months. Protein and starch digestibility were significantly affected at 45ºC whereas they remained unchanged at 10ºC. Nutritional quality of maize grains was adversely affected as a result of storage at elevated temperature. The protein digestibility of maize grains decreased by 15% following storage at 45ºC (Rehman *et al*., 2002).

Under sealed storage conditions, insects and fungi combine forces to deplete the oxygen of hermetically stored maize, creating an unfavourable atmosphere for their own survival (Moreno-Martinez *et al*., 2000). Seed deterioration may also occur in the absence of insects, mites and fungi (Pixton *et al*., 1975). Crop emergence failures are sometimes the result of poor quality of the seeds planted and sometimes the result of environmental stresses in the seedbed that are of such magnitude that even the highest quality seeds fail. Most often, however, they are the result of the interaction of seed quality and environmental stresses (Delouche, 2004).

2.5 Aspects of seed quality and vigour

2.5.1 Moisture Content

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). It is expressed as a percentage of the weight of the original sample. The methods prescribed for the calculation of moisture content of grain are designed to reduce oxidation, decomposition or the loss of other volatile substances while ensuring the removal of as much moisture as possible (ISTA, 2008). Moisture content is intimately associated with all aspects of physiological seed quality (Vertucci, 1989). Proven relationships exist between moisture content and seed maturity, optimum harvest time, longevity in storage, economies in artificial drying, injuries due to heat, frost, fumigation, insects and pathogens, mechanical damage and seed weight (Grabe, 1989). Problems in moisture measurement of seeds are imposed by the chemical composition of the seed and the interactions of seed and water. Water is held in the seed with varying degrees of strength, ranging from free water to chemically bound water (Hunt and Pixen, 1980 as cited in Grabe, 1989). During drying the free water is removed with difficulty. The moisture content is the amount of water in the seed and is usually expressed as a percentage. A small change in seed moisture content has a large effect on the effect on the storage life of the seeds (Spears, 2002). Seed moisture content and the temperature during testing are critical factors in many vigour tests. These are factors that require less precision during germination testing. Moisture content is important for results of standard germination and vigour tests (Grabe, 1989; Copeland and McDonald, 2001).

Seeds have to rehydrate to the critical moisture content for germination, which varies among the different kinds of seeds (Copeland and McDonald, 2001). Interestingly, the critical seed moisture content for germination appears to be the same as the moisture content of seeds when physiological maturity is attained. Seeds absorb moisture even in relatively "dry" seedbeds but very slowly and often they do not attain the critical moisture level for germination (Delouche, 2004). Unless and until the seedbed moisture is increased by rain or irrigation, the seeds are in effect "stored" in the soil at a relatively high and increasing moisture content in a micro-environment that can be warm or even hot, i.e., like

accelerated ageing. The performance of seeds under these conditions is determined by the interaction of the environmental stresses and seed quality, i.e. vigour (Delouche, 2004).

2.5.2 Germination

Germination is the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in soil (Copeland and McDonald, 2001). The object of the germination test is to determine the maximum germination potential of a seed lot as well as the evaluation of a particular seed lot under an ideal set of conditions (ISTA, 2008). In this process the seed's role is that of a reproductive unit (Copeland and McDonald, 2001). Different definitions of germination exist. A seed physiologist would define germination as *"the emergence of the radicle through the seed coat*" (AOSA, 1991 as cited in Copeland and McDonald, 2001), while a seed analyst's definition would be "*the emergence and development from the seed embryo of those essential structures, which for the kind of seed in question are indicative of the ability to produce a normal plant under favourable conditions*" (ISTA, 2008).

The percentage germination reported on the ISTA seed analysis certificate indicates the proportion by number of seeds that have produced seedlings classified as normal under the set conditions (ISTA, 2008). Normal seedlings are those that have all the essential structures (root system, coleoptile and leaves) well developed, complete and in proportion; also seedlings with slight defects are grouped under normal seedlings if they show an otherwise satisfactory and balanced development comparable to that of intact seedlings of the same test (Copeland and McDonald, 2001; ISTA, 2008) (Fig 2.2). Abnormal seedlings (Fig 2.2) do not show the potential to develop into normal plants when grown in good quality soil and under favourable conditions of moisture, temperature and light (ISTA, 2008)

Apart from the normal and abnormal seeds there are: damaged seedlings where any of the essential structures are missing or irreparable damaged; deformed seedlings with weak development or physiological disturbances or in which essential structures are deformed out of proportion (ISTA, 2008) (Fig 2.2). In a seed lot, at the end of the germination test

period there are also, hard seeds, those that have not absorbed water; dead seeds, those which at the end of the test period are neither hard nor fresh nor have produced any part of a seedling and; lastly, fresh seeds, those that have failed to germinate under conditions of the germination test but remain clean and firm and have the potential to develop into normal seedlings (ISTA, 2008).

There are two kinds of seed germination that occur; epigeal germination and hypogeal germination. Epigeal germination is characteristic of bean (*Phaseolus vulgaris* L.) and pine (*Pinus sp.*) seeds (Copeland and McDonald, 2001). During the germination the cotyledons are raised above the ground where they continue to provide nutritive support to the growing points. Hypogeal germination is characteristic of pea (*Pisum sativum* L.) seeds, all grasses e.g. maize and is characterised by the cotyledons or comparable storage organs that remain beneath the soil while the plumule pushes upwards and emerges above the ground (Copeland and McDonald, 2001; Spears, 2002).

Some of the major reasons that germination could drop include frost or freeze damage, mechanical damage during picking, harvested at a moisture that is too wet for the hybrid, not enough air circulation during drying, drying at too high a temperature, mechanical damage at drying time, mechanical damage during shelling, mechanical damage during transportation, mechanical damage during conditioning, improper storage, genetics and growing environment (Goggi, 2000). Several kernel characteristics contributing to reduced germination have been proposed to play important and interactive roles. These are solute leakage during germination, reduced kernel starch content, reduced storage protein reserves, reduced endosperm starch hydrolysis during germination and seedling growth due to reduced activity or amounts of α-amylase and susceptibility of kernels to infection by fungal pathogens (Young *et al*., 1997)

Fig 2.2: The different categories of seedlings at the end of the test period. Clockwise left to right 1) Abnormal seedlings as the root system is not developed, 2) Deformed seedlings with structures that would not develop normally, 3) Seedling with slight defect grouped under normal seedlings and 4) Normal seedlings with all essential structures in proportion – root systems, coleoptile and leaves.

Another factor that contributes to a lower germination is a host-parasite interaction. In a study on broomrape (*Orobanche* sp.) seeds, conducted by El-Halmouch and co-workers (2006), they found that germination of the root parasite seeds was triggered by host root exudates. Some of these germination stimulants have already been identified. The first was found on cotton root exudates and identified as strigol, a sesquiterpene (Cook *et al*., 1972). Other stimulants were identified as being produced by sorghum (Netzly *et al.*, 1988), red

clover (*Trifolium pratense* L.) (Yokota *et al.*, 1998) and sunflower (*Helianthus annuus* L.). However, an absence of or a reduced germination could be due to inhibitors or to an excess of stimulants in the root exudates (Brown *et al*., 1951 as cited in El-Halmouch *et al*., 2006). Other strategies to reduce the effects of pathogens is to plant late-maturing cultivars or delay planting of early maturing cultivars and this reduces seed infection by shifting seed maturation to cooler, drier conditions that are less favourable for most pathogens that infect seeds (Mengistu and Heatherly, 2006).

In seed germination, protein synthesis is one of the earliest and more important events required for seedling growth and establishment (Pérez-Méndez *et al*., 1993). Seed germination and early seedling growth involve an increase in several metabolic processes including, among others, oxygen consumption, ATP synthesis and storage mobilization (Grańa *et al*., 1993). The process of germination commences with imbibition of water, utilization of seed reserves and finishing with emergence and elongation of the embryonic tissues (Wahid *et al*., 1998). The transition of seeds from a dormant state to germination is associated with both an increase in respiratory enzymes and an increase in the activity of the enzymes involved in reserve mobilization (Grańa *et al*., 1993).

In a study conducted by Enríquez-Arredondo *et al*. (2005), the enzyme H^+-ATPase was under investigation for it role in maize seed germination. It was concluded that at the early stages of seed germination, H^+-ATPase is present: (1) in an active form in the nodal plate, the parenchyma cells, and in the vascular bundle of the scutellum, possibly facilitating the transport of stored nutrients from the scutellum to the rest of the embryo and, (2) in an inactive form after 2 h imbibition (to be further activated) in scutellum epidermis and root epidermal cells (which are involved in active transport of ions and nutrients) and in root and plumule cells (Enríquez-Arredondo *et al*., 2005).

In a study conducted by Pirovano *et al*. (1999), the inhibition by light or super-optimal temperature (30°C) of the germination of *Phacelia tanacetifolia* (Benth) seeds is suppressed if the covering structures of the radicle are removed by mechanical scarification. Chen and Thimann (1966) as cited in Pirovano *et al*. (1999), suggested that the inhibition of embryo growth is due to the mechanical constraint of the endosperm and

the integuments that light and super-optimal temperature inhibit the increase in the metabolically dependent 'expansive force' useful in promoting the protrusion of the radicle from the covering structures (Pirovano *et al*., 1999).

2.5.2.1 Environmental factors that affect germination

There are several abiotic factors that affect germination. Soil salinity, being a serious environmental hazard, greatly hampers germination and related processes. It primarily curtails hydration of the seed due to enhanced osmolality (Wahid *et al*., 1998). With the influx of water, the ions are inevitably taken up by the seed and become toxic to the physiological activities of the embryo (Wahid *et al*., 1998). Other factors are discussed below.

2.5.2.1.1 Water

Water is a basic requirement for germination. It is essential for enzyme activation, breakdown, translocation and use of reserve storage materials. As necessary as water is for the germination process, high moisture may inhibit germination (Copeland and McDonald, 2001). The early stages of the seeds imbibition represents a crucial period for seed germination. The seed coat permeability also influences water uptake (Copeland and McDonald, 2001).

2.5.2.1.2 Air

Air is composed of 20% oxygen, 0.03% carbon dioxide and almost 80% nitrogen. Oxygen is required for germination of most species although low oxygen levels are shown to stimulate the coleoptile growth while inhibiting root growth, in for example rice seeds (Bertani *et al*., 1981 as cited in Copeland and McDonald, 2001). Alternatively a very high carbon dioxide concentration retards the germination process. Nitrogen in the atmosphere has no influence (Spears, 2002).

2.5.2.1.3 Temperature

Three cardinal temperatures are recognized: the minimum, optimum and maximum. The optimum temperature allows for giving the greatest percentage of germination within the shortest time, while at the maximum temperature denaturation of proteins essential for

germination occurs (Copeland and McDonald, 2001). The response to temperature depends on species, variety, growing region, quality of the seed and duration of time from harvest. For most species the optimum temperature is between 15 and 30ºC and the maximum temperature for most species is between 30 and 40ºC (Scandalios *et al*., 2000). As the temperature increases above the optimum, germination/emergence slows down and the "weaker" seeds succumb to high temperature stress, i.e. the percentage germination/emergence decreases (Copeland and McDonald, 2001).

Because every species within the plant kingdom has an optimal temperature range at which growth and metabolic activity are accomplished, temperatures out of this range may cause increases and/or decreases in gene transcripts, proteins, and enzyme activities and this may create a diverse range of stresses, including oxidative stress (Scandalios, 1990 as cited in Scandalios *et al*., 2000). It was recently demonstrated, in maize, catalases might play a significant role during chilling stress. Three unlinked structural genes, *Cat*1, *Cat*2, and *Cat*3 encode the biochemically distinct catalase isozymes, CAT-1, CAT-2, and CAT-3. CAT-1 and are primarily expressed in the dry seed, and during the earliest stages of seed germination. In the scutellum, CAT-2 activity increases rapidly after 2 days post-imbibition (Scandalios *et al*., 2000). Maize seedling height and germination rate are reduced at 35°C, and even more so at 40°C, whereas scutellum fresh weight generally increases in the latter part of the developmental time period under higher temperatures (Scandalios *et al*., 2000).

2.5.2.1.4 Light

The influence of light intensities varies greatly for different species. Some seeds require moonlight (100 lux) while light intensities from indirect light (1080-2160 lux) from the average seed-testing laboratory are probably adequate for germination of most species (Copeland and McDonald, 2001). The influence of light is strongest immediately after harvest and diminishes with age of the seed and eventually disappears (Toole *et al*., 1957 as cited in Copeland and McDonald, 2001). In a study conducted by Thanos and Mitrakos (1979), it was found that the maize caryopses sown in water germinate equally in either darkness or under any light regime (Thanos and Mitrakos, 1979). Further results proved the existence and involvement of phytochrome in the germination of maize caryopses (Thanos and Mitrakos, 1979).

2.5.3 Vigour tests

Safe storage conditions were defined as those that maintain seed quality without loss of vigour for three years (Abba and Lovato, 1999). In 1979, the Association of Official Seed Analyst's Vigour committee defined seed vigour as *"those seed properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions"* (Copeland and McDonald, 2001). Seed vigour is defined by the ISTA as *"the sum total of those properties of the seed that determine the level of activity and performance of the seed during germination and seedling emergence"* (ISTA, 2006).

Seed vigour assesses the ability to germinate under a wide range of environmental conditions (Shah *et al*., 2002; http://www.ag.ohio-state.edu/~seedsci/svvto1.html). It remains not a single measurable property of physiological and physical quality like standard germination but a concept describing several characteristics associated with seed lot performance (Hampton, 1995; Copeland and McDonald, 2001). Vigour testing involves *direct* tests (e.g. cold test) where an environmental stress or other conditions are reproduced in the laboratory and the percentage and or rate of seedling emergence are recorded as well as *indirect* tests (conductivity) (Copeland and McDonald, 2001). Indirect tests are those tests, which measure other characteristics of the seed that have proved to be associated with some aspect of seedling performance (ISTA, 2008). This information can be used to make informed decisions regarding the value of different seed lots (Tekrony, 2003; ISTA, 2008).

Seed vigour testing has reached increasing importance to rank seed lots according to their physiological potential (Tekrony, 2003). Precision is an essential component in seed vigour testing. Vigour tests provide a more sensitive differentiation among seed lots than does the standard germination test (Lovato and Balboni, 2003). The object of a seed vigour test is to provide information about the planting value in a wide range of environments and or the storage potential of the seed lots (ISTA, 2008).

Precision is important during vigour testing. Loss of seed vigour precedes declines in seed germination, which occurs well before a seed loses viability (Hampton, 2002). Much of

seed viability depends upon storage conditions (Rindels, 1995). The ideal storage conditions for seeds are somewhere cool and dry. In a study conducted by Russin and co-workers (1987), it was found that although the germination percentages were high for soybean, the vigour in the field was low. This was due to the damage caused by the alfalfa hopper because most yield loss due to this insect in Louisiana results from late-season feeding when seeds are developing (Russin *et al*., 1987). In a study by Lovato *et al*. (2005), it was found that maize seed had a higher vigour after being incubated at 4.5ºC than at 10ºC, results showed that the cold test is a very reliable vigour test for maize (Lovato *et al*., 2005). Vigour tests give more reliable results than a germination test on its own as was found in a study done by Shah *et al*. (2002). Decline in seed quality and field emergence were observed after 12 months storage, this was more pronounced for subtropical maize hybrids than for temperate hybrids. The best predicators of these results were the cool test and the accelerated ageing test (Basu *et al*., 2004; Shah *et al*., 2005).

The characteristics of a vigour test is as follows: 1) inexpensive – it is important that the vigour tests are reasonably priced and require minimum investment in labour, equipment and supplies; 2) rapid – results must be available in a short period of time so that it can minimize the analyst time and germinator space; 3) uncomplicated - it should be simple so that it can be conducted in seed laboratories without requiring additional staff; 4) objective – a quantitative numerical index of quality that avoids subjectivity should be used; 5) reproducible – it should be able to be repeated in any laboratory for comparison and 6) correlated with field performance – the value of any vigour test should be its ability to predict field performance (McDonald, 1980 as cited in Copeland and McDonald, 2001).

2.5.3.1 Imbibition

When dry seeds are plunged into water, they imbibe water rapidly in the first few minutes, followed by a slower phase of imbibition until they become fully hydrated (Simon and Raja Harun, 1967 as cited in Wang *et al*., 2005). It is concluded that ultra-dry seed storage is beneficial for maintaining seed vigour and that starch mobilization proceeds regularly during germination (Wang *et al*., 2005). During the early stages of imbibition the seeds leak solutes such as organic and inorganic ions, sugars, amino acids and even proteins into the surrounding medium. As this loss means the loss of intracellular constituents, it

often results in extensive embryo damage and even its death (Duke and Kakefuda, 1981; Copeland and McDonald, 2001). Heat-killed seed embryos show significantly greater leakage during imbibition than do viable ones. Even though there are striking differences in leakage rates from viable and non-viable dry organisms, it appears that hydrating even the dead organisms from the vapour phase before they are plunged into water inhibits the leakage (Powell and Matthews, 1979).

The early stages of seed hydration, imbibition, mark the period when the seed changes from an anhydrous to a fully hydrated organism capable of growing and responding to environmental stimuli (Legesse and Powell, 1996). As seed hydrates, it becomes sensitive to cool temperatures and rapid imbibition may leak solutes and macromolecules profusely. Based on this, the stresses of imbibition interfere with the re-establishment of cellular organelles, particularly the membranes (Vertucci and Leopold, 1928 as cited in Saunders, 1930). Sensitivity of seeds to imbibition stress is controlled by three factors (i) initial moisture content of the seed; (ii) temperature of the medium and (iii) the rate at which water is taken up. The extent to which water imbibition occurs is dependent on three factors namely, composition of seed, seed coat permeability and water availability (Copeland and McDonald, 2001).

In order to test the above hypothesis, a study was undertaken by Wierzbicka and Obidzińska (1998) to examine the effect of lead on imbibition and germination of seeds of a number of plant species. Main focus of their study was on the effectiveness of the seed coat as a barrier to lead in various species, varieties and populations of plants, both crop plants and wild plants (Wierzbicka and Obidzińska, 1998). Seed coats are permeable to water to varying degrees. There are impermeable, semi-permeable and completely permeable seed coats, but most species seeds have one of the latter two types (Wierzbicka and Obidzińska, 1998).

Water uptake into dry maize somatic embryos is much more rapid than in a true seed because they lack a seed coat and endosperm (Senaratna *et al*., 1991). To maximize germination and vigour of seedlings from these dry somatic embryos, methods have been

developed which limit the rate of water uptake, maintain membrane integrity and prevent imbibitional injury (Senaratna *et al*., 1991).

2.5.3.2 Cold Test

This is the oldest method of stressing seeds and is most often employed for evaluating seed vigour in maize and soybeans (Copeland and McDonald, 2001). Seeds are placed in soil or paper towels lined with soil and exposed to cold for a specified period, during which stress from imbibition, temperature and moisture content occurs. Following the cold treatment, the seeds are placed under favourable growth conditions and allowed to germinate (Copeland and McDonald, 2001). The difficulty with standardization of this test is that soils differ in moisture, ph, particle composition and pathogen levels, thus contributing to divergent results (Nijënstein and Kruse, 2000). The Tray Cold Test is conducted on 200 seeds; a seven-day 10°C cold stress is imposed followed by a four-day 25°C warm period. Saturated Cold Test utilizes sub-irrigation to provide a saturated soil/towel media (100% WHC) condition throughout the test. The cold period is seven days at 10° C followed by a 64 hr 25° C warm period (Brix-Davis, 2000).

The well-known and widely used cold test for maize seeds evaluates the vigour or emergence potential of the seeds in the laboratory under simulated seedbed conditions of near minimal temperature and excessive moisture, conditions that are frequently encountered in the field in the temperate maize growing regions (Brix-Davis, 2000). The significant variables affecting emergence in the cold test are inheritance, mechanical damage and physiological quality of the seeds (Delouche, 2004).

2.5.3.3 Conductivity

Low vigour seeds have been shown to possess decreased membrane integrity as a result of storage deterioration and mechanical injury. During imbibition, seeds having poor membrane structure release cytoplasmic solutes into the imbibing medium (Oliveira *et al*., 1984). These solutes with electrolytic properties carry an electric charge that can be detected by a conductivity meter. Measurement of the conductivity of leachates from seeds is a rapid, precise, inexpensive and simple procedure (Powell *et al*., 1997). Initial seed moisture and seed size can affect the rate of solute leakage. Treated seeds may influence

conductivity measurements (ISTA, 2008). A conductivity meter is used to monitor the electrolyte leakage from each seed (Copeland and Mc Donald, 2001).

Measurement of the electrical conductivity of leachates provides an assessment of the extent of electrolyte leakage from plant tissues. Conductivity of the soak water of the sample gives an estimate of seed vigour (Powell *et al*., 1997). Seed lots that have high electrolyte leakage that is, having high leachate conductivity are considered as having low vigour. According to Barton (1961), the term conductivity was used to evaluate the viability of seeds by means of an electrical "after-current". Conductivity tests are based on the fact that the progression of seed deterioration results in loss of rigidity and water permeability, which lead to the cell contents to escape into solution and increase its electrical conductivity (Coolbear, 1995). Weaker seeds tend to have a higher loss. Hampton *et al*. (1992) revealed that a seed lot producing excessive amount of electrolytes after soaking, although having high germination, results in decreased vigour and poor field emergence. In many commercial seeds, the seed coat plays an important role in preventing or highly decreasing leakage from the embryo.

2.5.3.4 Tetrazolium test

The Tetrazolium test (TTZ) was developed in Germany in the early 1940s by Professor George Lakon (Copeland and McDonald, 2001) and is widely recognised as an accurate means of estimating seed viability. The principle of the TTZ distinguishes between viable and dead tissues of the embryo on the basis of their relative respiration rate in the hydrated state (Copeland and McDonald, 2001). Many enzymes are active during respiration and the test utilizes the activity of dehydrogenase enzymes as an index to the respiration rate and seed viability. Dehydrogenase enzymes react with substrates and releases H^+ to the oxidised colourless TTZ salt solution, which is changed into red formazan as it is reduced by H^+ (ISTA, 2008; Copeland and McDonald, 2001). Seed viability is interpreted according to the topographical staining pattern of the embryo and intensity of the colouration (Powell and Matthews, 1981; Copeland and McDonald, 2001).

