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.
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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.

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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.