The advantages of this technique are 1) results can be obtained within a space of hours, 2) it is useful to distinguish between dormant and non-dormant seeds and 3) can be used in combination with the germination test. The greatest disadvantage is that difficulty and experience are required to interpret the results (Copeland and McDonald, 2001). The standard germination test gives the percentage of immediate germination while the TTZ gives the percentage of live seeds.

2.5.3.5 Accelerated Ageing

The Accelerated Ageing (AA) test subjects unimbibed seeds to conditions of increased temperature and relative humidity for short periods (2 to 4 days). Seeds are removed from stress conditions and placed under optimum germination conditions. This test is rapid, inexpensive, simple and useful for all species and it can be used for individual seed evaluation (Copeland and McDonald, 2001).

The AA test exposes seeds for a short period to high temperature and high relative humidity (~95%). During the test, the seeds absorb moisture from the humid environment and the raised seed moisture content, along with the high temperature, causes rapid seed ageing (Rice and Dyer, 2001). High vigour seed lots will withstand these extreme stress conditions and age more slowly than low vigour seed lots. Thus after AA, high vigour lots retain a high germination, whilst that of low vigour lots is reduced (Basu *et al*., 2004). Aged seed that retain their capacity to germinate generally do so more slowly and with an enhanced sensitivity to external stress (Priestly, 1986). One of the most informative indications of the quality of a seed lot is its germinability. The longitivity of maize seed in particular has been reported to be highly dependent on its structural consistency. In studies conducted by Basu *et al*. (2004) maize parental lines were subjected to natural ageing and accelerated ageing. Results from this study showed a gradual decrease in germination following the initial four months due to fluctuating temperatures and humidity (Basu *et al*., 2004), especially following natural storage. However, the maize retained germination above minimum seed certification standards (80%) (Basu *et al*., 2004).

Ageing of dried seeds in storage is, thought to be, accompanied by changes in membranes and nucleic acids. The increased leakage of electrolytes and the decline in respiratory activity after accelerated ageing of soybeans has been interpreted as the result of membrane damage (Parrish and Leopold, 1978 as cited in Puntarulo and Boveris, 1990). This deterioration may be indicative of an inability to reform functionally competent membranes during rehydration of the seed, resulting in loss of vigour and lack of germination. The two most important environmental factors influencing seed longevity are ambient temperature and seed moisture. An increase in either during seed maturation and storage hastens senescence (Puntarulo and Boveris, 1990).

Most soybean ageing studies have been performed using systems of accelerated ageing, in which the symptoms of natural deterioration are induced over a relatively short period of time by exposing the seeds to conditions of high temperature and humidity. These procedures have lead to erratic information and it has been proposed that accelerated and natural ageing are biochemically different (Puntarulo and Boveris, 1990).

2.6 Ultrastructure of seeds

Plant cells are very complex (Smith, 1977) and considerable attention has been paid to the fine structure of viable and non-viable material. In a study by de Castro and Martinez-Honduvilla (1984), decline in germination of *Pinus pinea* L. seeds was confirmed by changes in the ultrastucture of the endosperm and embryo cells. Following stress in the form of heat shock, the nucleolus of the maize seed undergoes the most dramatic change, as loss of granular components (Fransolet *et al.,* 1979). Subjecting maize and wheat seeds to Cytochalasin B (CB), which is known to be an inhibitor of elongation growth of the roots, showed the accumulation of secretory vesicles in the vicinity of the dictyosomes. This study proved that CB did not inhibit elongation growth by interfering with cytoplasmic streaming (Pope *et al*., 1979). Treating seeds with either fungicide (Buadze *et al*., 1998) or metals such as zinc and cadmium (Jiang *et al*., 2007) may result in changes in the ultrastructure. In some studies, treating maize with metals (zinc, phosphorous) resulted in an increase in chlorophyll content in plants compared with those that did not receive external phosphorus (Jiang *et al*., 2007). Data from most studies where seeds have been treated are in agreement that increased concentrations of metals, above the recommended

dosage, will cause the deterioration of mitochondrial structure leading to the blocking of the energy processes, thus resulting in the complete destruction of the cell (Cao *et al.,* 2004; Crèvecoeur, *et al*., 1983**;** Berti and Cunningham, 1997; Neumann and Nieden, 2001; Jiang *et al*., 2007).

During hydration of most cereal crops, the total protein decreases in the first six hours, although the lipid composition remains the same until germination proceeds (Rost, 1972). However, the diacylglycerol, free fatty acid and total polar lipid content will decrease significantly with stress (Leech *et al*., 1973; Navari-izzo *et al*., 2005). Hydration also brings about a drastic change in the plasma membrane. The plasma membrane, which in dry seed, is disorganized and disrupted becomes intact and continuous (Webster and Leopold, 1977). The rapid alteration of membranes and organelles is a reflection of the seeds ability to recover from solute loss and solvent entry (Webster and Leopold, 1977).

Seed vigour has usually been determined by biochemical and physical methods. Changes on a cellular level during germination can now also confirm the results obtained with a standard germination test (de Castro and Martinez-Honduvilla, 1984). Apart from imbibition stress seeds are subjected to, other stress conditions (accelerated ageing, exposure of maize kernels to a low temperature). These condition may have an influence at the ultrastructural level and is shown during germination (Crèvecoeur *et al*., 1983). Due to the nature of treating maize seeds with fungicides in this study, i.e: via imbibition, this should not have a negative effect on high vigour seeds.

2.7 Literature Cited

Abba, E.J. and A. Lovato. 1999. Effect of seed storage temperature and relative humidity on maize (*Zea mays* L.) seed viability and vigour. *Seed Science and Technology*. 27: 101-114.

Abbas, H.K., Cartwright, R.D., Xie, W. and W.T. Shier. 2006. Aflatoxin and fumonisin contamination of maize (*Zea mays* L.) hybrids in Arkansas. *Crop Protection*. 25: 1-9.

Adenle, V.O. and K.F. Cardwell. 2000. Seed transmission of maize downy mildew (*Peronosclerospora sorghi*) in Nigeria. *Plant Pathology*. 49: 628-634.

Ahmed, N.E., Kanan, H.O., Inanaga, S., Ma, Y.Q. and Y. Sugimoto. 2001. Impact of pesticide seed treatments on aphid control and yield of wheat in the Sudan. *Crop Protection*. 20: 929-934.

Ajayi, S.A. and M.A.B. Fakorede. 2000. Physiological maturity effects on seed quality, seedling vigour and mature plant characteristic of maize in a tropical environment. *Seed Science and Technology*. 28: 301-319.

Allen, T.W., Enebak, S.A. and W.A. Carey. 2004. Evaluation of fungicides for control of species of *Fusarium* on longleaf pine seed. *Crop Protection*. 23: 979-982.

Anaso, A.B., Tyagi, P.D., Emechebe, A.M. and S.K. Manzo. 1989. Control of sorghum downy mildew (*Peronosclerospora sorghi*) of maize by seed treatment in Nigeria. *Crop Protection*. 8: 82-85.

Annecke, D.P. and V.C. Moran. 1982. Insects and mites of cultivated plants in South Africa. Butterworths, South Africa. Pages: 23-25.

Anonymous. 2004. CSIR completes study on speciality maize products. http://www.csir.co.za Accessed: 22/04/2006.

Appert, J. 1987. The Storage of food grains and seeds. Coste, R. (Ed). MacMillan Publishers, Hong Kong. Pages: 51-55.

Agrios, G.N. 2005. Plant Pathology – 4th Edition. Academic Press, San Diego. Pages: 209-211.

Arny, D.C. and R.R. Nelson. 1971. *Phyllosticta maydis* species nova, the incident of yellow leaf blight of maize. *Phytopathology*. 61: 1170-1172.

Asiedu, E.A. and A.A. Powell. 1998. Comparisons of the storage potential of cultivars of cowpea (*Vigna unguiculata*) differing in seed coat pigmentation. *Seed Science and Technology*. 26: 211-221.

Association of Official Seed Analysts (AOSA). 1991. Rules for testing seeds. *Journal of Seed Technology.* 12: 1-109.

Babadoost, M. and S.Z. Islam. 2003. Fungicide seed treatment effects on seedling damping-off of pumpkin caused by *Phytophthora capsici*. *Plant Disease.* 87: 63-68.

Bant, M.U. 2007. Tropical maize for biofuels. http://www.aces.uiuc.edu/news/stories/news4169.html. Accessed: 19/09/2007.

Barton, L.V. 1961. Seed presentation. Leonard Hill, London. Page: 62.

Barrow, M.R. 1992. Development of maize hybrids resistant to maize streak virus. *Crop Protection*. 11: 261–267.

Basu, S., Sharma, S.P. and M. Dadlani. 2004. Storability studies on maize (*Zea mays* L.) parental line seeds under natural and accelerated ageing conditions. *Seed Science and Technology*. 32: 239-245.

Berti, W.R. and S.D. Cunningham. 1997. In-place inactivation of Pb in Pb-contaminated soils. *Environmental Science and Technology*. 31: 1359-1364.

Blaney, B.J., Ramsey, M.D. and A.L. Tyler. 1984. Mycotoxins and toxigenic fungi in insect-damaged maize harvested during 1983 in far North Queensland. *Australian Journal of Agricultural Research*. 37: 235-244.

Buadze, 0., Sadunishvili, T. and G. Kvesitadze. 1998. The effect of 1,2_benzanthracene and 3,4-benzpyrene on the ultrastructure of maize cells. *International Biodeterioration and Biodegradation.* 41: 119-125.

Buchanan, R.E. and N.E. Gibbons. 1974. Bergeys Manual of Determinative Bacteriology (8th Ed.). William and Wilkins, Baltimore. Pages: 125-127.

Brix-Davis, K. 2000. Corn testing services. http://www.mwseed.com. Accessed: 10/02/2005.

Calub, A.G., Compton, W.A., Gardner, C.O. and M.L. Schuster. 1974. Reaction of 113 corn (*Zea mays* L.) genotypes to leaf freckles and wilt. *Plant Disease*. 58: 956-960.

Cao, X.D., Ma, L.Q., Rhue, D.R. and C.S Appel. 2004. Mechanisms of lead, copper and zinc retention by phosphate rock. *Environmental Pollution*. 131: 435-444.

Christensen, J.J. and R.D. Wilcoxson. 1966. Stalk rot of maize. Monograph No. 3. American Phytopathological Society: St Paul, Minnesota. Page: 16.

Christensen, C.M and H.H. Kaufmann. 1969. Grain Storage: The role of fungi in quality loss. Oxford University Press, London. Page: 5.

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.

Cook, C.E., Whichard, L.P., Wall, M.E., Egley, G.H., Coggon, P., Luhan, P.A. and A.T. McPhail. 1972. Germination stimulants. Part II: the structure of strigol—a potent seed germination stimulant for witchweed (*Striga lutea* Lour.). *Journal of American Chemistry Society*. 94: 6198–6199.

Coolbear, P. 1995. Mechanisms of seed deterioration. Pages: 223-318 *In*: Basra, A.S. (Ed). Seed quality: basic mechanisms and agricultural implications. Food Product Press, New York.

Copeland, L.O. and M.B. McDonald. (Eds.) 2001. Pages: 124-128 *In*: Principles of Seed Science and Technology (4th Edition). Kluwer Academic Publishers, Massachusetts.

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.

Dale, J.L. 1963. Maize anthracnose. *Plant Disease*. 47: 245-249.

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

Delouche, J.C. 2004. Seed quality is not always the problem. Seed News: The international seed magazine. http://www.seednews.inf.br. Accessed: 22/01/2006.

Dredge, R.D. 2004. Assessment of current economic and physical environment of agriculture. Executive Summary. http://www.nda.agric.za. Accessed: 24/07/2005.

Drinkwater, T.W. 1994. Comparison of imidacloprid with carbamate insecticides, and the role of planting depth in the control of false wireworms, *Somaticus* species, in maize. *Crop Protection*. 13: 341-345.

Duke, S.H. and G. Kakefuda. 1981. Role of the testa in preventing cellular rupture during imbibition of legume seeds. *Plant Physiology*. 67: 449-456.

Eddins, A.H. 1930. Dry rot of maize caused by *Diplodia macrospore* Earle. *Phytopathology*. 20: 439-448.

Egal, S., Hounsa, A., Gong, Y.Y., Turner, P.C., Wild, C.P., Hall, A.J., Hell, K. and K.F. Cardwell. 2005. Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa. *International Journal of Food Microbiology*. 104: 215-224.

El-Halmouch, Y., Benharrat, H. and P. Thalouarn. 2006. Effect of root exudates from different tomato genotypes on broomrape (*O. aegyptiaca*) seed germination and tubercle development. *Crop Protection*. 25: 501-507.

Enríquez-Arredondo, C., Sánchez-Nieto, S., Rendón-Huerta, E., González-Halphen, D., Gavilanes-Ruíz, M. and D. Díaz-Pontones. 2005. The plasma membrane H^+ ATPase of maize embryos localizes in regions that are critical during the onset of germination. *Plant Science*. 169: 11-19.

Falloon, R.E. 1982. Fungicide seed treatment of maize to improve establishment and control of pathogens. *New Zealand Journal of Agriculture*. 10: 197-202.

Furtell, M.C. and M. Kilgore. 1969. Poor stands of maize and reproduction of root growth caused by *Fusarium monililforme*. *Plant Disease*. 53: 213-215.

Fransolet, S., Deltour, R., Bronchart, R. and C. Van de Walle. 1979. Changes in ultrastructural and transcription induced by elevated temperature in *Zea mays* embryonic root cells. *Planta*. 146: 7-18.

Frederickson, D.E. and K. Leuschner. 1997. Potential use of Benomyl for control of ergot (*Claviceps africana*) in Sorghum A-lines in Zimbabwe. *Plant Disease.* 81: 761-765.

Goggi, S. 2000. Tetrazolium (TZ) Testing: Seed health testing! Accessed: 09/02/2006.

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.

Grańa, X., Broceño, C., Garriga, J., Pérez de la Ossa, P. and F. Climent. 1993. Phosphoglycerate mutase activity and mRNA levels during germination of maize embryos. *Plant Science*. 89: 147-151.

Hampton, J.G., Johnston, K.A. and V. Eua-Unipon. 1992. Bulk conductivity test variables for mungbean, soybean, and french bean seed lots. *Seed Science and Technology.* 20: 677-686.

Hampton, J.G. 1995. Methods of viability and vigour testing – a critical appraisal. Pages: 81-117 *In*: Basra, A.S. (Ed.). Seed Quality – Basic mechanisms and agricultural implications. The Haworth Press, New York.

Hell, K., Cardwell, K.F., Setamou, M. and H.M. Poehling. 2000. The influence of storage practices on aflatoxin contamination in maize in four agro-ecological zones of Benin, West Africa. *Journal of Stored Products Research.* 36: 365-382.

Hewett, P.D. and D.C Griffiths. 1986. Biology of seed treatment. Pages: 4-10 *In*: K.A Jeffs (Ed). Seed Treatment. 2nd edition. Lavenham Press Limited, England.

Hols, T. 2006. Monsanto's maize research efforts in Africa. http://www.monsanto.co.za. Accessed: 17/10/2005.

http://www.africancrops.net/crops1/maize/index.htm. Biotechnology, Breeding and Seed systems for African crops.Accessed: 24/04/2006.

http://www.cornell.edu. Accessed: 17/10/2005.

http://www.fao.org. Highlights within Southern Africa. Accessed: 24/08/2005.

http://www.idrc.ca. Accessed: 17/10/2005.

http://www.nda.agric.za/docs/Maize.htm. Background to maize industry of South Africa. Accessed: 24/04/2006.

http://www.omafra.gov.on.ca. 2006. Corn seedling diseases. Seed Rot, Seedling Blight and root rot. Accessed: 20/08/2006.

http://www.pannarseed.co.za/maizeprod.htm. Counter Season Maize Production. 2004. Accessed: 24/05/2005.

http://www.syngenta.com/en/products_services/seed_treat.aspex. Accessed: 20/08/2005.

International Seed Testing Association (ISTA). 2006. International Rules for Seed Testing. Seed Science and Technology. Seed Vigour Testing. ISTA Handbook, 2006.

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

Jayas, D.S. and N.D.G. White. 2003. Storage and drying of grain in Canada: Low cost approaches. *Food Control.* 14: 255-261.

Jeffs, K.A. 1986. A Brief history of seed treatment. Pages: 1, 15, 25-30 *In*: Seed Treatment. 2nd Edition. The Lavenham Press Limited, England.

Jiang, H.M., Yang, J.C. and J.F. Zhang. 2007. Effects of external phosphorus on the cell ultrastructure and the chlorophyll content of maize under cadmium and zinc stress. *Environmental Pollution*. 147: 750-756.

Justice, O.L. and L.N. Bass. 1979. Principles and practices of seed storage. Castle House Publications Ltd, London. Pages: 2, 7, 9, 11.

Kendrick, J.B. 1926. Holcus bacterial spot of *Zea mays* and Holcus species. Iowa Agricultural Experimental Station. *Bull*. 100: 303-334.

Khalil, I.A., Mercer, E.I. and Z.X. Wang. 1990. Effect of triazole fungicides on the growth, chloroplast pigments and sterol biosynthesis of maize (*Zea mays* L.). *Plant Science*. 66: 21-28.

Kidane, A. 2001. Quarantine precautions for maize seed imported into Ethiopia. Proceedings of the Second National Maize Workshop of Ethiopia, Addis Ababa, Ethiopia. Pages: 176-178.

Kloppers, R. 2005. Maize diseases: Reflection on the 2004/2005 season. http://www.saspp.org. Accessed: 27/09/2006.

Kommedahl, T. and C.E. Windels. 1986. Treatment of maize seeds. Pages: 163-182. *In*: K.A. Jeffs (Ed). Seed Treatment. 2nd edition. Lavenham Press Limited, England.

Latterell, F.M. and A.E. Rossi. 1977. Further evidence for the genetic relationship between *Cercospora zeae-maydis* and a species of *Mycosphaerella*. Proceedings of the International Mycological Congress II, Tampa. Florida.

Leech, R.M., Rumsby, M.G. and W.W. Thomson. 1973. Plastid differentiation, acyl lipid, and fatty acid changes in developing green maize leaves. *Plant Physiology*. 52: 240-245.

Legesse, N. and A.A. Powell. 1996. Relationship between the development of seed coat pigmentation, seed coat adherence to the cotyledons and the rate of imbibition during the maturation of grain legumes. *Seed Science and Technology*. 24: 23-32.

Lewis, G.C. 1988. Improvements to newly sown ryegrass by use of combined fungicide and insecticide treatment. *Crop Protection*. 7: 34-38.

Liu, Z., Gao, J. and J. Yu. 2006. Aflatoxins in stored maize and rice grains in Liaoning Province, China. *Journal of Stored Products Research*. 42: 468-479.

Lovato, A. and N. Balboni. 2003. Seed vigour in maize (*Zea mays* L.): Two-Year Laboratory and field test compared. *Italian Journal of Agronomy*. 1: 1-6.

Magan, N., Hope, R., Colleate, A. and E.S. Baxter. 2002. Relationship between growth and mycotoxin production by *Fusarium* species, biocides and environment. *European Journal of Plant Pathology*. 108: 685-690.

Maienfisch, P., Angst, M., Brandl, F., Fischer, W., Hofer, D., Kayser, H., Kobel, W., Rindlisbacher, A. and R. Senn. 2001. Chemistry and biology of thiamethoxam: a second generation neonicotinoid. *Pest Management Science* 57: 906-913.

Mao, W., Lumsden, R.D., Lewis, J.A and P.K. Hebbar. 1998. Seed treatment using pre-infiltration and biocontrol agents to reduce damping-off of maize caused by species of *Pythium* and *Fusarium. Plant Disease.* 82:294-299.

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.

Martinez, C., Lévesque, A.A., Bélanger, R.R. and R.J. Tweddell. 2005**.** Evaluation of fungicides for the control of carrot cavity spot. *Pest Management Science.* 61: 767-771.

Mathre, D.E, Callan, N.W., Johnston, R.H., Miller, J.B. and A. Schwend. 1994. Factors influencing the control of *Pythium ultimum* induced seed decay by seed treatment with *Pseudomonas aureofaciens* AB254. *Crop Protection*. 13: 301-307.

Maude, R.B. 1977. Results in practice – iii. Vegetable crops. Pages: 259-260. *In*: Marsh, R.W, Byrde, R.J.W. and D. Woodcock (Eds.). Systemic fungicides. Longman Group Limited, London.

Mc Gee, D.C. 1988. Maize Diseases: A Reference source for seed technologist. CPL Scientific Publishing Services Limited, California. Page 150.

Mc Govern, R.J., Mc Slorley, R. and M.L. Bell. 2002**.** Reduction of landscape pathogens in Florida by soil solarization. *Plant Disease.* 86 1388-1395.

McKevith, B. 2004. Nutritional aspects of cereals. *Nutrition Bulletin*. 29: 111-142.

McKim, F. 1994. Fungicide resistance re-examined. *Plant Science*. 102: 221-223.

Mduduzi, M.P., Chelule, P.K. and G. Nceba. 2005. Reduction of Fumonisin B1 and Zearalenone by latic acid bacteria in fermented maize meal. *Journal of Food Protection*. 68: 2095-2099.

Mengistu, A. and L.G. Heatherly. 2006. Planting date, irrigation, maturity group, year, and environment effects on *Phomopsis longicolla*, seed germination, and seed health rating of soybean in the early soybean production system of the mid-southern USA. *Crop Protection*. 25: 310-317.

Mikkelson, M., Riesselman, J., Mathre, D., Johnston, B. and S.Blodgett. 2003. Small Grain Seed Treatment Guide. http://www.montana.edu/publications. Accessed: 25/08/2007.

Mislivec, P.B. 2000. Training in mould identification, handling and evaluation of conditions leading to mycotoxin production. http://www.usda.gov/UNDP.htm. Accessed: 24/08/2005.

Modi, A.T. 2004. Short-term preservation of maize landrace seed and taro propagules using indigenous storage methods. *South African Journal of Botany*. 70: 16-23.

Moreno-Martinez, E., Jiménez, S. and M.E. Vázquez. 2000. Effect of *Sitophilus zeamais* and *Aspergillus chevalieri* on the oxygen level in maize stored hermetically. *Journal of Stored Product Research.* 36: 25-36.

Munkvold, G.P., Martinson, C.A., Shriver, J.M. and P.M. Dixon. 2001. Probabilities for profitable fungicide use against grey leaf spot in hybrid maize. *Phytopathology*. 91: 477-484.

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.

Munkvold, G.P. 2003. Epidemiology of *Fusarium* diseases and their Mycotoxins in maize ears. *European Journal of Plant Pathology*. 109: 705-713.

Nansen, C., Phillips, T.W., Parajulee, M.N. and R.A. Franqui. 2004. Comparison of direct and indirect sampling procedures for *Plodia interpunctella* in a maize storage facility. *Journal of Stored Products Research.* 40: 151-168.

Nault, L.R. and O.E. Bradfute. 1979. Maize stunt: involvement of a complex of leafhopper-borne pathogens. Pages: 561-586. *In*: Maramorosch, K. and K. Harris (Eds.) Leafhopper Vectors and Plant Disease Agents. Academic Press, New York.

Navari-izzo, F., Quartacci, M.F and R Izzo. 2005. Lipid Changes in maize seedlings in response to field water deficits. *Journal of Experimental Botany*. 40: 675-680.

Netzly, D.H., Riopel, J.L., Ejeta, G. and L.G. Butler. 1988. Germination stimulants of witchweed (*Striga asiatica*) from hydrophobic root exudate of Sorghum. *Weed Science*. 36: 441–446.

Neumann, D. and U. Nieden, U. 2001. Silicon and heavy metal tolerance of higher plants. *Phytochemistry*, 56: 685-692.

Nijënstein, J.H. and M. Kruse. 2000. The potential for standardization in cold testing in maize (*Zea mays* L.). *Seed Science and Technology*. 28: 837-851.

Ngoko, Z., Cardwell, K.F., Marasas, W.F.O., Wingfield, M.J., Ndemah, R. and F. Schulthess. 2002. Biological and physical constraints on maize production in the humid forest and western highlands of Cameroon. *European Journal of Plant Pathology*. 108: 893-902.

Nuninger, C., Watson, G., Leadbitter, N. and H. Ellgehansen. 1996**.** Introduction of the enantionmeric form of the fungicide metalaxyl. *In*: Proceeding of the British Crop Protection Conference. Pest and disease, Brighton. Pages 41-46.

Okada, A., Bannos, S., Ichiishi, A., Kimura, M., Yamaguchi, I. and M. Fujimura. 2005. Pyrrolnitrin interferes with osmotic signal transduction in *Neurospora crassa*. *Journal of Pesticide Science* 30: 378-383.

Olakojo, S.A. and T.A. Akinlosotu. 2004. Comparative study of storage methods of maize grains in South Western Nigeria. *African Journal of Biotechnology*. 3: 362-365.

Oliveira, M de A., Matthews, S. and A.A. Powell. 1984. The role of split seed coats in determining seed vigour in commercial seed lots of soybean, as measured by the electrical conductivity test. *Seed Science and Technology*. 12: 659-668.

Oswald, A. and J.K. Ransom. 2004. Response of maize varieties to *Striga* infestation. *Crop Protection*. 23: 89-94.

Pope, D.G., Thorpe, J.R., Al-Azzawi, M.J. and J.L. Hall. 1979. The effect of cytochalasin B on the rate of growth and ultrastructure of wheat coleoptiles and maize roots. *Planta*. 144: 373-383.

Powell, A.A. and S. Matthews. 1979. The influence of testa condition on the imbibition and vigour of pea seeds. *Journal of Experimental Botany*. 30: 193-197.

Powell, A.A. and S. Matthews. 1981. A physical explanation for solute leakage from dry pea embryos during imbibition. *Journal of Experimental Botany*. 32: 1045-1050.

Powell, A.A., Fergusson, A.J. and S. Matthews. 1997. Identification of vigour differences among combining pea (*Pisum sativum*) seed lots. *Seed Science and Technology*. 25: 443-464.

Pedersen, W.L., Kline, J.D., Bradley, C.A. and D.S. Mueller. 2003. Influence of metalaxyl fungicide seed treatment on severity of rootworm (*Diabrotica* spp.) damage to maize (*Zea mays* L.) under no-tillage conditions. *Crop Protection*. 22: 647-652.

Pérez-Méndez, A., Aguilar, R., Briones, E. and E. Sánchez-de-Jiménez. 1993. Characterization of ribosomal protein phosphorylation in maize axes during germination. *Plant Science*. 94: 71-79.

Petch, G.M., Maude, R.B. and J.G. White. 1991. Effect of film-coat layering of metalaxyl on the germination of carrot seeds, their emergence and the control of cavity spot. *Crop Protection*. 10: 117-120.

Pirovano, L., Morgutti, S., Espen, L. and S.M. Cocucci. 1999. Light or high (30°C) temperature effects on L-[U-14C] leucine incorporation and protein patterns in embryos and endosperm during the early phase of germination of the negatively photoblastic and thermosensitive seeds of *Phacelia tanacetifolia. Plant Science.* 140: 137-144.

Pixton, S.W., Warburton, S. and S.T. Hill. 1975. Long-term storage of wheat observed during 16 years of storage. *Journal of Stored Products Research*. 11: 177-185.

Placinta, C.M., D'Mello, J.P.F. and A.M.C. MacDonald. 1999. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Animal Feed Science and Technology*. 78: 21-37.

Pommer, E.H. and G. Lorenz. 1982. Resistance of *Botrytis cinerea* Pers. to dicarboximide fungicides-a literature review. *Crop Protection*. 1: 221-230.

Priestly, D.A. 1986. Seed ageing: implications for seed storage and persistence in the soil. University Press, London. Pages: 17, 71.

Puntarulo, S. and A. Boveris. 1990 Effect of natural and accelerated ageing on the hydroperoxide metabolism of soybean embryonic axes. *Plant Science*. 68: 27-32.

Quezada, M.Y., Moreno, J., Vázquez, M.E., Mendoza, M., Méndez-Albores, A. and E. Moreno-Martínez. 2006. Hermetic storage system preventing the proliferation of *Prostephanus truncatus* Horn and storage fungi in maize with different moisture contents. *Postharvest Biology and Technology*. 39: 321-326.

Rane, K and G. Ruhl. 2002. Crop diseases in maize, soybean and wheat. http://www.btny.purdue.edu/Extension/Pathology/Crop.htm. Accessed: 24/05/2006.

Rehman, Z., Habib, F. and S.I. Zafar. 2002. Nutritional changes in maize (*Zea mays* L.) during storage at three temperatures. *Food Chemistry*. 77: 197-201.

Rehman, Z. 2006. Storage effects on nutritional quality of commonly consumed cereals. *Food Chemistry*. 95: 53-57.

Reynolds, C. 2004. South Africa: Planted maize area at historic lows. http://www.pecad.fas.usda.gov/cropexplorer. Accessed: 24/06/2006.

Rhodes, L.H. and D.K. Myers. 1989. Effect of seed treatment with metalaxyl or pyroxyfur on damping-off of alfalfa caused by *Phytophthora megasperma* f.sp. *medicaginis. Crop Protection.* 8: 369-372.

Ribeiro, R.L.D., Durbin, R.D., Arny, D.C. and T.F. Uchytil. 1977. Characterization of the bacterium inciting chocolate spot of maize. *Phytopathology*. 67: 1427-1431.

Rice, K.J. and A.R. Dyer. 2001. Seed ageing, delayed germination and reduced competitive ability in *Bromus tectorum*. *Vegetatio*. 155: 237-243.

Rindels, S. 1995. Seed Viability. http://www.ipm.iastate.edu/ipm/hortnews/1995/3-3-1995/seedv.html. Accessed: 09/02/2006.

Ronchi, A., Farina, G., Gozzo, F. and C. Tonelli. 1997. Effects of a triazolic fungicide on maize plant metabolism: modifications of transcript abundance in resistance-related pathways. *Plant Science*. 130: 51-62.

Rost, T. L. 1972. The ultrastructure and physiology of protein bodies and lipids from hydrated dormant and non-dormant embryos of *Setaria lutescens*. *American Journal of Botany*. 59: 607-616.

Russin, J.S., Newsom, L.D., Boethel, D.J and A.N. Sparks, jr. 1987. Multiple pest complexes on soybean: influences of three cornered alfalfa hopper injury on pod and stem blight and stem anthracnose diseases and seed vigour. *Crop Protection*. 6: 320-325.

Salvador, R.J. 1997. History of maize. http://maize.agron.iastate.edu/maizearticle.html. Accessed: 25/02/2005.

Sauer, J.D. 1993. *Zea Mays In*: Historical geography of crop plants-a select roster. CRC Press, Florida. Pages: 10-15.

Saunders, A.R. 1930. Maize in South Africa. Central News Agency Limited, South Africa. Pages: 7-34.

Scandalios, J.G., Acevedo, A. and S. Ruzsa. 2000. Catalase gene expression in response to chronic high temperature stress in maize. *Plant Science*. 156: 103-110.

Senaratna, T., McKersie, B.D., Kasha, K.J. and J.D. Procunier. 1991. Direct DNA uptake during the imbibition of dry cells. *Plant Science*. 79: 223-228.

Shah, F.S., Watson, C.E. and E.R. Cabrera. 2002. Seed Vigour testing of subtropical maize hybrids. Research report – Mississippi Agricultural and Forestry Experiment Station. 23. Pages: 1-5.

Shaw, C.G. 1978. *Peronospora* species and other downy mildews of the *Gramineae*. *Mycologia*. 70: 594-604.

Shurtleff, M.C. 1980. Compendium of maize diseases- 2nd edition. The American Phytopathological Society, Minnesota. Pages: 12-34.

Singh, I.P. and S. Lal. 1985. Effective spray schedule of metalaxyl formulations for the therapy of sugarcane downy mildew of maize. *Tropical Pest Management*. 31: 327-328.

Smith, H. 1977. The Molecular Biology of Plant Cells.University of California Press, Berkley. Pages: 1, 7, 20.

Sparg, S.G., Kulkarni, M.G., Light, M.E. and J. Van Staden. 2005. Improving seedling vigour of indigenous medicinal plants with smoke. *Bioresource Technology*. 96: 1323-1330.

Sparrow, F.K. 1974. Observations on *Chytridiaceous* parasites of phanerogams XXI. The endobiotic stage of *Physoderma maydis*. *Mycologia*. 66: 693-697.

Spears, J.F. 2002. Small grain seed production. http://www.smallgrains.ncsu.edu.html. Accessed: 16/02/2005.

Sumner, D.R. and N.W. Schaad. 1977. Epidemiology and control of bacterial leaf blight of maize. *Phytopathology*. 67: 1113-1118.

Suryanarayana, D. 1978. Seed Pathology. Vikas Publishing House, New Delhi. Pages: 4-7.

Tekrony, D.M. 2003. Precision is an essential component in seed vigour testing. *Seed Science and Technology*. 31: 435-447.

Thamaga-Chitja, J.M., Hendriks, S.L., Ortmann, G.F. and M. Green. 2004. Impact of maize storage on rural household food security in Northern Kwazulu-Natal. *Journal of Family Ecology and Consumer Science*. 32: 8-15.

Thanos, C.A. and K. Mitrakos. 1979. Phytochrome-mediated germination control of maize caryopsis. *Planta*. 146: 415-417.

Tigar, B.J., Osborne, P.E., Key, G.E., Flores-s, M.E and M. Vazquez-a. 1994. Insect pests associated with rural maize stores in Mexico with particular references to *Prostephanus truncatus* (*Coleoptera:Bostrichidae*). *Journal of Stored Products Research.* 30: 267-281.

Uremis, I., Bayat, A., Uludag, A., Bozdogan, N., Aksoy, E., Soysal, A. and O. Gonen. 2004. Studies on different herbicide application methods in second-crop maize fields. *Crop Protection*. 23: 1137-1144.

Vázquez-arista, M., Ramírez-Flores, A and A. Blanco-Labra. 1995. Maize and bean storage and their use by rural farmers in a central state of Mexico. *Journal of Stored Product Research*. 31: 325-333.

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.

Vidaver, A.K. and R.R. Carlson. 1978. Leaf spot of field maize caused by *Pseudomonas andropogonis. Plant Disease*. 62: 213-216.

Wahid, A., Javed, I., Ali, I., Baig, A. and E. Rasul. 1998. Short term incubation of sorghum caryopses in sodium chloride levels: changes in some pre- and post-germination physiological parameters. *Plant Science*. 139: 223-232.

Wang, X., Jing, X and J. Lin. 2005. Starch mobilization in ultradried seed of maize (*Zea mays* L.) during germination. *Journal of Integrative Plant Biology*. 47: 443-451.

Ward, J.M.J., Laing, M.D. and D.C. Nowell. 1997. Chemical control of maize grey leaf spot. *Crop Protection*. 16: 265-271.

Ward, J.M.J., Stromberg, E.L., Nowell, D.C. and F.W. Nutter. 1999. Gray Leaf Spot: A disease of global importance in maize production. *Plant Disease*. 83: 884-895.

Webster, B.D and A.C Leopold. 1977. The ultrastructure of dry and imbibed cotyledons of soybean. *American Journal of Botany*. 64: 1286-1293.

Wierzbicka, M. and J. Obidzińska. 1998. The effect of lead on seed imbibition and germination in different plant species. *Plant Science*. 137: 155-171.

Wu, Y.S., Lee, H.K. and S.F.Y. Li. 2001. High-performance chiral separation of fourteen triazole fungicides by sulphated ß- cyclodextrin mediated capillary electrophoresis. *Journal of Chromatography*. 912: 171-179.

Wyckhuys, K.A.G. and R.J. O'Neil. 2006. Population dynamics of *Spodoptera frugiperda* Smith (Lepidoptera: *Noctuidae*) and associated arthropod natural enemies in Honduran subsistence maize. *Crop Protection*. 25: 1180-1190**.**

Yates, I., Widstrom, N., Bacon, C., Glenn, A., Hinton, D., Sparks, D. and A. Jaworski. 2005. Field performance of maize grown from *Fusarium verticillioides* inoculated seed. *Mycopathologia.* 159: 65-73.

Yokota, T., Sakai, H., Okuno, K., Yoneyama, K. and Y. Takeuchi. 1998. Alectrol and orobanchol, germination stimulants for Orobanche minor, from its host red clover. *Phytochemistry.* 40: 1967–1973.

Young, T.E., Juvik, J.A. and D.A. De Mason. 1997. Changes in carbohydrate composition and ά-amylase expression during germination and seedling growth of starch-deficient endosperm mutants of maize. *Plant Science*. 129: 175-189.

CHAPTER THREE

THE EFFECT OF TRADITIONAL STORAGE METHODS ON GERMINATION AND VIGOUR OF MAIZE (*Zea mays* L.)

Published as:

V. Govender, T.A.S. Aveling and Q. Kritzinger. 2008. The effect of traditional storage methods on germination and vigour of maize (*Zea mays* L.) from nothern KwaZulu-Natal and southern Mozambique. *South African Journal of Botany*. 74: 190-196.

ELSEVIER

Available online at www.sciencedirect.com
ScienceDirect

South African Journal of Botany 74 (2008) 190–196

SOUTH AFRICAN JOURNAL OF BOTANY

www.elsevier.com/locate/sajb

The effect of traditional storage methods on germination and vigour of maize (*Zea mays* L.) from northern KwaZulu-Natal and southern Mozambique

V. Govender *, T.A.S. Aveling, Q. Kritzinger

Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, South Africa

Received 31 July 2007; received in revised form 1 October 2007; accepted 4 October 2007

Abstract

In sub-Saharan Africa, maize (*Zea mays* L.) is one of the most nutritional crops and proper storage of seeds continues to be a challenge for subsistence farmers. Storage fungi, which reduce seed quality, become active in seeds when moisture levels are 14% or higher and this is influenced by the way seeds are stored. The aim of the present study was to test germination and vigour of maize seeds that were subjected to traditional storage during 2005 and to test germination of the maize seeds after storage for one year under conditions of fluctuating temperature. A preliminary survey was conducted and maize samples (white and yellow) were collected from small-scale subsistence farmers in northern KwaZulu-Natal (South Africa) and Mozambique. Seeds that were left in the field to dry and seeds that were commercially treated with Celest® XL served as controls. Germination was measured according to the International Seed Testing Association (ISTA) rules. The maize that was left in the field (*NHS*) to dry gave 100% germination in 2005. The treated control had a germination of 94.0%. Seeds that were imbibed for 40 h had the highest percentage weight increase following rapid imbibition but four of the six samples maintained germination above 70% following slow imbibition. The conductivity of the solute was read following imbibition. Field stored maize had the lowest solute leakage (1181 μS) and this correlated with the high percentage seeds with living tissue as indicated by the tetrazolium staining following rapid (94.4%) and slow (95.8%) imbibition. The number of fungi isolated from the samples reflected the initial condition of the samples with the fungicide treated control having the lowest percentage infection (11%), *NHS* had 33% and yellow maize that was stored on the cob and had with insect damage (*SIH*) had the highest, namely 71%. After the first set of experiments, samples were stored at 26–28 °C to simulate the fluctuating original storage conditions. A year later the samples were subjected to the standard germination test. The decline in seed viability during the storage period was exhibited by results of the standard germination test. Maize that was left in the field had a 74.7% decrease in germination while the sample stored in potato bag (*PHEL*) and the treated control maintained germination above 80%. Two of the six samples failed to germinate. This study also showed that fungicide seed treatment is a viable option to maintain viability of the seeds, especially when the maize is to be stored until the next season.

Keywords: Germination; Maize; Seed treatment; Traditional storage

1. Introduction

Maize (*Zea mays* L.) is important as a source of energy and protein in the human diet throughout the world (Rehman, 2006). Proper crop storage plays an integral part in ensuring domestic food supply (Thamaga-Chitja et al., 2004) and that seed quality and vigour is maintained (Joao Abba and Lovato, 1999). Fluctuations in temperature, humidity and prolonged storage result in considerable nutrient losses (Shah et al., 2002). Despite significant advances in food storage methods, many African and South African communities still rely on traditional storage methods for seed to be used as food and fodder (Olakojo and Akinlosotu, 2004; Thamaga-Chitja et al., 2004). Storage facilities not only offer the opportunity to provide a supply of food between staple crop harvests but farmers are able to improve farm incomes by storing crops and selling at premium prices when demand outstrips supply later in the post-harvest period (Florkowski and Xi-Ling, 1990). The most important factors that influence storage are temperature, moisture, carbon dioxide (CO_2), oxygen (O_2), grain characteristics, micro-organisms, insects, mites, rodents, birds, geographical location and storage facility structure (Jayas and White, 2003).

* Corresponding author.
E-mail address: velo@student.up.ac.za (V. Govender).

0254-6299/$ - see front matter © 2007 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2007.10.006

Insect pests are one of the major organisms that are responsible for the decline in quantity, quality and germination potential of maize seeds in storage (Jayas and White, 2003). A common strategy in many African countries is to sell maize grains immediately after harvest, to avoid losses to insect pests (Olakojo and Akinlosotu, 2004). Farmers in sub-Saharan Africa generally store their un-husked maize on wooden posts (Thamaga-Chitja et al., 2004). In most situations, maize is traditionally left to dry in the fields prior to harvesting. Other storage structures include a traditional silo that is made of mud and twigs. This structure is relatively inexpensive but it is not airtight and often exposes the stored maize to harsh environmental conditions such as sun and rain (Olakojo and Akinlosotu, 2004). Other storage facilities include the use of iron tanks, re-used maize-meal sacks to store maize on the cob and in addition, polyethylene, polypropylene and cotton sacks are frequently used (Thamaga-Chitja et al., 2004).

In 1979, the Association of Official Seed Analyst's vigour committee defined seed vigour as "those seed properties, which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions" (Copeland and McDonald, 2001). Seed vigour is defined by the International Seed Testing Association (ISTA) as "the sum total of those properties of the seed that determine the level of activity and performance of the seed during germination and seedling emergence" (ISTA, 2006). Vigour testing involves direct tests (e.g. cold test) where an environmental stress is reproduced in the laboratory and the percentage and or rate of seedling emergence are recorded. In indirect tests (e.g. conductivity) other characteristics of the seed are measured, that have proved to be associated with some aspect of seedling performance (ISTA, 2006).

When dry seeds are plunged into water, they imbibe water rapidly in the first few minutes, followed by a slower phase of imbibition until they become fully hydrated (Copeland and McDonald, 2001). It is concluded that ultra-dry seed storage is beneficial for maintaining seed vigour and that starchy mobilization proceeds regularly during germination (Wang et al., 2005). During the early stages of imbibition the seeds leak solutes such as organic and inorganic ions, sugars, amino acids and even proteins into the surrounding medium. Depending on the condition of the seed this loss means the loss of intracellular constituents and often results in extensive embryo damage and even its death (Duke and Kakefuda, 1981; Copeland and McDonald, 2001). Conductivity of the soak water of the sample gives an estimate of seed vigour. Seed lots that have high electrolyte leakage that is, having high leachate conductivity are considered as having low vigour (Barton, 1961; Coolbear, 1995).

Proper and safe storage conditions are defined as those that maintain seed quality without loss of vigour for three years (Joao Abba and Lovato, 1999). The loss of quality of maize seeds is not only visually observed by the poor condition of the seeds (Hell et al., 2000) but also by the poor performance of this seed when it is planted for the next season (Bellon, 2001). Seeds cannot retain their viability indefinitely and after a period of time, the seeds deteriorate (Pascual et al., 2006). In a study conducted on wheat (*Triticum aestivum* L.), by Gilbert et al. (1997), it was shown that germination after storage at temperatures −10, 2.5 and 10 °C decreased with length of storage. This occurred because most of the stored seeds were infected with *Fusarium graminearum* Schwabe and although they were stored at an acceptable temperature (10 °C) there were lowered germination percentages (Gilbert et al., 1997). Tekrony et al. (2005) studied the effects of storage of maize on germination and vigour in an "uncontrolled" warehouse and in a controlled environment, where the temperature and humidity were monitored. Their results showed that all seed lots had 87–99% germination prior to storage but a range in seed vigour as measured by the accelerated ageing test (ISTA, 2006). After eight months storage in the "uncontrolled" warehouse, the germination declined to 50–80% (Tekrony et al., 2005). Germination and vigour tests information can be used to make informed decisions regarding the value of different seed lots (Copeland and McDonald, 2001; Tekrony, 2003; ISTA, 2006).

The aim of the present study was to test germination and vigour of maize seeds that were subjected to traditional storage during 2005 and to evaluate the vigour of fungicide treated maize seed when stored for one year under conditions of fluctuating temperature.

2. Materials and methods

2.1. Collection of samples

Maize samples were obtained from small-scale subsistence farmers in Pongola and Kosi Bay area (northern KwaZulu-Natal, South Africa) and Ponto Molangane (southern Mozambique) in 2005 (Table 1). The quantity of the maize seeds that were stored by these farmers was enough to sustain those households that they were obtained from and most gave a small sample of their supply for this study. Of the seed that was kept for food, a small percentage was kept for planting the next season.

Maize that was left in the field to dry (*NHS*) [10 km from Nsalamanga High School — Kosi Bay area] prior to harvesting served as one of the controls. These seeds were in a good condition. The other control was seeds commercially treated with Celest® XL ([*fludioxonil (25 g ai/L)+mefenoxam (10 g ai/L)*] obtained from Syngenta (SA) Pty. Ltd, Midrand).

After collection, all samples were stored in plastic bags and brown paper bags (depending on the original storage condition), under cool conditions and transported back to the Department of Microbiology and Plant Pathology laboratories (University of Pretoria, South Africa) for tests. The moisture content of the seeds (11%) that were collected in 2005 was within the percentage acceptable for maize (10–14%) (ISTA, 2006). After the tests that were conducted in 2005, all seed samples were stored in the laboratory in brown paper bags at temperatures ranging from 26–28 °C to simulate the conventional storage conditions that the seeds originally came from.

2.2. Standard germination tests

Standard germination tests were conducted on all samples according to the between-paper (BP) method of the International Seed Testing Association (ISTA, 2006). Due to the quantity

Table 1
Storage conditions and characteristics of the treated control and the maize seeds collected in 2005 from the small-scale subsistence farmers

Sample[a] code	Colour of maize seeds	Storage structure	Storage container[b]	Other characteristics
Treated Control	Yellow	Commercial store	Commercially packed in plastic sack	Good condition (seeds were healthy, free of insect damage and the kernels were whole)
NHS	White with variegated kernels	Left in field to dry	Not applicable	Good condition (seeds were healthy, free of insect damage and the kernels were whole)
BHEK	White	Cement floors and walls	Maize-meal bags	Visually in good condition
JOZ	White	Commercial store	Commercially packed in plastic bags (originally obtained from Vryheid), available in general store in Jozini	Seeds had a small degree of insect damage
MOL	Yellow		Maize-meal bag	Good condition (seeds were healthy, free of insect damage and the kernels were whole)
R22	Yellow with variegated kernels	Hut with clay walls and thatch roof	Potato bags	Maize was stored on the cob, fair condition (seeds were healthy, had a bit of insect damage and some of the kernels were not whole)
SIH	Yellow	Cement and stone walls with a plastic sheet serving as a roof	Maize-meal bag	Maize stored on the cob, severe insect damage
PHEL	Yellow	Cement room	Potato bag	Good condition (seeds were healthy, free of insect damage and the kernels were whole)

[a] Treated control: maize that was treated with Celest® XL [*Fludioxonil (25 g ai/L) + Mefenoxam (10 g ai/L)*] *NHS:* Maize that was left in the field to dry [10 km from Nsalamanga High School — Kosi Bay], *BHEK*: Bhekamangwane [Pongola], *JOZ*: Jozini [Jozini], *MOL*: Molongane [Mozambique], *R22*: Area 4 km from Jozini [Jozini], *SIH*: Sihadla west gate [Pongola], *PHEL*: Phelandaba [Pongola]. The samples were stored to be either planted in the next season or sustain the household.
[b] The maize-meal bags originally contained a powdered form of the maize used for making porridge. The potato bags were thick brown paper bags that originally contained potatoes.

of the samples collected, only two hundred maize seeds could be randomly chosen from each sample. Four replicates of fifty seeds were placed equidistance apart on moist germination paper (containing one sheet of paper towel and four sheets of germination paper) {Anchor Paper 54 × 30 cm, [Agricol (Pty) Ltd, (South Africa)]} at 25 seeds per paper towel. Paper towels were rolled up and placed individually in polythene bags. Bags were sealed with an elastic band and incubated in an upright position at 25 ± 1 °C. Percentage germination was determined after seven days and rated for normal/abnormal seedlings at 11 days. Seeds were visually assessed according to the ISTA rules (ISTA, 2006). Results were presented as the percentage of seedlings that had germinated by the end of the test period.

2.3. Imbibition test

The imbibition tests were conducted according to the rules outlined by ISTA (2006). For rapid imbibition, sterile distilled water (4 mL) was placed into each well of a 24-well plastic ice-cube tray. These trays were chosen so that each tray represented an experimental unit. Seeds were imbibed for the following time intervals: 6, 24 and 40 h, with one seed per well. Seeds were weighed individually prior to imbibition. At the end of the time intervals, seeds were removed from the wells and left on paper towels and once air-dried were weighed again and planted onto germination paper as described for the standard germination test. Ratings were done after seven and 11 days as described for the standard germination test. In contrast, for slow imbibition, seeds were initially weighed individually and were planted onto germination paper as described for the standard germination test. After 6, 24 and 40 h imbibition, the paper towels were unrolled and seeds weighed and replaced onto the germination sheets, left to germinate and rated at seven and 11 days as described for the standard germination test. For each time interval a different sample of seeds was used so that at the end of the incubation times the germination of the seeds could be compared following 6, 24 or 40 h imbibition.

The percentage weight increase was calculated as:

$$\%\,\text{Weight increase} = (\text{Weight after imbibition} - \text{Initial weight}) \div \text{Initial weight} \times 100$$

2.4. Conductivity and tetrazolium test

The conductivity of the solution, after seeds were subjected to rapid and slow imbibition, was read using an E215 Conductivity meter (Hanna Instruments, South Africa). With rapid imbibition, the seeds were placed in wells of a 24-well plastic ice-cube tray containing 4 mL sterile distilled water for 24 h. Thereafter the conductivity was measured. For slow imbibition, seeds were planted onto germination paper, as described for the standard germination test, for 40 h, and then placed in plastic ice-cube trays containing sterile distilled water for 6 h. Thereafter the conductivity was read.

Seeds from the conductivity test were used for the tetrazolium staining test. A 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) (Labretoria, Pretoria) (10 g of TTC dissolved in a small quantity of hot water in a beaker) was transferred to a 1 L flask and tap water was added to make it up to 1 L. The seed coats of the seeds were removed and an incision was made longitudinally through the embryo and 3/4 of the endosperm as outlined in the ISTA rules (ISTA, 2006). Each seed was placed in

Table 2
Percentage germination and vigour of maize seed following storage under stress conditions

Germination (%)			Aspects of vigour measured in 2005		
Sample[#]	2005	2006	Conductivity (μS)	Tetrazolium staining [t] (% seeds with living tissue)	
				Slow imbibition	Rapid imbibition
Treated control	94.0*de**y	86.0ex	2536[@]cd	79.2[$]c^x	75.0cx
NHS	100gy	25.3bx	1181a	95.8ex	94.4fx
BHEK	92.0dy	56.0dx	3518e	74.4bcy	62.3bx
JOZ	88.7cy	0.0ax	2626cd	76.4bcx	75.0bx
MOL	82.0b	33.3cx	3006de	78.0bcx	74.7dex
R22	96.7efy	24.7bx	2030bc	89.0dy	72.2cx
SIH	18.7ay	0.0ax	6164f	49.6ay	28.3ax
PHEL	99.3fgy	92.0ex	1727ab	76.4bcx	73.1dex

[#] Treated control: maize that was treated with Celest® XL [*Fludioxonil (25 g ai/L) + Mefenoxam (10 g ai/L)*] *NHS:* Maize that was left in the field to dry [10 km from Nsalamanga High School — Kosi Bay], *BHEK*: Bhekamangwane [Pongola], *JOZ*: Jozini [Jozini], *MOL:* Molongane [Mozambique], *R22*: Area 4 km from Jozini [Jozini], *SIH*: Sihadla west gate [Pongola], *PHEL*: Phelandaba [Pongola].
[t] Triphenyl tetrazolium chloride test, a mean of 24 seeds expressed as percentage cotyledons with living tissue.
* Each value is a mean percentage of four replicates of 50 seeds. Means within a COLUMN for percentage germination not followed by the same letters are significantly different from each other ($P=0.05$).
** Each value is a mean percentage of four replicates of 50 seeds. Means within a ROW for the percentage germination not followed by the same letters are significantly different from each other ($P=0.05$).
[@] Each value is a mean percentage of four replicates of 24 seeds. Means within a COLUMN for conductivity values not followed by the same letters are significantly different from each other ($P=0.05$).
[$] Each value is a mean percentage of four replicates of 24 seeds. Means within a COLUMN for slow imbibition not followed by the same letters are significantly different from each other ($P=0.05$).
^ Each value is a mean percentage of four replicates of 24 seeds. Means within a ROW for the percentage germination not followed by the same letters are significantly different from each other ($P=0.05$).

an individual well of a 24-well plastic ice-cube tray and covered with the TTC solution. The trays were covered and incubated at 30 °C for 2 h in the dark. The seeds were then removed from the stain, cut into two halves and the cut surface was examined under a stereo-microscope (Nikon/SMZ-1, Japan). The seeds were rated as 1 — entire embryo was stained (seeds containing living tissue), 2 — part of the seed not stained and 3 — seed totally unstained (e.g. hard seed). Results were expressed as the percentage seeds containing living tissue.

2.5. Isolation of fungi

A hundred seeds from each batch of the samples and the controls were surface sterilized in 1% sodium hypochlorite (NaOCl) for 1 min. Thereafter they were rinsed three times in sterile distilled water. Seeds were directly plated onto potato dextrose agar (PDA) (Merck, Johannesburg) and malt extract agar (MEA) (Merck, Johannesburg) supplemented with rifampicin (Calbiochem®, Johannesburg). Five seeds were plated onto one plate with one seed placed in the centre and one in each quadrant. Plates were incubated at 25 °C for seven days with light/dark cycles. Plates were examined for fungal growth. Fungi were isolated and cultured onto PDA for identification purposes. Identification of some genera was done according to Nelson et al. (1983). The fungi that occurred in most of the samples were noted and recorded at genus level.

2.6. Statistical analysis

Two-way analysis of variance (ANOVA) was performed on all data and least significant differences ($P=0.05$) were determined according to the student's t test.

3. Results and discussion

The sample descriptions for the abbreviations *SIH, PHEL, NHS, JOZ, R22, BHEK* and *MOL* are outlined in Table 1. In 2005, with the exception of *SIH* (18.7%), all the samples had percentage germination above 80% (Table 2). *NHS*, which gave 100% germination, differed significantly from all of the samples except *PHEL* (99.3%). However, after storage under suboptimum conditions for one year the percentage germination of all the samples decreased significantly. Two samples, *JOZ* and *SIH* failed to germinate in 2006 (Table 2).

In this study the treated control had a germination of 94.0%, which decreased to 86.0% due to storage under sub-optimum conditions (Table 2). This was still an acceptable decrease in germination, compared to most of the other samples, 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. This was also confirmed by the results of the vigour tests. Following rapid imbibition this sample had a 55.7% weight increase and maintained germination above 85% after 40 h rapid imbibition (Table 3). Likewise, following 40 h slow imbibition there was a 46.0% weight increase of these treated seeds, but a percentage germination of 95.8% (Table 3). Slow imbibition is the natural way that seeds imbibe water (Copeland and McDonald, 2001; ISTA, 2006) and the increase in weight did not have an effect on the percentage germination. This sample had a leachate conductivity value of 2536 μS and was lower than some of the samples (Table 2). This result was reflected by the percentage seeds with living tissue in the tetrazolium staining test. Results for rapid (75%) and slow (79%) imbibitions did not differ significantly and is an indication of the good condition of the seeds, relative to some of the other samples (Table 2). The treated control had the lowest percentage of storage fungi (11%) with the genus *Rhizopus* (5%) predominating (Table 4).

Researchers from Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) have been helping small-scale farmers in Mexico with training in proper storage of maize since 1997 (Bellon, 2001). The training helped farmers to rather make use of a storage pesticide (fostoxin) and silos for their grain so that the stored seed could be kept for a longer period of time (Bellon, 2001). In this current study, results confirmed that treating seeds prior to storage was advisable, as the treated control maintained acceptable germination percentages even after storage under sub-optimum conditions.

Table 3
Percentages weight increase and germination following fast and slow imbibitions of maize seeds that were subjected to stress conditions

		Samples[#]							
		Treated control	*NHS*	*BHEK*	*JOZ*	*MOL*	*R22*	*SIH*	*PHEL*
% Weight increase									
Slow imbibition	6	13.9*ab**x	20.7cx	11.2ax	43.0ex	11.3ax	17.6bcx	37.7dx	13.1abx
	24	31.8by	35.5cdy	36.1dy	52.7fy	27.1ay	33.4cby	42.8ex	31.9by
	40	46.0cz	38.1by	51.9dz	51.1dy	33.3ayz	39.4by	60.2ey	45.7ez
Rapid imbibition	6	23.7cx	17.7bx	21.2cbx	42.9dx	13.5ax	23.0cx	40.0dx	19.0bx
	24	40.9cy	32.4by	41.4cy	51.2dy	32.4by	35.7by	51.0dy	24.2ax
	40	55.7dz	47.5cbz	44.2by	57.0dy	30.0ay	52.1cdz	64.2ez	42.0by
% Germination									
Slow imbibition	40	95.8dey	100ey	91.7dx	41.6by	84.8cy	97.2dex	2.8ay	100ey
Rapid imbibition	40	88.9ex	93.1efx	95.8fx	23.6bx	73.0cx	97.2fx	0.0ax	79.3dx

[#] Treated control: maize that was treated with Celest® XL [*Fludioxonil (25 g ai/L)+Mefenoxam (10 g ai/L)*], *NHS*: Maize that was left in the field to dry [10 km from Nsalamanga High School — Kosi Bay], *BHEK*: Bhekamangwane [Pongola], *JOZ*: Jozini [Jozini], *MOL*: Molongane [Mozambique], *R22*: Area 4 km from Jozini [Jozini], *SIH*: Sihadla west gate [Pongola], *PHEL*: Phelandaba [Pongola].
* Each value is a mean percentage of four replicates of 24 seeds. Means within a ROW for the weight increase not followed by the same letters are significantly different from each other ($P=0.05$).
** Each value is a mean percentage of four replicates of 24 seeds. Means within a COLUMN for percentage germination not followed by the same letters are significantly different from each other ($P=0.05$).

The field sample (*NHS*) that was used as a second control represented freshly harvested seed and gave 100% germination in 2005. As soon as these seeds were stored in brown paper bags at between 26 and 28 °C, they were placed under the same stress conditions as the other samples, and germination dropped to 25.3% in 2006 (Table 2). Generally maize left in the field has more time to dry so that it has a lowered moisture content percentage (Appert, 1987). Results from the vigour test confirmed that the seeds had high vigour in 2005 with a low leachate conductivity value of 1181 μS and high percentage seeds with living tissue (95.8% following slow imbibition and 94.4% following rapid imbibition) (Table 2). The weight increase following slow and rapid imbibitions was lower when compared to some of the other samples (Table 3). The percentage weight increase following slow and rapid imbibitions for 40 h was 38.1 and 47.5%, respectively and percentage germination was 100 and 93.1%, respectively (Table 3). These results mirrored the germination in 2005 (Table 2). This seed sample was infected with predominantly *Cladosporium* spp. (16%) and *Fusarium* spp. (10%) (Table 4). Although this control had 100% germination when initially tested, storage under sub-optimum conditions would allow for storage fungi to become a major threat to the quality of the grain. 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%.

With the samples that still had maize on the cob, *R22* (96.7%) did not differ significantly from *PHEL* (99.3%) in the standard germination test. In 2005, *R22* did not differ significantly from the treated control (94.0%). However, in 2006 the germination of this sample (*R22*), decreased by 72%. *R22* had a 39.4% weight increase and 97.2% germination following slow imbibition for 40 h and had a conductivity value of 2030 μS. This was reflected by the percentage seeds with living tissue (89 and 72.2%), following slow and rapid imbibitions (Table 2). In contrast, *PHEL* had a 7.3% decrease in germination and did not differ significantly from the treated control in 2006. This sample had a conductivity value of 1727 μS and had percentage seeds with living tissue above 70% following both slow and rapid imbibitions (Table 2). Most subsistence farmers prefer to store maize on the cob over the fire and the smoke helps to prevent seeds from spoiling and from pest infestation but most of the time quality of the maize was found to be inferior, leading to a low germination rate and lower yields (Modi, 2004). Sparg et al. (2005) found that in many other crops the application of smoke stimulated seed germination.

Table 4
Percentage of fungi found in the maize seed controls and samples that were subjected to stress conditions

Fungal genera	Samples[a] [b]							
	Treated control	*NHS*	*BHEK*	*JOZ*	*MOL*	*R22*	*SIH*	*PHEL*
Aspergillus spp.	2	–	14	10	12	6	14	2
Cladosporium spp.	–	16	25	–	20	16	10	12
Fusarium spp.	–	10	3	–	–	–	30	5
Penicillium spp.	–	–	–	–	–	–	2	–
Rhizopus spp.	5	–	5	20	–	–	15	2
Stenocarpella spp.	–	–	–	2	–	–	–	–
Other	4	7	2	4	8	20	–	–
Total fungi	11	33	49	36	40	42	71	21

[a] Treated control: maize that was treated with Celest® XL [*Fludioxonil (25 g ai/L)+Mefenoxam (10 g ai/L)*], *NHS*: Maize that was left in the field to dry [10 km from Nsalamanga High School — Kosi Bay], *BHEK*: Bhekamangwane [Pongola], *JOZ*: Jozini [Jozini], *MOL*: Molongane [Mozambique], *R22*: Area 4 km from Jozini [Jozini], *SIH*: Sihadla west gate [Pongola], *PHEL*: Phelandaba [Pongola].
[b] For the percentage of fungi for each sample, four replicates of 25 seeds were used per sample.

The germination of *BHEK* was 92.0% and did not differ significantly from the treated control in 2005. The germination decreased to 56% in 2006 (Table 2). This sample had a high leachate conductivity value (3518 µS) and percentage seeds with living tissue below 75% following slow (74.4%) and rapid (62.3%) imbibitions (Table 2). In contrast to all the trends noticed with the other samples, the high conductivity value was not an indication of the condition of the seed lot as percentage germination following slow and rapid imbibitions was 91.7 and 95.8% respectively (Table 3). In this sample, the high conductivity did not necessarily indicate a low vigour seed lot. Decreased membrane integrity could be as a result of storage deterioration (most of the samples in this study) and mechanical injury (Copeland and McDonald, 2001), however, other factors could play a role in increased leachate conductivity, such as initial seed moisture and seed size (Tao, 1978; Gras et al., 1990).

JOZ and MOL had percentages germination of 88.7 and 82.0%, respectively and differed significantly from each other and from the other samples, as most of the other samples had percentages above 90% except *SIH* (18%) (Table 2). *JOZ* decreased from 88.7% (2005) to 0.0% (2006). The vigour tests in 2005 showed that this sample had percentage weight increase above 50% for both slow and rapid imbibitions after 40 h. The percentage germination was lower following slow (41.6%) and rapid (23.6%) imbibitions compared to the other samples (Table 3). *JOZ* had a conductivity value of 2626 µS (Table 2). The deterioration in the *JOZ* sample could be explained by the fact that these seeds had mild insect damage. Even though they were commercially packaged in plastic bags, they were in a general store without air-conditioning and the temperature during summer (when the seed was collected) reached 35–37 °C during midday. This was not conducive to maintaining the quality of this sample. Combined with the temperature, insect damage and storage stress, these seeds failed to germinate in 2006.

In a study conducted by Casini (1999), the advantages of storing dry grain in plastic bags were evaluated. Dry grain (maize, soybean and wheat) can be stored in plastic bags for 24 months, if certain guidelines are followed. Ideally grain should be kept with low oxygen content and a high concentration of carbon dioxide (CO_2). This gives control of insects and fungi that are the major causes of increases in the temperature of the grains. In this sample, such guidelines were not followed in contrast to the treated control. However, storage in plastic bags is better than storage in paper bags but the original condition and an optimum temperature needs to be taken into account (Casini, 1999).

For the *SIH* sample, decline in germination from 18.7% to failure to germinate the following year was expected as this sample had severe insect damage. The standard germination results of this sample differed significantly from the other treatments and the control in 2005 as all the other samples had germination percentages above 80%. With the exception of *JOZ* (0.0% germination), *SIH* differed significantly by failing to germinate (0.0%) in 2006 when compared to the other samples. This was comparable to a study by Thamaga-Chitja et al. (2004) who found that storing maize seeds in sacks provided little protection against insects and maize stored in this manner absorbed moisture from the floor (typically mud, sealed with cow dung or cement). Following slow and rapid imbibitions, *SIH* had a percentage weight increase that was above 60% (Table 3) and had a 2.8 and 0.0% germination, respectively. The weakened initial condition of the *SIH* seeds was indicated by the high leachate conductivity value (6164 µS) and was mirrored by the low percentage seeds with living tissue following the tetrazolium test (49.6 after slow imbibition and 28.3% after fast imbibition) (Table 2). The standard germination test should ideally provide the seeds with optimum conditions to germinate (Copeland and McDonald, 2001; Tekrony et al., 2005), however even with these optimum conditions *SIH* did not germinate well and adding stress (vigour tests) in addition to the insect damage, illustrated the extremely low vigour potential of this seed lot. Most of the vigour tests give an indication of the field performance of the seed lot, however, there are other factors to consider as well, such as environmental conditions (Copeland and McDonald, 2001). This sample when exposed to a "controlled stress environment" failed to germinate so the chances of this sample producing any seedlings in an uncontrolled field environment is very low to non-existent. The storage fungi isolated from this sample totalled 71% positive incidence with *Aspergillus* (14%), *Fusarium* (30%) and *Rhizopus* (15%) species predominating (Table 4).

Of the storage conditions that were presented in this study the two samples (*R22* and *PHEL*) that were still on the cob and in potato bags had a high germination, above 95%, in 2005. The difference in the decrease in their germination in 2006 (24.7 and 92.0%, respectively) can be explained by the initial condition of those seeds. *R22* was in fair condition in contrast to *PHEL*, which was in good condition. Field stored maize is useful as fresh seed for immediate use and for planting. Long-term storage as indicated in this study is not feasible as the moisture content of the seed will increase to above 14% and as the subsistence farmers may not have the knowledge and equipment to get those seeds back to an acceptable moisture content, those seeds will deteriorate. Modi (2004) showed the limitations of the conventional storage structures, where structures are made very weak and allow insects to enter and provide an environment for storage fungi to thrive. Bags stored in either very cold temperatures or in cement structures work very well in terms of protecting seeds from most pest and insects. Sealed plastic bags, as was the case in the treated control, are the best as indicated by Gras et al. (1990), but seeds need to be in a good condition (mechanically and insect damaged seed must be removed) and storage temperatures must be kept as low as possible (4–10 °C).

This study reiterated the importance of proper storage techniques and their impact on germination and vigour of maize seeds. Apart from correct storage, the original condition of the seeds needs to be taken into account before they are stored as insect damage could aggravate the problem as shown in this study. Seed treatments have a major role in protecting the seed during storage (Chen and Burris, 1993) and can also play an important role in achieving uniform seedling emergence under certain conditions (Rane and Ruhl, 2002). This study confirmed that using a fungicide such as Celest® XL protected the seed and was effective even after storing seeds at 26–28 °C.

Small-scale farmers that may not have facilities to store their seed at 4–10 °C, will benefit from protecting their seed with a fungicide so as to provide undamaged seed for planting the following season.

References

Appert, J., 1987. Water Content. In: Coste, R. (Ed.), The Storage of Food Grains and Seeds. MacMillan Publishers, Hong Kong, pp. 59–60.

Barton, L.V., 1961. Seed presentation. Leonard Hill, Londo, p. 62.

Bellon, M., 2001. Empowering Farmers to Save Seed and Diversity. http://www.cimmyt.org. Accessed: 24/01/2006.

Casini, C., 1999. Guidelines for Storing Grains in Plastic Bags. http://www.cosechaypostcosecha.org. Accessed: 20/11/2006.

Chen, Y., Burris, J.S., 1993. Effect of seed treatment emulsifiers on seed quality and membrane function in maize. Postharvest Biology and Technology 2, 231–239.

Copeland, L.O., McDonald, M.B. (Eds.), 2001. Principles of Seed Science and Technology, 4th ed. Kluwer Academic Publishers, Massachusetts, pp. 124–128.

Coolbear, P., 1995. Mechanisms of seed deterioration. In: Basra, A.S. (Ed.), Seed Quality: Basic Mechanisms and Agricultural Implications. Food Product Press, New York, pp. 223–318.

Duke, S.H., Kakefuda, G., 1981. Role of the testa in preventing cellular rupture during imbibition of legume seeds. Plant Physiology 67, 449–456.

Florkowski, J., Xi-Ling, W., 1990. Simulating the impact of pecan storage technology on farm price and grower's income. Southern Journal of Agricultural Economics 22, 217–222.

Gilbert, J., Tekauz, A., Woods, S.M., 1997. Effect of storage on viability of *Fusarium* head blight-affected spring wheat seed. Plant Disease 81, 159–162.

Gras, P.W., Bason, M.L., Esteves, L.A., Sabio, G.C., Annis, P.C., Van, S., Gravers, J.E., 1990. Quality changes in maize stored in sealed bag stacks. Journal of Stored Product Research 26, 199–208.

Hell, K., Cardwell, K.F., Setamou, M., Poehling, H.M., 2000. The influence of storage practices on aflatoxin contamination in maize in four agro ecological zones of Benin, west-Africa. Journal of Stored Products Research 36, 365–382.

International Seed Testing Association (ISTA), 2006. International rules for seed testing. Seed Science and Technology. Basserdorf, Switzerland.

Jayas, D.S., White, N.D.G., 2003. Storage and drying of grain in Canada: low cost approaches. Food Control 14, 255–261.

Joao Abba, E., Lovato, A., 1999. Effect of seed storage temperature and relative humidity on maize (*Zea mays* L.) seed viability and vigour. Seed Science and Technology 27, 101–114.

Modi, A.T., 2004. Short-term preservation of maize landrace seed and taro propagules using indigenous storage methods. South African Journal of Botany 70, 16–28.

Nelson, P.E., Tousson, T.A., Marasas, W.F.O., 1983. Fusarium Species: An Illustrated Manual for Identification. The Pennsylvania State University Press, University Park, pp. 104–111.

Olakojo, S.A., Akinlosotu, T.A., 2004. Comparative study of storage methods of maize grains in South Western Nigeria. African Journal of Biotechnology 7, 362–365.

Pascual, B., San Bautista, A., López-Galarza, S., Alagarda, J., Maroto, J.V., 2006. Germination behaviour after storage of caper seeds. Seed Science and Technology 34, 151–159.

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

Rane, K., Ruhl, G., 2002. Crop Diseases in Corn, Soybean and Wheat. http://www.btny.purdue.edu/Extension/Pathology/Crop Diseases/Corn. Accessed: 24/05/2006.

Rehman, Z.U., 2006. Storage effects on nutritional quality of commonly consumed cereals. Food Chemistry 95, 53–57.

Shah, W.H., Rehman, Z.U., Kausar, T., Hussain, A., 2002. Storage of wheat with ears. Pakistan Journal of Scientific and Industrial Research 17, 206–209.

Sparg, S.G., Kulkarni, M.G., Light, M.E., Van Staden, J., 2005. Improving seedling vigour of indigenous medicinal plants with smoke. Bioresource Technology 96, 1323–1330.

Tao, K.J., 1978. Factors causing variations in conductivity test for soybean seeds. Journal of Seed Technology 3, 10–18.

Tekrony, D.M., 2003. Review: precision is an essential component of seed vigour testing. Seed Science and Technology 31, 435–447.

Tekrony, D.M., Shande, T., Rucker, M., Egli, D.B., 2005. Effect of seed shape on corn germination and vigour during warehouse and controlled environmental storage. Seed Science and Technology 33, 185–197.

Thamaga-Chitja, J.M., Hendriks, S.L., Ortmann, G.F., Green, M., 2004. Impact of maize storage on rural household food security in northern KwaZulu-Natal. Journal of Family Ecology and Consumer Science 32, 8–15.

Wang, X., Jing, X., Lin, J., 2005. Starch mobilization in ultradried seed of maize (*Zea mays* L.) during germination. Journal of Integrative Plant Biology 47, 443–451.

Edited by P Berjak

CHAPTER FOUR

THE EFFECT OF FUNGICIDE SEED TREATMENTS ON GERMINATION AND VIGOUR OF UNSTORED MAIZE (*ZEA MAYS* L.) SEEDS.

Abstract

The availability of good quality seed is dependent on two very broad aspects, how healthy (disease-free) a seed is and the viability of the seed (field performance). The objective of the study was to evaluate the efficiency of fungicide seed treatments on maize (*Zea mays* L.) by comparing germination, vigour and greenhouse emergence of treated seeds. Maize seeds were treated with four fungicides: Apron® XL (metalaxyl), Thiram (thiram), Celest® XL (fludioxonil, metalaxyl) and Apron® Star (thiamethoxam, metalaxyl, difenoconazole). After seeds were treated the moisture content of the seeds were calculated according to the rules outlined by the International Seed Testing Association (ISTA). The standard germination and vigour tests were conducted according to the rules outlined by ISTA. Thereafter seeds were planted under greenhouse conditions. The control consisted of seeds that were untreated. In the standard germination test, results were expressed as percentage seedlings that have germinated at the end of the test period. All treated seed maintained germination above 75%. Seeds treated with Apron® XL had the highest germination of 83%. This higher percentage germination compared to the other treatments was maintained following the cold test and the greenhouse trial. Thiram had 82% germination which decreased following imbition.. In this study none of the treated seeds had major imbibition damage as indicated by the percentage weight increase and the low leachate conductivity (1012-1271 $\mu Scm^{-1}g^{-1}$). The results from the vigour test gave an indication of the emergence of the seedlings following the greenhouse trial.

4.1 Introduction

Fungicide seed treatments are the most economical and easiest method to protect important seeds (Anaso *et al*, 1989) and young vulnerable seedlings (Rane and Ruhl, 2002). Seeds treatments that use pesticides can protect seeds from insect damage (Chen and Burris, 1993) and those seeds that are mechanically damaged at harvesting (Kommedahl and Windels, 1986). Most of commercially produced seed of maize (Zea mays L.) is almost universally treated with a fungicide prior to sale to protect the seed from fungal infection after planting (Kommedahl and Windels, 1986; Munkvold and O' Mara, 2002).

Protection of the seeds against pathogens has a direct impact on the germination of seeds (Gange *et al*., 1992; Jonitz and Leist, 2003). Pathogens can be successfully controlled through the use of suitable seed treatments, with a corresponding increase in the number of seeds that can germinate normally (Jonitz and Leist, 2003). A study conducted by Gange *et al*., (1992) hypothesized that pesticides used on seeds could either have a phytotoxic effect of a stimulatory effect on seed germination. Of the pesticides that were tested in their study, two had few significant effects on germination (Gange *et al*., 1992). Vigour tests are designed specifically to simulate the conditions in the field (Copeland and McDonald, 2001; Noli *et al*., 2008). Results obtained with the cold test gives an indication as to how seeds will germinate under conditions of increased pathogen density (Lovato *et al*., 2005). In a study by Lovato *et al*. (2005), it was found that maize seed had a higher vigour after being incubated at 4.5ºC than at 10ºC, results showed that the cold test is a very reliable vigour test for maize (Lovato *et al*., 2005). Subjecting treated seed to vigour tests gives an indication as to how specific treatments can indirectly increase germination (Nijënstein and Kruse, 2000; Noli *et al*., 2008).

Thiram is an organic sulphur fungicide, classified under dithiocarbamate. It is an excellent protectant compound registered for a large number of important crops (Frederickson and Leuschner, 1997; Agrios, 2005). Celest® XL includes two active ingredients, namely fludioxonil and mefenoxam. This is a water-based odourless chemical (http://www.syngenta.com). The fungicide fludioxonil is used as a seed treatment providing

protection during germination and early growth stages of the development. If the seeds are protected from biotic factors such as fungal infection then the chances of those seeds being viable is increased (Errampalli, 2004; http://www.syngenta.com).

The aim of the current study was to treat maize seeds with fungicides (Apron® XL, Apron® Star 42 WS, Thiram and Celest® XL) and to assess the effect these treatments have on germination, vigour and greenhouse emergence.

4.2 Materials and Methods

4.2.1 Treatment of the seed

Untreated seeds were obtained from Syngenta Pty. Ltd (Midrand, South Africa). All the chemicals 1) Celest® XL [*fludioxonil (25 g ai/L) + mefenoxam (10 g ai/L*]; 2) Apron® Star [*thiamethoxam (20% w/w) + metalaxyl – M (20% w/w) + difenoconazole (2% w/w)*]; 3) Apron® XL [*metalaxyl – M (350 g ai/L*] and 4) Thiram [*thiram (50.0% m/m)*] were also supplied by Syngenta. Seeds were placed in a flat-bottomed bowl and the recommended amount of fungicide and water were added to the seeds. The seeds were mixed thoroughly with the fungicide until all the seeds were covered with the fungicide, which took 5-10 min. After treatment, the seeds were left on paper towels in a laminar flow cabinet to dry. Once the seeds had dried, they were divided into three batches (1: immediate use; 2: 2 and 4 day accelerated ageing and 3: 3 and 6 months storage). This chapter focuses on the batch that was processed immediately.

4.2.2 Moisture content

Prior to proceeding with the rest of the tests, the moisture content was measured for the treated seeds. Two samples of 10 g were used. Two metal containers of a diameter of more than 8 cm were weighed. The samples were ground individually, using a grinding mill, and placed into the metal containers. The resultant maize powder in the containers was then weighed (initial weight). The containers were placed in an oven at 130ºC for 4 hr. At the end of the 4 hr the samples were placed in a dessicator for 30 min to cool. The samples were then reweighed. The percentage moisture content was calculated according to the formula outlined in the International Seed Testing Association rules (ISTA, 2008).

(M2-M3) x 100/(M2-M1)

Where M1 – is the weight in grams of the containers and the cover

M2 – is the weight in grams of the container, its cover and its contents before drying

M3 – is the weight in grams of the container, cover and contents after drying

4.2.3 Standard germination test

Standard germination tests were conducted for all samples according to the between-paper (BP) method of the ISTA rules (ISTA, 2008). Two hundred maize seeds were randomly chosen from each sample and were placed on moist germination paper (containing 4 sheets of germination paper and 1 sheet of paper towel) {Anchor Paper 54x30 cm, (Agricol (Pty) Ltd, South Africa)} equidistant apart. Paper towels were rolled up and placed individually in polythene bags. These bags were sealed with an elastic band. They were incubated in an upright position at 25 ± 1°C. Four replicates of 50 seeds were used. Percentage germination was determined after seven days and ratings for normal/abnormal seedlings were done at eleven days. Seeds were visually assessed according to the ISTA rules (ISTA, 2008). Results were presented as the percentage seedlings that had germinated at the end of the test period.

4.2.4 Vigour tests

4.2.4.1 Imbibition

Seeds were subjected to slow and rapid imbibition as outlined in the ISTA (2006) rules. For rapid imbibition, seeds were weighed individually and placed in 4 ml water in a 24 well ice-cube tray. Seeds were incubated for 6, 24 and 40 hr. At the end of the incubation times, seeds were removed, left to dry and then reweighed. The percentage weight increase was calculated according to the formula:

$$\% \text{ Weight increase} = \frac{\text{weight of 6hr imbibition}}{\text{initial weight of seed}}$$

Thereafter the seeds were placed in germination paper and left to germinate as described for the standard germination test. In contrast, with slow imbibition the seeds were weighed and then placed on germination paper as described for the standard germination test. The seeds were incubated for 6, 24 and 40 hr as described for the rapid imbibition. At the end of the

incubation times, the seeds were reweighed and put back onto the germination paper. The percentage seedlings were noted as described for the standard germination test. The percentage seeds were compared with that of the results from the standard germination test.

4.2.4.2 Conductivity

With rapid imbibition, the seeds were placed in wells of the ice-cube tray for 24 hr. Thereafter the conductivity of the solution was read on an E215 Conductivity meter (Hanna Instruments). After the conductivity was read, the same seeds were used in the tetrazolium staining test. Slow imbibition consisted of seeds being placed on germination paper for 40 hr. At the end of the 40 hr, seeds were placed in the trays for 6 hr, thereafter the conductivity of the solution was read and the same seeds were used in the tetrazolium staining test. The conductivity of the fungicide solutions in the absence of seeds was also tested.

4.2.4.3 Tetrazolium test

Seeds from the conductivity test were used for the tetrazolium staining. A 1% solution of the 2,3,5-triphenyl tetrazolium chloride (TTC) (Labretoria, Pretoria) (10 g of TTC dissolved in a small quantity of hot water in a beaker) was transferred to a 1 L flask and tap water was added to make it up to 1 L The seed coats of the seeds were removed and the seeds were slit longitudinally through the embryo and ¾ of the endosperm. The slit seeds were placed in individual wells and covered with the TTC. The trays were incubated at 30ºC for 2 hr. The seeds were then removed from the stain, cut into two halves and the cut surface was examined using a stereo-microscope (Nikon/SMZ-1, Japan). The seeds were rated as 1 – totally stained seed, 2 – part of the seed was not stained and 3 – if the seed is totally unstained (e.g. hard seed). Results were expressed as the percentage of seeds containing living tissue.

4.2.4.4 Cold Test

Soil was obtained from a maize field and brought back to the Plant Pathology laboratories (University of Pretoria). The germination paper was prepared as for the standard germination test with one difference, soil was included onto the paper and seeds were planted onto the soil. Paper towels were rolled up and placed individually in polythene

bags. They were incubated in an upright position at 5°C for a week, thereafter incubated at 25°C. Four replicates of 50 seeds were used. Percentage germination was determined after 7 d following incubation at ambient temperature and rating for normal / abnormal seedlings were done at 11 d. Seeds were visually assessed according to the ISTA rules (ISTA, 2008). Results were presented as the number of seedlings that had germinated at the end of the test period.

4.2.5 Greenhouse trial

The seedling trays were cleaned using 2% sodium hypochlorite and left to dry for 24 hr before the trays were filled with pasteurised soil (Braaks, Pretoria). The trays were watered until run-off. This was done a day before the seeds of the different treatments were sown. Four replicates of 25 seeds were used per treatment. Each tray had three different treatments in a randomised block design. The temperature within the greenhouse ranged from 25-30ºC. The trays were monitored regularly and were watered daily. The trial was terminated three weeks after planting and the trial was repeated. Results were expressed as the percentage seedlings that have emerged at the end of the test period.

4.2.6 Statistical analysis

Two-way analysis of variance (ANOVA) was performed on all data and least significant differences (P= 0.05) were determined according to the student's t test.

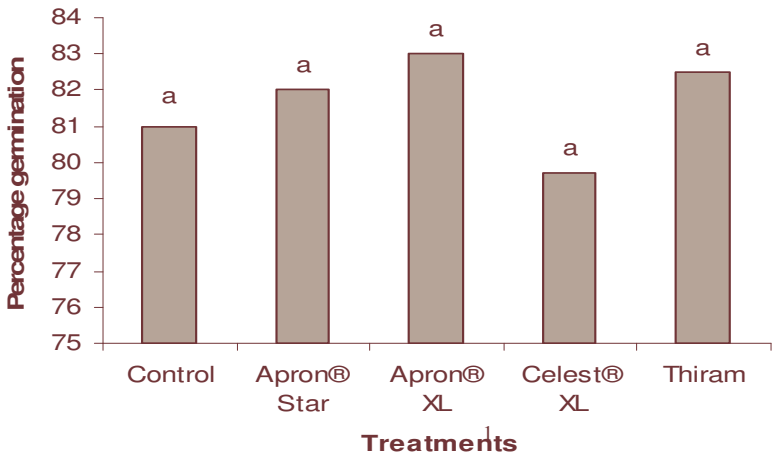
4.3 Results

4.3.1 Moisture content

The average percentage moisture content for each of the treatments and the control was between 11.0 to 11.5%. All the seed samples were within tolerance (< 0.3%) for maize as stipulated according to ISTA (ISTA, 2008).

4.3.2 Standard germination test

All the seed treatments had germination percentages above 75% and did not differ significantly from the control and each other (Fig 4.1). Seeds treated with Apron® XL had the highest percentage (83%) followed by Apron® Star and Thiram (82 and 82.5%, respectively).



[1] Each value is a mean percentage of four replicates of 50 seeds
Bars containing the same letters above them did not differ significantly (*P = 0.05*)

Fig 1: Standard germination results of treated and untreated maize seeds.

4.3.3 Vigour test

4.3.3.1 Imbibition

Results show that the different treatments had a high percentage weight increase following 40 hr rapid imbibition and did not differ significantly from each other (Table 4.1). Within the different treatments the lowest weight increase was seen following 6 hr slow imbibition (Table 4.1).

The untreated control had a lower percentage (46.7%) weight increase following slow imbibition compared to some of the treatments (Table 4.1) and this was reflected in the germination following 40 hr slow imbibition (83%). The percentage weight increase of seeds treated with Apron® Star did not differ significantly following 40 hr rapid and slow imbibition (58.5 and 57.3%). Percentage germination results for seeds treated with Apron® Star following slow imbibition was 63.6% but it did not differ significantly from the germination following rapid imbibition (56.2%)

Table 4.1: The percentage weight increase and germination following imbibition of the treated and untreated maize seeds

	Time (hr)	Treatments[1]				
		Control	Apron® Star	Apron® XL	Celest® XL	Thiram
		Weight increase (%)				
Rapid imbibition	6	34.3*a**w	34.0aw	31.5aw	34.7av	34.8ay
	24	47.7ax	51.7ay	48.1ay	52.2ay	51.4az
	40	56.4ay	58.5az	59.2az	56.0ay	58.4az
Slow imbibition	6	16.3av	18.3av	19.0av	21.0au	19.3ax
	24	35.2aw	44.2bx	39.4abx	40.0abw	40.0aby
	40	46.7ax	57.3byz	41.7ax	46.0aw	48.2az
		Germination (%)				
Rapid imbibition	40	60.4*b**x	56.2ax	59.4bx	67.7bx	52.1ax
Slow imbibition	40	83.4by	63.6ax	80.2by	64.0ax	77.1by

[1]- Each value is a mean percentage of four replicates of 24 seeds
*Means within a ROW not followed by the same letter are significantly different ($P = 0.05$)
**Means within a COLUMN not followed by the same letter are significantly different ($P = 0.05$)

Seeds treated with Apron® XL had the highest percentage weight increase following rapid imbibition however it did not differ significantly from all the other treatments (Table 4.1). This treatment had the lowest percentage weight increase (41.7%) following slow imbibition and the highest percentage germination (80.2%) of the four treatments.

Seeds treated with Celest® XL had 56.0% weight increase and was similar to the untreated control (56.4%) but did not differ significantly from the other treatments. This treatment recorded the second lowest germination (64.0%) following 40 hr slow and did not differ significantly from Apron® Star (63.6%). In contrast to the trend of the other treatments, Celest® XL had the highest percentage germination (67.7%) following 40 hr rapid imbibition.

Thiram had 48.2% weight increase after slow imbibition and did not differ significantly from the other treatments. When comparing with the other treatments, it differed from weight increase following rapid imbibition (58.4%). The percentage germination following slow imbibition was 77.1% and did not differ significantly from the untreated control (83.4%) and Apron® XL (80.2%) (Table 4.1).

4.3.3.2 Conductivity and Tetrazolium test

Compared to the water control, the conductivity of the fungicide solutions did not differ from control. Following imbibition all treatments had low leachate conductivity values with Thiram having the lowest (1012 $\mu Scm^{-1}g^{-1}$), although there were no significant differences between treatments (Table 4.2). These low conductivity values were mirrored in the percentage seeds with living tissue following the tetrazolium test. Thiram had the highest percentage seeds with living tissue (82%) and differed from all treatments (Table 4.2). Following rapid imbibition the percentage of the seeds with living tissue was lower, with Thiram having the highest (66%) and differed significantly from the other treatments (Table 4.2).

Table 4.2: The effect of seed treatments on the seed coat permeability of the maize seeds as measured by leachate conductivity

	Treatments				
	Control	**Apron® Star**	**Apron® XL**	**Celest**	**Thiram**
[1]Conductivity ($\mu Scm^{-1}g^{-1}$)	1233 *a	1271a	1053a	1242a	1012a
	Living tissue (%)				
[t]TTZ: slow imbibition	52a**y	60by	62by	74cy	82dy
TTZ: rapid imbibition	33ax	40ax	36ax	33ax	66bx

[1]- Each value is a mean percentage of four replicates of 24 seeds
*Means within a ROW not followed by the same letter are significantly different (*P = 0.05*)
**Means within a COLUMN not followed by the same letter are significantly different (*P = 0.05*)
[t] – triphenyl tetrazolium chloride test, a mean of 24 seeds expressed as percentage cotyledons with living tissue

4.3.3.3 Cold Test

The percentage seedlings that have germinated following the cold test were all below 70% (Table 4.3, Column A) and did not differ from each other and the control. Seeds treated with Apron® XL did have the highest percentage germination (66%) (Table 4.3).

Table 4.3: Percentage germination following the cold test on treated and untreated maize seeds

	Cold Test	Greenhouse emergence	
Treatments	A Germination (%)[1]	B Emergence (%)[2]	C Emergence (%)[3]
Control	60*a	67ab**x	66.7ax
Apron® Star	58a	65abx	65.3ax
Apron® XL	66a	71bcx	78.7bx
Celest® XL	61a	73bcy	65.3ax
Thiram	59a	77cx	76.0bx

[1]Each value is a mean percentage of four replicates of 25 seeds that have germinated in the cold test.
[2]Each value is a mean percentage of four replicates of 25 seeds that have emerged in the greenhouse.
[3]Each value is a mean percentage of four replicates of 25 seeds that have emerged in the greenhouse.

*Means within a COLUMN not followed by the same letter are significantly different (*P = 0.05*)
**Means within a ROW not followed by the same letter are significantly different (*P = 0.05*)

4.3.4 Greenhouse trial

The percentage seedlings that emerged following the first greenhouse trial showed that all the treatments and the untreated control had percentages emergence above 65%. Of the treatments, seeds treated with Thiram had the highest percentage emergence (77%) and differed significantly from the untreated control (67%) and seeds treated with Apron® Star (Table 4.3, Column B). Similar results were obtained in the second trial with all the treatments and the untreated control having percentage emergence above 65% (Table 4.3, Column C). Seeds treated with Apron® XL recorded the highest percentage emergence (78.8%) and did not differ from Thiram (76%).

Comparison of the two greenhouse trials, columns B and C in Table 4.3, results for the untreated control were similar in both trials. A similar trend was seen with the treatments, with the exception of seeds treated with Celest ® XL, where the percentage emergence for both trials did differ significantly from each other. With seeds treated with Celest ® XL, this treatment gave a higher emergence in trial one (73%) compared to trial two (65.3%).

4.4 Discussion

In this study none of the fungicides tested reduced the germination, vigour or caused any phytotoxic effects as reflected by the greenhouse emergence results. This is in agreement with a study conducted by Smith (1969), where the effect of fungicides tested on the germination and emergence of maize showed that all the fungicides increased germination of the maize by 5-6% (Smith, 1969). Khan (1992) reported similar results on wheat, where two systemic fungicides increased percentage germination by a small margin, germination was increased from 9.9 to 14.0% (Khan, 1992). Metalaxyl seed treatment also proved to be successful in controlling downy mildew in sorghum and increasing the yield of this crop (Anaso *et al*., 1989) and similarly increasing yield of maize (Pedersen *et al*., 2003). Seeds treated with Apron® XL, had one of the higher percentage germination and its results were consistent for most of the vigour tests, as indicated in this study.

The fungicide and pesticide treatment did not have any effect on the moisture content of the seeds and all the seeds had a moisture content below 14% and was within tolerance (<0.3%), which is the acceptable percentage range for maize (ISTA, 2008). Imbibition was not effected by the fungicide treatment; this was confirmed with the germination of the seeds. This was in agreement with methods in the study done on the uptake of Triticonazole by wheat (Quérou *et al*., 1997.). The fungicide did not affect the pathways needed for the uptake of water and the wheat seeds tested were able to germinate. The percentages germination following 40 hr rapid imbibition confirmed in this study that there was no imbibition damage.

The germination of the fungicide treated seeds did not differ significantly from the untreated control, the treated seeds were processed shortly after treatment and a major effect on the seeds would not be expected. Fungicides containing both t thiram and metalaxyl was tested against eleven fungal species including *Fusarium* and *Ulocladium* on lettuce seeds. Results showed that the fungicide treatment increased seed germination by 64.5% when seeds were incubated at 35ºC (Jin and Tylkowska, 2005). In this current study, seeds treated with Thiram and Apron® XL (metalaxyl) gave germination results that were higher that the other treatments. Seeds treated with Apron® XL gave better results than Apron® Star as indicated by standard germination and emergence percentages.

The vigour tests gave promising results in that none of the fungicides tested negatively affected the vigour of the seeds. Fungicide seed treatments do not affect vigour and viability of maize seeds (Crozier, 1890, Bradley *et al*., 2001). Studies where fungicides were used proved that if the fungicides are used at the recommended dosage then the treatment will not have an affect on the functioning of the seeds (Crozier, 1890). Other studies confirmed that maize seeds treated with thiram did not negatively effect the germination as long as it was used at the recommended dosage (Tort *et al*., 2006). In this study the dosage at which these fungicides were tested did not have an effect on the germination.

Conductivity values were relatively low for seeds that were treated and the untreated control, this was confirmed with the relatively low percentage weight increase and the high percentage seeds with living tissue Zhang and Hampton (1999) tested the effects of three systemic fungicide based products on peas and legumes. Thiram and Apron TZ were among the fungicides tested. At the recommended dosage, the conductivity of the treated seeds did not differ from the untreated control (Zhang and Hampton, 1999). Similar results were obtained when maize seeds were treated with three fungicides and two insectides (Marchi and Cicero, 2003). The fungicides contained thiram and fludioxonil, which are two of the active ingredients found in the fungicides tested in this current study. Treating the maize seeds did not affect conductivity of the seeds (Marchi and Cicero, 2003) and seeds treated with fludioxonil had increased radicle length as indicated by Munkvold and O'Mara (2002).

Germination following the cold test mirrored results of the standard germination test and gave an indication of the results for the greenhouse emergence trial. The greenhouse emergence showed that Thiram and Apron® XL were able to protect the seeds in an *in vivo* environment and allowed the seeds to germinate. Nijënstein and Kruse (2000) reported that with all the problems associated with standardising the cold test, it remains a test that has been used on maize to simulate field conditions and to predict field behaviour. This was confirmed by Noli *et al.* (2008). In their study they found that the cold test was the most accurate vigour test to predict field performance as long as the conditions for the laboratory

test were kept at a low temperature and the soil microflora was similar to that of the field as was indicated in this current study where soil from a maize field was used in the cold test.

In this study all seeds had a low degree of imbibition damage as this was indicted by the low conductivity values and the high percentage of seeds with living tissue. Results from this study conclude that treating maize seeds will not affect germination the functioning of the seed. The next chapter addresses whether the fungicide seed treatments will continue to sustain germination of the seeds when they are subjected to storage under stress conditions.

4.5 Literature cited

Agrios, G.N. 2005. Plant Pathology – 4th Edition. Academic Press, San Diego. Pages: 209-211.

Anaso, A.B., Tyagi, P.D., Emechebe, A.M. and S.K. Manzo. 1989. Control of sorghum downy mildew (*Peronosclerospora sorghi*) of maize by seed treatment in Nigeria. *Crop Protection*. 8: 82-85.

Chen, Y. and J.S. Burris. 1993. Effect of seed treatment emulsifiers on seed quality and membrane function in maize. *Postharvest Biology and Technology*. 2: 231-239.

Copeland, L.O. and M.B. McDonald. (Eds.) 2001. Pages: 124-128 *In* Principles of Seed Science and Technology (4th Edition). Kluwer Academic Publishers, Massachusetts.

Crozier, A.A. 1890. On the effects of certain fungicides upon the viability of seeds. *The Journal of Mycology*. 6: 8-11.

Errampalli, D. 2004. Effect of fludioxonil on germination and growth of *Penicillium expansum* and decay in apple cvs. Empire and Gala. *Crop Protection*. 23: 811-817.

Frederickson, D.E. and K. Leuschner. 1997. Potential use of benomyl for control of ergot (*Claviceps africana*) in sorghum A-lines in Zimbabwe. *Plant Disease.* 81: 761-765.

Gange, A.C., Brown, V.K. and L.M. Farmer. 1992. Effects of pesticides on germination of weed seeds: implications for manipulative experiments. *The Journal of Applied Ecology*. 29: 303-310.

http://www.syngenta.com/en/products_services/seed_treat.aspex.
Accessed: 20/08/2005.

International Seed Testing Association. 2006. International Rules for Seed Testing. Seed Science and Technology. Seed Vigour Testing. ISTA Handbook, 2006.

International Seed Testing Association. 2008. International Rules for Seed Testing. Seed Science and Technology.

Jin, H. and K. Tylkowska. 2005. Effects of priming in combination with fungicides on germination and infestation of lettuce (*Lactuca sativa* L.). *Agricultural Sciences in China.* 4: 449-454.

Jonitz, A. and N. Leist. 2003. Seed testing and the effect of insecticidal active ingredients on the germination and emergence of hybrid maize seed Pages: 186-202. *In*. International rules for Seed Testing., ISTA. Switzerland.

Khan, S.A.J. 1992. Studies on fungi causing seed-borne diseases of wheat and rice and their control. MSc Thesis. Department of Botany, University of Karachi. Pages: 203-211.

Kommedahl, T. and C.E. Windels. 1986. Treatment of maize seeds. Pages: 163-182. *In*: Seed Treatment. K.A. Jeffs (Ed) 2nd edition. Lavenham Press Limited, England.

Lovato, A., Noli, E. and A.F.S. Lovato. 2005. The relationship between three cold test temperatures, accelerated ageing test and field emergence of maize seed. *Seed Science and Technology*. 33: 249-253.

Marchi, J.L. and S.M Cicero. 2003. Influence of chemical treatment of maize seeds with different levels of mechanical damage on electrical conductivity values. *Seed Science and Technology*. 31: 481-486.

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.

Nijënstein, J.H. and M. Kruse. 2000. The potential for standardization in cold testing in maize (*Zea mays* L.). *Seed Science and Technology*. 28: 837-851.

Noli, E., Casarini, E., Urso, G and S. Conti. 2008. Suitability of three vigour test procedures to predict field performance of early sown maize. 36: 168-176.

Pedersen, W.L, Kline, J.D., Bradley, C.A and D.S. Mueller. 2003. Influence of metalaxyl fungicide seed treatment on severity of rootworm (*Diabrotica* spp.) damage to corn (*Zea mays*) under no-tillage conditions. *Crop Protection*. 22: 647-652.

Quérou, R., Euvrard, M. and C. Gauvrit. 1997. Uptake of Triticonazole, during imbibition, by wheat caryopses after seed treatment. *Pesticide Science*. 49: 284-290.

Smith, A.M. 1969. Effect of fungicides on the germination of maize seed after storage. Plant Disease Reporter. 43: 174-175.

Tort, N., Dereboylu, A.E. and B. Turkyilmaz. 2006. Morphological and Physiological effects of a fungicide with a thiram agent on some corn culture forms. *Journal of the Faculty of Science*. 29: 67-79.

Rane, K. and G. Ruhl. 2002. Crop diseases in maize, soybean and wheat. http://www.btny.purdue.edu/Extension/Pathology/Crop.htm. Accessed: 24/05/2006.

Zhang, T. and J.G. Hampton. 1999. Does fungicide seed treatment affect bulk conductivity test results? *Seed Science and Technology*. 27: 1041-1045.

CHAPTER FIVE

THE EFFECT OF ACCELERATED AGEING AND LONG-TERM STORAGE ON UNTREATED AND FUNGICIDE TREATED MAIZE (*ZEA MAYS* L.) SEED

Abstract

Seed aging is a natural process that occurs during storage and it is expressed as a reduction in germination and this process can be accelerated by unfavourable environmental conditions (high humidity and temperatures). The aim of the current study was to test the effect of accelerated ageing [2 and 4 days accelerated ageing (AA)] and long-term storage (3 and 6 months) on germination and vigour of treated maize seeds. Maize seeds were treated with four fungicides: Apron® XL (metalaxyl); Thiram (thiram) and Celest® XL (fludioxonil, metalaxyl) and (Apron® Star (thiamethoxam, metalaxyl, difenoconazole). The control consisted of seeds that were untreated. After treatment the moisture content of the seeds was determined according to the rules outlined by the International Seed Testing Association (ISTA). Seeds were then subjected to 2 and 4 d accelerated ageing at 45ºC and high RH (100%). Concurrently seeds were stored for 3 and 6 months at 30ºC and 75% RH. Following the accelerated ageing and storage, seeds were subjected to standard germination and vigour tests according to the rules outlined by ISTA. In the standard germination test, results were expressed as percentage seedlings that had germinated at the end of the test period. The control and the treated seeds still germinated after 2 and 4 d AA. Seeds treated with thiram had the highest germination (69%) following 2 d AA. There was a gradual decrease in germination following ageing and storage of the treated seeds and the control. Seeds treated with Apron® XL failed to germinate at 3 months. The decrease in germination was mirrored by the leachate conductivity readings. Germination following the cold test linked well with the results of the standard germination. Seed treated with thiram was the only treatment to maintain germination after 6 months, all the other treatments and the control failed to germinate.

5.1 Introduction

The accelerated ageing (AA) test was initially proposed as a method to evaluate seed storability (Copeland and McDonald, 2001; Rice and Dyer, 2001) and indirectly field emergence (Jensen, 2002). This test exposes seeds for a short period of time (2 to 4 days) to conditions of high temperature (45ºC) and high humidity (100%) (Copeland and McDonald, 2001). The seeds absorb moisture from the humid environment and the raised seed moisture content, along with the high temperature, causes rapid seed ageing (Rice and Dyer, 2001; Sowiak, 2004). High vigour seed lots will withstand these extreme stress conditions and age more slowly than low vigour seed lots (Basu *et al*., 2004).

In situations where maize seed is not sold immediately after harvest and due to delays in processing, this seed is required to be stored for up to 8-10 months under ambient conditions (Basu *et al*., 2004). Safe storage is defined as storage in which the seed quality and vigour is maintained for at least three years. (Harrington, 1958, as cited in Basu *et al*., 2004). Many changes occur in the lipid composition of most seed types during storage (Kadlag *et al.*, 1995) but one of the most devastating changes occur when conditions are favourable for mould development and that seed is no longer usable (Appert, 1987).

In a study by Lovato *et al*. (2005) a comparison was done on the standard germination, cold temperature and accelerated ageing tests of maize. Germination was high for most of the maize lots tested but this percentage decreased following the vigour tests (cold and AA tests). The findings were that the AA test was just as effective as the 10ºC cold test for assessing maize seed lot vigour as the results obtained for both tests were similar (Lovato *et al*., 2005).

Exposing seeds to conditions of high temperature, even for a short amount of time gives valuable information about the internal condition of the seed (Wang *et al*., 2005). In a study by Dreyer and van de Venter (1992), an investigation was carried out on the mitochondrial activity of the etiolated shoots of freshly harvested and aged kernels of maize. Impaired mitochondrial activity was not evident at 25ºC (favourable temperature) but was detected at 13 and 46ºC.

In the previous chapter (Chapter Four), results proved that treating maize seeds did not affect germination and vigour of unstored seeds. As was expected most seed treatments had percentages germination that did not differ from the untreated control and maintained greenhouse emergence over two seasons. In this chapter subjecting treated maize seeds to stress conditions before germination gives an indication as to which fungicide seed treatment may affect viability or vigour when treated maize seeds have to be stored for a longer period of time i.e 3-6 months. The aim of the current study, therefore, was to test the effect of accelerated ageing (2 and 4 d AA) and long-term storage (3 and 6 months) on germination and vigour of fungicide treated maize seeds.

5.2 Materials and methods

5.2.1 Treatment of the seeds

Untreated seeds were obtained from Syngenta® Pty. Ltd (Midrand, South Africa). All the chemicals: 1) Celest® XL [*fludioxonil* (*25 g ai/L*) + *mefenoxam* (*10 g ai/L*)]; 2) Apron® Star [*thiamethoxam* (*20%w/w*) + *metalaxyl – M* (*20%w/w*) + *difenoconazole* (*2%w/w*)]; 3) Apron® XL [*metalaxyl – M* (*350 g ai/L*)] and 4) Thiram [*thiram* (*50.0% m/m*)] were also supplied by Syngenta® Pty. Ltd. Seeds were treated as described in Chapter Four. After treatment, the seeds were left on paper towels in a laminar flow cabinet to dry. Once the seeds had dried, they were divided into three batches (1: immediate use (Chapter Four; 2: 2 and 4 day accelerated ageing and 3: 3 and 6 months storage). This chapter focuses on the batches that were subjected to accelerated ageing and long term storage.

5.2.2 Moisture content

Prior to proceeding with the tests, the moisture content of two samples of 10 g of the treated seeds was measured. Two metal containers of a diameter of 10 cm were weighed. The samples were ground individually, using a grinding mill, and placed into the metal containers. The resultant maize powder in the containers was then weighed (initial weight). The containers were placed in an oven at 130ºC for 4 hr and then placed in a dessicator for 30 min to cool. The samples were then reweighed. The percentage moisture content was calculated according to the formula outlined in the International Seed Testing Association (ISTA) rules (ISTA, 2008).

(M2-M3) x 100/(M2-M1)

Where M1 – is the weight in grams of the containers and its cover

M2 – is the weight in grams of the container, cover and contents before drying

M3 – is the weight in grams of the container, cover and contents after drying

5.2.3 Accelerated ageing (AA) and long term storage

The seed batch that was subjected to accelerated ageing at high relative humidity (90-100%) and temperature (45ºC) was divided in half, with one half being used for 2 d AA and the other half for 4 d AA. A humid environment was created by placing seeds on a grid above a salt solution in a sealed chamber which was incubated at 45ºC. At the end of a 2 d or 4 d incubation period the seeds were removed and subjected to the standard germination and vigour test. The third batch to be used for the storage test was also divided into two halves with one half being subjected to 3 months and the other half 6 months storage at 30ºC and 75% RH. Incubation in the humid environment was created as described for the 2 and 4 d AA. At the end of the 3 months and 6 months, seeds were removed and subjected to the standard germination and vigour tests.

5.2.4 Standard Germination

Standard germination tests were conducted for all samples according to the between-paper (BP) method of the ISTA rules (ISTA, 2008). Two hundred maize seeds were randomly chosen from each sample and were placed equidistant apart on moist germination paper (containing four sheets of germination paper and one sheet of paper towel) {Anchor Paper 54x30 cm, (Agricol (Pty) Ltd, South Africa)}. Paper towels were rolled up and placed individually in polythene bags. These bags were sealed with an elastic band. They were incubated in an upright position at 25 ± 1°C. Four replicates of 50 seeds were used. Percentage germination was determined after seven days and ratings for normal/abnormal seedlings were done at 11 d. Seeds were visually assessed according to the ISTA rules (ISTA, 2008). Results were presented as the percentage seedlings that had germinated at the end of the test period.

5.2.4 Vigour tests

5.2.4.1 Imbibition

Seeds were subjected to slow and rapid imbibition as outlined in the ISTA (2008) rules. For rapid imbibition, seeds were weighed individually and placed in 4 ml water in a 24-well ice-cube tray. Seeds were incubated for 6, 24 and 40 hr. At the end of the incubation times, seeds were removed, left to dry and then reweighed. The percentage weight increase was calculated according to the formula:

$$\% \text{ Weight increase} = \frac{\text{weight of 6hr imbibition}}{\text{initial weight of seed}}$$

Thereafter the seeds were placed on germination paper and left to germinate as described for the standard germination test. In contrast, with slow imbibition the seeds were weighed and then placed on germination paper as described for the standard germination test. The seeds were incubated for 6, 24 and 40 hr as described for the rapid imbibition. At the end of the incubation times, the seeds were reweighed and returned to the germination paper. The percentage seedlings were noted as described for the standard germination test.

5.2.4.2 Conductivity test

With rapid imbibition, the seeds were placed in wells of the ice-cube tray for 24 hr. Afterwards the conductivity of the solution was read on an E215 conductivity meter (Hanna Instruments). After the conductivity of the solution was read, the same seeds were used in the tetrazolium staining test. In slow imbibition, seeds were placed on germination paper for 40 hr and then in ice-cube trays for 6 hr. Thereafter the conductivity of the solution was read and the same seeds were used in the tetrazolium staining test.

5.2.4.3 Tetrazolium test

Seeds from the conductivity test were used for tetrazolium staining. A 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) (Labretoria, Pretoria) (10 g of TTC dissolved in a small quantity of hot water in a beaker) was transferred to a 1 L flask and tap water was added to make up 1 L. The seed coats of the seeds were removed and the seeds were slit longitudinally through the embryo and ¾ of the endosperm. The slit seeds were placed individually in ice-cube wells and covered with the TTC. The trays were incubated at 30ºC

for 2 hr after which the seeds were removed from the stain, cut into two halves and the cut surface was examined using a stereo-microscope (Nikon/SMZ-1, Japan). The seeds were rated as 1 – totally stained seed, 2 – part of the seed was not stained and 3 – if the seed was totally unstained (e.g. hard seed). Results were expressed as the percentage of seeds containing living tissue.

5.2.4.4 Cold test

The germination paper was prepared as for the standard germination test with one difference, soil from a cultivated maize field was included onto the paper and seeds were placed equidistant on the soil. Four replicates of 50 seeds were used. Paper towels were rolled up and placed individually in polythene bags. They were incubated in an upright position at 5°C for 7 d and then at 25°C for a further 7 d. Percentage germination was then determined and rating for normal/abnormal seedlings was done at 11 d. Seeds were visually assessed according to the ISTA rules (ISTA, 2008). Results were presented as the number of seedlings that had germinated by the end of the test period.

5.2.5 Statistical analysis

Two-way analysis of variance (ANOVA) was performed on all data and least significant differences (P= 0.05) were determined according to the student's t test.

5.3 Results

5.3.1 Moisture content

The average percentage moisture content for each of the fungicide treatments and the control was between 11.7 to 12.5%. All the seed samples were within tolerance (< 0.3%) for maize as stipulated by the ISTA rules (ISTA, 2008).

5.3.2 Standard Germination

Table 5.1 reflects the effect of accelerated ageing and storage on germination of maize seeds that have been treated with fungicides. Comparing the different treatments and the untreated control within the 2 d AA column in Table 5.1 shows that seeds treated with Thiram had the highest percentage germination (69%) followed by seeds treated with Celest® XL (68.5%). The untreated control had the lowest germination (61%) and differed

from both Thiram and Celest® XL treatments. After 4 d AA seeds treated with Apron® Star had the highest percentage germination and differed significantly from the rest of the fungicide treatments and the control. Seeds treated with Apron® XL had a significant decrease in percentage germination to 9% (Table 5.1). After 3 months storage, the untreated control had the highest germination percentage (71.5%) and differed from the other fungicide/insecticide treatments. Seeds treated with Thiram had the second highest (58%) but did not differ from the Apron® Star (56%) treatment. Seeds treated with Celest® XL decreased to 15% and seeds treated with Apron® XL failed to germinate (Table 5.1). After 6 months storage, only seeds treated with Thiram had 12.5% germination with the untreated control and the other fungicide treated seeds failing to germinate.

Table 5.1: Impact of accelerated ageing and storage on germination of maize that has been untreated or treated with fungicides/insecticide

	Germination (%)			
	Accelerated ageing		Storage	
Treatment	**2 d AA**	**4 d AA**	**3 month**	**6 month**
Control	61*b**y	46.5bx	71.5ayz	0bw
Apron® Star	62.5by	49ax	56bxy	0bw
Apron® XL	65aby	9dx	0w	0bw
Celest® XL	68.5az	42by	15cx	0bw
Thiram	69ay	31.5cx	58by	12.5aw

AA = Accelerated ageing.

* Means within a column not followed by the same letter are significantly different (P = 0.05)

** Means within a row not followed by the same letter are significantly different (P = 0.05)

5.3.3 Vigour tests

5.3.3.1 Imbibition

Table 5.2 shows the percentage weight increase following rapid and slow imbibition. Following 24 hr imbibition there are major differences between the treated seeds and the untreated control. An increase in the period of ageing and storage showed an increase in percentage weight of the seeds, especially following rapid imbibition. Comparing the percentage weight increase of seeds for slow imbibition showed that at 2 d and 4 d AA

there were differences between the treatments and the untreated control but not significantly (Table 5.2).

Table 5.2: Percentage weight increase of untreated and fungicide/insecticide treated maize seeds following imbibition

			Weight increase (%)			
			Accelerated ageing		Storage	
	Time (hr)	Treatments	2 d AA	4 d AA	3 month	6 month
Rapid imbibition	**24**	Control	42.4*b**x	51.3cxy	56.0by	70.2az
		Apron® Star	45.6bx	49.7bcx	64.7cy	75.3bz
		Apron® XL	44.5bw	50.0cwx	62.8cy	74.9bz
		Celest® XL	46.0bx	45.1ax	74.4dz	76.8bz
		Thiram	45.0x	47.4bx	59.0by	70.0az
Slow imbibition	**24**	Control	33.1ax	43.5ay	46.9ay	79.0bz
		Apron® Star	38.0bw	40.8ax	50.1aby	71.6abz
		Apron® XL	33.3aw	41.7ax	59.3by	66.4az
		Celest® XL	35.7aw	48.3bx	54.6by	67.5az
		Thiram	33.7aw	40.6aw	49.2ay	65.2az

AA= Accelerated ageing

* Means within a column not followed by the same letter are significantly different (P = 0.05)

** Means within a row not followed by the same letter are significantly different (P = 0.05)

After 3 months storage the percentage weight increase reflected the standard germination results. The untreated control had the lowest percentage weight increase of the seeds (46.9%) (Table 5.2) and this is reflected in the standard germination test where the control had the highest percentage germination (71.5%) (Table 5.1). After 3 months storage seeds treated with Apron® XL and Celest® XL showed a higher percentage weight increase (59.3 and 54.6%) compared to the other treatments. There was an increase in imbibition damage following 6 months storage, and therefore increased water uptake (Table 5.2). For slow imbibition, seeds treated with Thiram had the lowest percentage weight increase (65.2%)

and this is the only fungicide treatment that maintained germination following 6 months storage.

5.3.2.2 Conductivity test

The trend shown in Table 5.3 indicates that as the period of ageing and storage of the seeds increases so does the leachate conductivity values. At 2 d AA, the untreated control had the highest (2404 $\mu Scm^{-1}g^{-1}$) conductivity reading. Seeds treated with Celest® XL had a lower conductivity value following 4 d AA and 3 months storage. After 6 months storage the trend with the leachate conductivity values mirrored that of the germination results in Table 5.1 where seeds treated with Thiram had the lowest value 1252 $\mu Scm^{-1}g^{-1}$ compared to the other fungicide treatments and the untreated control. From the standard germination results seeds treated with Thiram were the only treatment to have germinated when all the other treated seeds and the untreated control failed to germinate which is reflected by the high leachate conductivity values.

Table 5.3: The effect of accelerated ageing and storage on conductivity of untreated and fungicide/insecticide treated maize seed

	Conductivity ($\mu Scm^{-1}g^{-1}$)			
	Accelerated ageing		Storage	
	2 d AA	4 d AA	3 month	6 month
Treatments				
Control	2404*a**y	2412ay	2819ay	3455az
Apron® Star	1643cx	2049ay	2756ay	3041az
Apron® XL	1219dy	1170by	2139abz	2336bz
Celest® XL	1134dx	1571by	1464cy	2475bz
Thiram	2186abz	1796by	2556az	1252cy

AA= Accelerated ageing

* Means within a column not followed by the same letter are significantly different (P = 0.05)

** Means within a row not followed by the same letter are significantly different (P = 0.05)

5.3.2.3 Tetrazolium test

For the untreated control, there were no significant differences between the percentage seeds with living tissue following slow and rapid imbibition (Table 5.4). A similar trend shown by the leachate conductivity values was seen in the control and the treated seeds. There was a decrease in the percentage of seeds with living tissue following the ageing of the seeds and storage. After 6 month storage, seeds treated with Thiram had the highest percentage seeds with living tissue following slow (36%) and rapid (20%) imbibition.

Table 5.4: The percentage seeds with living tissue following accelerated ageing and storage of untreated and fungicide/insecticide treated maize seed

		Seeds with living tissue (%)			
		Accelerated ageing		Storage	
	Treatments	2d AA	4d AA	3 month	6 month
Slow imbibition	Control	54*cd**z	36y	20x	4aw
	Apron® Star	30bz	25byz	21bxy	17bx
	Apron® XL	64ez	40cdy	25cx	13bw
	Celest® XL	58dz	34cy	33dy	13bx
	Thiram	42bcy	42dy	25cx	36cy
Rapid imbibition	Control	52cdz	20by	8ax	4ax
	Apron® Star	21ayz	25bz	17by	4ax
	Apron® XL	36bz	20by	13aby	4ax
	Celest® XL	46cz	33cy	20bcx	4aw
	Thiram	50cz	12ax	25cy	20by

AA = Accelerated ageing

* Means within a column not followed by the same letter are significantly different (P = 0.05)

** Means within a row not followed by the same letter are significantly different (P = 0.05)

5.3.2.4 Cold test

Results from the cold test (Table 5.5) mirrored results from the standard germination test (Table 5.1). Seeds treated with Celest® XL had the highest percentage germination at 2 d AA (57%), however, it decreased to 20% at 4 d AA and failed to germinate following

storage at 3 and 6 months. Seeds treated with Apron® XL failed to germinate (Table 5.5) following 3 months storage as found in the standard germination test. After 6 months storage the results from the cold test reflected the standard germination results where seeds treated with Thiram was the only treatment that germinated (10%) (Table 5.5).

Table 5.5: The effect of accelerated ageing and storage on untreated and fungicide/insecticide treated maize seed subjected to the cold test

	Germination (%)			
	Accelerated ageing		Storage	
	2d AA	4d AA	3 month	6 month
Treatments				
Control	33*b**y	54dz	22bx	0aw
Apron® Star	40by	43cy	15bx	0aw
Apron® XL	20ay	10ax	0aw	0aw
Celest® XL	57dy	20bx	0aw	0aw
Thiram	38bz	19by	16bxy	10bx

AA= Accelerated ageing

* Means within a column not followed by the same letter are significantly different (P = 0.05)

** Means within a row not followed by the same letter are significantly different (P = 0.05)

5.4 Discussion

In contrast to findings of the previous study (Chapter Four), this study showed that ageing and storage of treated maize seeds does have an effect on their viability and vigour. In this current study the moisture content was higher for seeds that were subjected to stress conditions, where it increased from a range of 11.0-11.5% (results of Chapter Four) to a range of 11.7-12.5%. Albeit it within tolerance, higher moisture content of the seeds does influence germination. Abba and Lovato (1999) subjected maize seeds that were treated with a fungicide to accelerated ageing and storage at temperatures that ranged from 20-30ºC. Following AA moisture content of the seeds (both treated and untreated) increased from 10.5 to 17% (Abba and Lovato, 1999).

Following 3 months storage, results from this study showed that seeds treated with Apron® XL failed to germinate compared to the other seed treatments. Previous studies have confirmed that storage under stress conditions will result in decline in germination of low vigour seeds (Basu *et al*., 2004.). This is in agreement with Lugo and Leopold, (1992) and Simić *et al*. (2004). Lugo and Leopold (1992) showed that decline in maize seed vigour is closely related to the decline of content of several sugars in the embryo under accelerated conditions. In the study conducted by Simić and co-workers, the vigour test following accelerated ageing test has proven its potential for predicting seed storability.?

In a study conducted by Basu *et al*., (2004) physiologically mature maize seeds were subjected to accelerated ageing and natural ageing. Results showed that accelerated ageing was effective in predicating the influence of natural ageing over time on the maize seeds (Basu *et al*., 2004). This was seen in this current study in the case of seeds treated with Apron® XL. The higher decrease in germination following 4 d AA (standard germination and the cold test) was indicative of the extreme decrease in germination following 6 months storage. None of the other treatments and the untreated control showed that trend.

Following the harsh conditions that the seeds were stored under, the other seeds have deteriorated (visually) and failed to germinate. In contrast seeds treated with Thiram consistently proved (germination following imbibition, cold test and the leachate conductivity value) to have higher vigour than the other seed treatments. The function of Thiram functioned was to protecting the seed from loosing vigour and viability to a greater extent than the other treatments. Treated maize seeds are expected to withstand the cold test as the soil for the test is obtained from a maize field. However, as the storage of these seeds favour deterioration and proliferation of storage fungi (Qasem and Christensen, 1958), these seeds are already damaged before the onset of this test. In this study all the seeds were exposed to the same pathogen density. It is possible that as Thiram is a broad-spectrum contact fungicide (http://www.syngenta.com), it may have protected the seeds from damage by storage fungi and hence from pathogens found in the soil.

For 2d AA, the percentage germination after 40 hr following slow imbibition ranged from 53-58%. Interestingly seeds treated with thiram and the untreated control had the lowest

percentage germination. Following 3 month storage, seeds treated with thiram were the only seeds that germinated following both slow and rapid imbibition. The low germination results under the low water stress (slow imbibition) was explained by Perissé and co-workers (2002) as the threshold of water content required in the embryo as a requisite for the initiation of cell elongation and radicle emergence. The ageing process is accompanied by alterations in the mitochondrial activity of the cells, which in turn affects respiration and consequently germination (Dreyer and van de Venter, 1992).

The cold test is normally considered to be the best for predicting field emergence (Lovato and Balboni, 2003, Basu *et al*., 2004). Results from this current study showed that the cold test results corresponded to the standard germination results. Woltz *et al*. (1998) conducted standardization studies on the cold test for maize. The test was conducted in 20 laboratories where five seed lots were tested. Some of these seed samples tested consisted of Thiram treated maize and untreated seeds. Thiram was able to protect the seeds and was not affected by the range of species of soil-borne pathogens present (Woltz *et al*., 1998). Although the cold test is recommended for maize, difficulties in standardizing the test occur with differences in pathogen levels, pH, moisture etc (Nijënstein and Kruse, 2000). Suggestions from their study included conducting a standard germination test with the cold test (Nijënstein and Kruse, 2000). Results from this current study proved that that is a good comparison where cold test results linked well with the standard germination results.

In this study there was a distinct influence of AA and long-term storage on the viability and vigour of fungicide treated seeds. Wilson *et al*. (1992) showed that the accelerated ageing, leachate conductivity and other vigour tests were combined to develop a prediction of final stand in sweet corn (Wilson *et al*., 1992). Due to climatic stress high vigour lots do not necessarily give high yield (http://www.ag.ohio-state.edu) and storage for more than 4 months is not recommended according to findings of Abba and Lovato (1999). Seeds treated with Thiram, as in this study, maintained viability even after accelerated ageing and storage under sub-optimum condition. The conditions tested in this study were very harsh and even if subsistence farmers treated their seeds and stored them at sub-optimum conditions (which are less harsh than the conditions tested here), a small percentage of the seeds would still germinate and could be used for planting. The next step is to test these

seed treatments and the untreated control under greenhouse conditions. The vigour test results can then be compared to the emergence in the greenhouse. In addition, the seed treatments can be tested against a pathogen of maize under greenhouse conditions.

5.5 Literature Cited

Abba, E.J. and A. Lovato. 1999. Effect of seed storage temperature and relative humidity on maize (*Zea mays* L.) seed viability and vigour. *Seed Science and Technology*. 27: 101-114.

Appert, J. 1987. The Storage of food grains and seeds. Coste, R. (Ed). MacMillan Publishers, Hong Kong. Pages: 51-55.

Basu, S., Sharma, S.P. and M. Dadlani. 2004. Storability studies on maize (*Zea mays* L.) parental line seeds under natural and accelerated ageing conditions. *Seed Science and Technology*. 32: 239-245.

Copeland, L.O. and M.B. McDonald. (Eds.) 2001. Pages: 124-128 *In*: Principles of Seed Science and Technology (4th Edition). Kluwer Academic Publishers, Massachusetts.

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.

Harrington, J.F. 1958. Moisture proof packaging of seeds. *Seed World*. 83: 8-9

http://www.ag.ohio-state.edu~seedsci/svvt02.html. Seed vigour and vigour tests. Accessed: 15/05/2006.

http://www.syngenta.com/en/products_services/seed_treat.aspex. Accessed: 20/08/2005.

International Seed Testing Association. 2008. International Rules for Seed Testing. Seed Science and Technology.

Jensen, M. 2002. Seed vigour testing for predicting field seedling emergence in *Fagus sylvatica* L. *Drendrobiology*. 47: 37-54.

Kadlag, R.V., Chavan, J.K. and D.P. Kachare. 1995. Effects of seed treatments and storage on the changes in lipids of pearl millet. *Plant Foods and Human Nutrition*. 47: 279-285.

Lovato, A and N. Balboni. 2003. Seed vigour in maize (*Zea mays* L.): Two-year laboratory and field test compared. *Italian Journal of Agronomy*. 1: 1-6.

Lovato, A., Noli, E. and A.F.S. Lovato. 2005. The relationship between three cold test temperatures, accelerated ageing test and field emergence of maize seed. *Seed Science and Technology*. 33: 249-253.

Lugo, I.B and A.C. Leopold. 1992. Changes in soluble carbohydrates during seed storage. *Plant Physiology*. 98: 1207-1210.

Nijënstein, J.H and M. Kruse. 2000. The potential for standardisation in cold testing of maize (*Zea mays* L.). *Seed Science and Technology*. 28: 837-851.

Perissé, P., Aiazzi, M.T. and A.M. Planchuelo. 2002. Water uptake and germination of *Lupinus albus* L. and *Lupinus angustifolius* L under water stress. *Seed Science and Technology*. 30: 289-298.

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.

Rice, K.J. and A.R. Dyer. 2001. Seed aging, delayed germination and reduced competitive ability in *Bromus tectorum*. *Vegetatio*. 155: 237-243.

Simić, A., Sredojević, S., Todorović, M., Dukanović, L. and C. Radenović. 2004. Studies on the relationship between the content of total phenolics in exudates and germination ability of maize seed during accelerated aging. *Seed Science and Technology*. 32: 213-218.

Sowiak, V. 2004. Seed vigour – What is it? Agricultural, Food and Rural development. http://www.seedvigour.ualn.gov. Accessed: 09/02/2006.

Wang, X.F, Jing, X.M and J. Lin. 2005. Starch mobilization in ultradried seed of maize (*Zea mays* L.) during germination. *Journal of Integrative Plant Biology.* 47: 443-451.

Wilson, D.O., Jr., Alleyne, J.C., Shafii, B. and S. Krishna-Mohan. 1992. Combining vigour test results for prediction of final stand of shrunken-2 sweet corn seed. *Crop Science*. 32: 1496-1502.

Woltz, J.M., Tekrony, D.M., Egli, D.B. and P.Vincelli.1998. Corn cold test germination as influenced by soil moisture, temperature and pathogens. *Seed Technology*. 20: 56-70.

CHAPTER SIX

GREENHOUSE EMERGENCE OF UNTREATED AND TREATED MAIZE SEED AND CONTROL OF *FUSARIUM GRAMINEARUM* (SCHWABE)

Abstract

Seed treatments are important in protecting seeds from diseases and insects prior to and after planting and during storage. Apart from being the casual agent of seedling blight, *Fusarium graminearum* (Schwabe) is also a serious storage fungus, producing mycotoxins, which have deleterious health implications. The objective of the study was to investigate the effect of seed treatments on maize (*Zea mays* L.) seedling emergence and against *F. graminearum* under greenhouse conditions. Maize seeds were treated with Celest® XL [fludioxonil + mefenoxam], Apron® Star [thiamethoxam + metalaxyl–M + difenoconazole], Apron® XL [metalaxyl–M] and Thiram [thiram]. The control consisted of untreated seeds. Following treatment, seeds were subjected to 2 and 4 d accelerated ageing (AA) and 3 and 6 months storage. In the un-inoculated trial following 2 d AA, seeds treated with Thiram had the highest percentage emergence (70.7%) followed by Celest® XL (68%) and the untreated control (62.7%). Subjecting the treated seeds to stress conditions resulted in a decrease in emergence. Following the 6 months storage, only the control and seeds treated with Thiram germinated and had 1.3 and 6.7% emergence, respectively. Following inoculation, a similar trend was seen for seeds treated with Thiram and the untreated control. Seeds treated with Celest® XL had among the lowest percentage diseased seedlings (1, 2 and 10%) but failed to germinate at 6 months.

6.1. Introduction

Maize (*Zea mays* L.) is important as a source of energy and protein in the human diet throughout the world (Rehman, 2006). The loss of quality of maize seed is not only visually observed by the poor condition of the seed (Hell *et al*., 2000) but also by poor emergence (Cardwell *et al*., 2000).

One of the challenges facing the resource-poor smallholder farmers who produce the bulk of Nigeria's maize is how to preserve the quality of the grains in storage. Maize can be contaminated in the field and in the store where kernels are subject to infection by a variety of toxigenic fungi (Cardwell *et al*., 2000). The most common genera are *Aspergillus*, *Penicillium* and *Fusarium* that produce the aflatoxins, fumonisins and other mycotoxins that have important economic impact on the grain industry and risks to human and animal health (Bradley *et al*., 2001). Fungi of the genus *Fusarium* colonize various host plants, including crops that are essential for human nutrition such as maize and wheat (Klix *et al*., 2007). Within the *Fusarium* complex, *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* ([Schw.] Petch) has been reported to be the dominant species (Klix *et al*., 2007).

Fungicide seed dressings were found to reduce deterioration of seeds that are stored (Adebisi *et al*., 2004). Fungicide formulations that are not compatible with the seeds, could alter membrane function, altered membrane function can result in reduced seed and seedling performance (Chen and Burris, 1993). Fungicide seed treatments have been used with success. In a study conducted by Leishman *et al*. (2000), two climatic conditions were chosen (wet winter and summer) , treated and untreated *Medicago* seeds were buried in the test field in those conditions. Fungicide treated seeds remained viable longer in soil (Leishman *et al*., 2000). The active ingredients of the fungicides used in this study have previously been effective in improving germination. Difenoconazole has previously been shown to improve seed germination of wheat (*Triticum aestivum* L.) (Allen *et al*., 2004) and maize (Munkvold and O'Mara, 2002). Maize seeds treated with Apron® XL increases yield and vigour and therefore fewer seeds are destroyed (www.syngenta.com). With Thiram, improved emergence and high plant stand in the fungicide/insecticide mixture treatments compared to the untreated control could have resulted from control of seed rot and pre-emergence damping-off diseases reported earlier (Ahmed *et al*., 2001).

Fungicide seed treatments do not affect germination and plants develop as normal (Tort *et al*., 2006). This was confirmed in this study where the effect of fungicides were tested on unstored seeds. The objective of the current study was to investigate the effect of pesticide

seed treatments on maize (*Zea mays* L.) seed emergence and their effectiveness against *Fusarium graminearum* (Schwabe) under greenhouse conditions.

6.2. Materials and methods

6.2.1 Treatment of seeds

Untreated seed and the chemicals, Celest® XL [*fludioxonil (25 g ai/L) + mefenoxam (10 g ai/L*], Apron® Star [*thiamethoxam (20% w/w)+ metalaxyl–M (20% w/w) + difenoconazole (2% w/w)*], Apron® XL [*metalaxy–M (350 g ai/L*] and Thiram [*thiram (50.0% m/m)*] were supplied by Syngenta® Pty. Ltd (Midrand, South Africa). Seeds were treated as discussed in Chapter Four (4.2.1). After treatment, the seeds were left on paper towels in a laminar flow cabinet to dry. Once the seeds had dried, they were divided into three batches (1: immediate use; 2: 2 and 4 day accelerated ageing (AA) and 3: 3 and 6 months storage). This chapter focuses on the seeds that were aged and stored.

6.2.2 Greenhouse trial

6.2.2.1 Preparation of the pathogen

Fusarium graminearum (CAMS 1256) was obtained from the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. This fungus was initially isolated from a maize plant. The fungus was cultured on potato dextrose agar (PDA) and incubated at 25°C for seven days (12 hr day/night cycle).

6.2.2.2 Inoculation of the pasteurised soil

The seedling trays were cleaned using 2% sodium hypochlorite and left to dry a day before they were filled with pasteurised soil (Braaks, Pretoria). The filled trays were watered until run-off the day before inoculation. A cork borer (diameter of 5 mm) was used to remove mycelial plugs from the actively growing cultures. Two mycelial plugs were inoculated per cell of the seedling tray with the mycelial plugs being placed on opposite ends of a single cell. Inoculation was done prior to planting. Maize seeds were sown the next day in the space between the two plugs. Four replicates of 25 seeds were used per treatment. Each tray had three different treatments in a random block design. The temperature within the greenhouse ranged from 25-30ºC. The trays were monitored regularly and were watered daily. The trial was terminated three weeks after planting and the results were expressed as

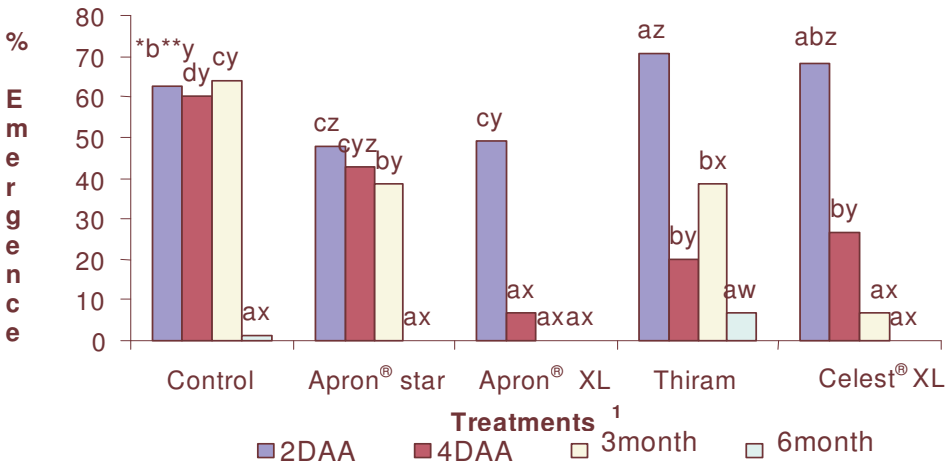
the percentage seedling emergence and percentage diseased seedlings at the end of the test period.

6.2.3 Statistical analysis

Two-way analysis of variance (ANOVA) was performed on all data and least significant differences (P= 0.05) were determined according to the student's t test.

6.4 Results

Comparing the 2 d AA, seeds treated with Thiram had the highest percentage emergence (70.7%) (Fig. 6.1). This was followed by seeds treated with Celest® XL (68%). The untreated control had the third highest emergence and did not differ significantly from Celest® XL. It did, however, differ significantly from the other treatments. For 4 d AA, seeds treated with Thiram had a 50% decrease in emergence followed by Celest® XL which had a 41.3% decrease in emergence (Figure 6.1).



[1]Each value is a mean percentage of four replicates of 25 seeds that have emerged in the greenhouse; Means above the bars not followed by the same letter are significantly different (*P = 0.05*). *Means are being compared amongst treatments. **Means are being compared within storage conditions (2 and 4d accelerated ageing or 3 and 6 months long-term storage).

Fig. 6.1: Emergence of un-inoculated untreated and treated maize seeds following accelerated ageing and long- term storage.

The untreated control had the highest (60%) emergence and differed significantly from the treatments. Apron® XL had the lowest emergence, already decreasing to 6.6% after 4 d AA. After 3 months storage, the control once again had the highest percentage emergence (64%). Seeds treated with Apron® Star and Thiram had a 38.7% emergence and did differ significantly from the other treatments and the control. Apron® XL failed to germinate (Fig. 6.1). After 6 months storage, with the exception of seeds treated with Thiram, the rest of the treatments failed to germinate and had 0% emergence. The control and seeds treated with Thiram had emergence of 1.3 and 6.7%, respectively (Fig. 6.1).

Table 6.1: Emergence of untreated and treated maize seeds following inoculation with *Fusarium graminearum*

	Emergence (%)[1]			
	2d AA	4d AA	3 month	6 month
Treatments				
Control	57.3 *bc**z	52.0 dz	44.0 dy	2.7 abx
Apron® Star	46.7 abz	34.7 cy	22.7 bx	0.0 aw
Apron® XL	40.0 ay	5.3 ax	0.0 ax	0.0 ax
Celest® XL	64.0 cz	28.0 bcy	4.0 ax	0.0 ax
Thiram	58.7 bcz	17.3 bx	33.3 cy	4.0 bw

[1]Each value is a mean percentage of four replicates of 25 seeds that have emerged in the greenhouse; *Means within a COLUMN not followed by the same letter are significantly different ($P = 0.05$); **Means within a ROW not followed by the same letter are significantly different ($P = 0.05$)

Emergence following inoculation with *.F. graminearum* showed a difference in the results. After 2 d AA, seeds treated with Celest® XL had the highest percentage emergence (64%) (Table 6.1). Seeds treated with Thiram and the control had the second and third highest percentage emergence (58.7 and 57.3%, respectively) and did not differ significantly from seeds treated with Celest® XL. Following 4 d AA, control had the highest percentage emergence and differed from all the treatments. Seeds treated with Thiram once again, had a decrease in the percentage emergence of 41.4%, although Apron® XL dropped to 5.3% emergence (Table 6.1). After 3 and 6 months, a similar trend was seen, with the control having the highest percentage emergence (44%) and seeds treated with Apron® XL failing

to germinate after 3 months. Only the control and seeds treated with Thiram had germinated after 6 months storage (Table 6.1).

In the inoculated trial following 2 d AA, the untreated control had the highest percentage diseased seedlings (17%) (Table 6.2) and there was no significant differences between the treatments. Following the 4 d AA, the control once again had the highest percentage diseased seedlings (11%). Both seeds treated with Apron® XL and Celest® XL had lower percentage diseased plants (2.0%) and only differed significantly from the control (Table 6.2). After 3 months storage the untreated control had the highest percentage diseased seedlings (10.0%), followed by Apron® Star (6.0%). Seeds treated with Celest® XL had the lowest percentage diseased seedlings (1.0%). After 3 months storage, seeds treated with Apron® XL failed to emerge. Following 6 months storage only the control and seeds treated with Thiram had seedlings that emerged. The control had 1.0% diseased seedlings, with Thiram having no diseased seedlings (0.0%).

Table 6.2: Percentage of diseased seedlings, following inoculation with *Fusarium graminearum*

	Diseased seedlings (%)[1]			
	2d AA	4d AA	3 month[2]	6 month[2]
Treatments				
Control	17.0 *by	11.0 by	10.0 by	1.0ax
Apron® Star	10.0 abx	7.0 abx	6.0 abx	-
Apron® XL	9.0 ay	2.0 ax	-	-
Celest® XL	10.0 aby	2.0 ax	1.0 ax	-
Thiram	10.0 abx	6.0 abx	4.0 abx	0.0 a

[1]Each value is a mean percentage of four replicates of 25 seeds that have emerged in the greenhouse; [2] - = no emergence. *Means within a COLUMN not followed by the same letter are significantly different (*P = 0.05*); **Means within a ROW not followed by the same letter are significantly different (*P = 0.05*).

6.5 Discussion

In this study the trend in emergence followed a similar pattern as was noted with germination in the previous study (Chapter Five). Seeds that were treated and processed immediately (Chapter Four), showed no significant differences from the control. Following accelerated ageing and storage, a decline in emergence was seen. This was in contradiction to what Adebisi *et al.* (2004) found in their study where fungicide treated soybean seeds had a significantly longer storage life than untreated seeds. The results obtained for the 4 d AA, 3 and 6 months storage could be as a consequence of the way in which the seeds were treated.

Although the control had no protection by any fungicide following inoculation with *F. graminearum,* it had the highest percentage emergence after 2 and 4 d AA and 3 months storage. After 6 months storage, seeds treated with Thiram had the highest percentage emergence. The results for the untreated control can be explained by the vigour test of the previous chapter. Following imbibition, the untreated control and the seeds treated with Thiram had a lower percentage weight increase, and thus less imbibition damage, compared to the other treatments and were able to emerge despite the stress conditions the seeds were subjected to.

Emergence of seeds treated with Apron® XL decreased following ageing, but already after 3 months storage there was no emergence. These results mirrored those of the germination tests in the previous chapter (Chapter Five). Apron® XL is known to increase yield and vigour (www.syngenta.com), which was evident in the samples that were processed immediately. Results for the inoculated trial for the aged and stored seeds showed a similar trend as the un-inoculated seed where following 3 and 6 months storage Apron® XL failed to germinate.

Thiram has proved to be an effective fungicide providing protection to vegetable crops (Maude, 1977) and was the most important alternative to captan as a seed treatment fungicide for maize (Kommedahl and Windels, 1986). In this study Thiram treated seed still germinated and emerged even after 6 months storage in comparison to the other treatments with the exception of the untreated control. The thiram treatment alo had a

lower level of disease than the inoculated untreated control after being subjected to the harsh 6 month storage period. something about controlling storage fungi. This reiterated the protective nature of Thiram (Maude, 1977, Falloon, 1982). Some fungicides are able to improve germination, not emergence (Gilbert *et al.*, 1997). In this current study Thiram improved emergence and protected the seed in the inoculated trial.

With seeds treated with Celest® XL there was an obvious decrease in emergence following ageing and storage. A similar trend was noted in the inoculated trial. The trend in the control of *F. graminearum* found in this study with Celest® XL confirmed results obtained from other studies. In a study by Broders *et al*. (2007) seed treatment fungicides azoxystrobin, trifloxystrobin, fludioxonil and captan were tested for their effectiveness against *F. graminearum* on soybean seeds and seedlings. Of the fungicides tested, it was only fludioxonil that provided sufficient inhibition of mycelial growth *in vitro* (Broders *et al*., 2007). A similar result was found against *F. graminearum* on maize (Munkvold and O' Mara, 2002).In another study, fludioxonil was found to reduce certain parameters associated with the disease in barley, including incidence, severity, and deoxynivalenol concentration, while increasing the percentage of plump kernels and yield (Jones, 2000).

In terms of disease control, fungicides that are registered for use on maize differ in their effectiveness against certain diseases, depending on their active ingredients and will protect the seeds under field conditions and allow for emergence of the seedlings (Van Dyk, 2000). In terms of fungicide performance, Thiram, a broad-spectrum fungicide, effectively controlled *F. graminearum* whilst insuring some emergence. In the greenhouse trials, Thiram was found to be the best treatment in this study. The results for the vigour tests of the previous chapter have been substantiated by the results from this chapter. .

6.6 Literature cited

Adebisi, M.A., Daniel, I.O and M. O. Ajala. 2004. Storage life of soybean (*Glycine max* L. Merril) seeds after seed dressing. *Journal of Tropical Agriculture.* 42: 3-7.

Allen, T.W., Enebak, S.A. and W.A. Carey. 2004. Evaluation of fungicides for control of species of *Fusarium* on longleaf pine seed. *Crop Protection*. 23: 979-982.

Ahmed, N.E, Kanan, H.O., Inanaga, S., Ma, Y.Q. and Y. Sugimoto. 2001. Impact of pesticide seed treatments on aphid control and yield of wheat in the Sudan. *Crop Protection*. 20: 929-934.

Anaso, A.B., Tyagi, P.D., Emechebe, A.M. and S.K. Manzo. 1989. Control of sorghum downy mildew (*Peronosclerospora sorghi*) of maize by seed treatment in Nigeria. *Crop Protection*. 8: 82-85.

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

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.

Cardwell, K.F., King, J.G., Maziya-Dixon, B and N.A. Bosque-Perez. 2000. Interactions between *Fusarium verticillioides*, *Aspergillus flavus*, and insect infestation in four maize genotypes in Lowland Africa. *Phytopathology*. 90: 276–284.

Chen, Y. and J.S. Burris. 1993. Effect of seed treatment emulsifiers on seed quality and membrane function in maize. *Postharvest Biology and Technology*. 2: 231-239.

Falloon, R.E. 1982. Fungicide seed treatment of maize to improve establishment and control seedling pathogens. *New Zealand Journal of Experimental Agriculture*. 10:197-202.

Gilbert, J., Tekauz, A. and S.M. Woods.1997. Effect of storage on viability of Fusarium Head blight-affected spring wheat seed. *Plant Disease*. 81: 159-162.

Hell, K., Cardwell, K.F., Setamou, M. and H.M. Poehling. 2000. The influence of storage practices on aflatoxin contamination in maize in four agro ecological zones of Benin, West Africa. *Journal of Stored Products Research.* 36: 365-382.

http://www.syngenta.com/en/products_services/seed_treat.aspex. Accessed: 20/08/2005.

Jones, R.K. 2000. Assessments of Fusarium head blight of wheat and barley in response to fungicide treatment. *Plant Disease*. 84: 1021-1030.

Klix, M.B., Verreet, J.A and M. Beyer. 2007. Comparison of the declining triazole sensitivity of *Gibberella zeae* and increased sensitivity achieved by advances in triazole fungicide development. *Crop Protection*. 26: 683-690.

Marchi, J.L. and S.M. Cicero. 2003. Influence of chemical treatment of maize seeds with different levels of mechanical damage on electrical conductivity values. *Seed Science and Technology*. 31: 481-486.

Maude, R.B. 1977. Results in practice – iii. Vegetable crops. Pages: 259-260. *In*: Marsh, R.W, Byrde, R.J.W. and D. Woodcock (Eds.). Systemic fungicides. Longman Group Limited, London.

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.

Rehman, Z. 2006. Storage effects on nutritional quality of commonly consumed cereals. *Food Chemistry*. 95: 53-57.

Tort, N., Dereboylu, A.E. and B. Turkyilmaz. 2006. Morphological and physiological effects of a fungicide with a Thiram agent on some corn culture forms. *Journal of the Faculty of Science*. 29: 67-79.

Van Dyk, J. 2000. Controlling leaf diseases in seed corn in 2000. http://www.ipm.iastate.edu/ipm/icm.html. Accessed: 24/02/2006.

Zhang, T. and J.G. Hampton. 1999. Does fungicide seed treatment affect bulk conductivity test results? *Seed Science and Technology*. 27: 1041-1045.

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.

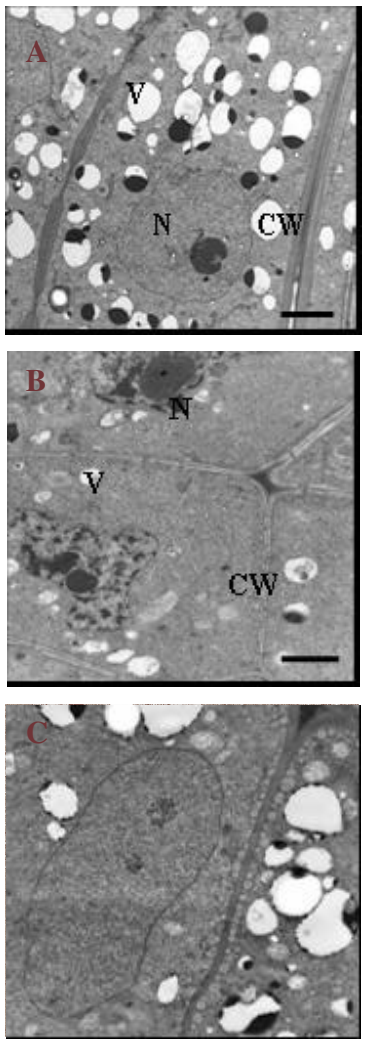


Figure 7.1: TEM micrographs of embryonic tissue of untreated and treated maize seeds following 2 d accelerated ageing and 48 hr rapid imbibition, (Bar = 1 µm) a) Untreated control, b) Apron® XL and c) Celest® XL (CW = cell wall, N = nucleus, V = vacuole).

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).

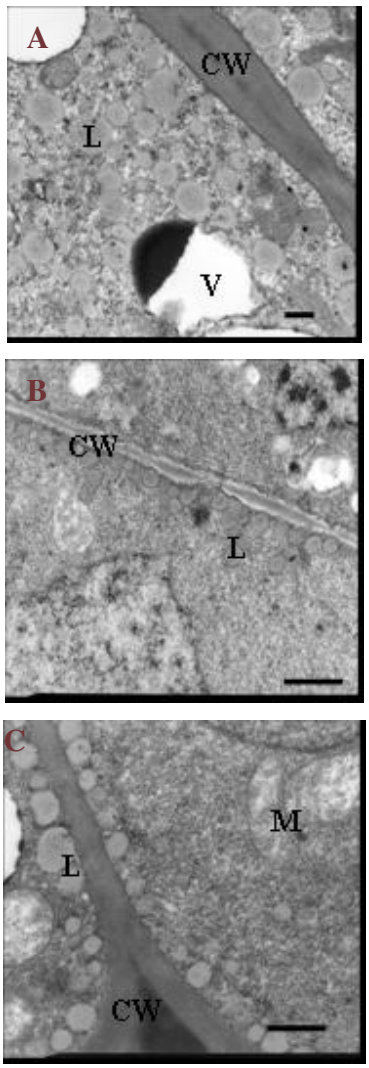


Figure 7.2: TEM micrographs of embryonic tissue of untreated and treated maize seeds following 2 d accelerated ageing and 48 hr rapid imbibition showing differences in the lipid body arrangement, (Bar = 1 µm) a) Untreated control, b) Apron® XL and c) Celest® XL (CW = cell wall, L = lipid, V = vacuole,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

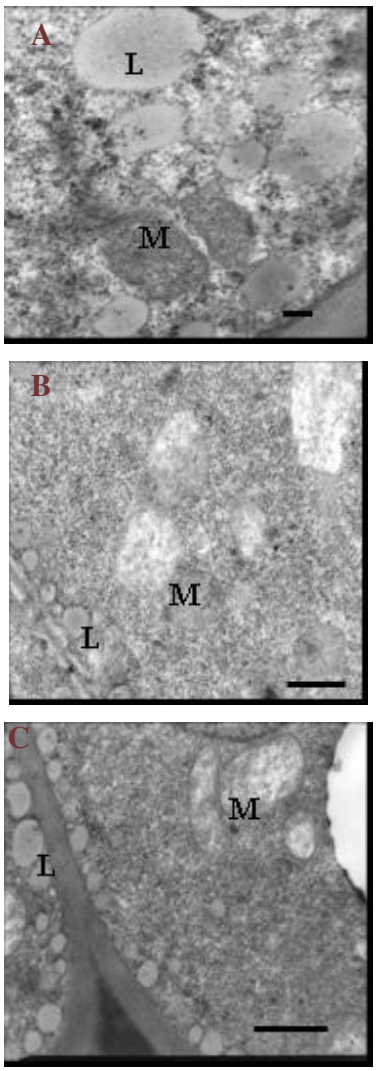


Figure 7.3: TEM micrographs of embryonic tissue of untreated and treated maize seeds following 2d accelerated ageing and 48 hr rapid imbibition showing differences in the mitochondrial structure, (Bar = 1 µm) a) Untreated control, b) Apron® XL and c) Celest® XL. (L = lipid, M = mitochondrium).

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.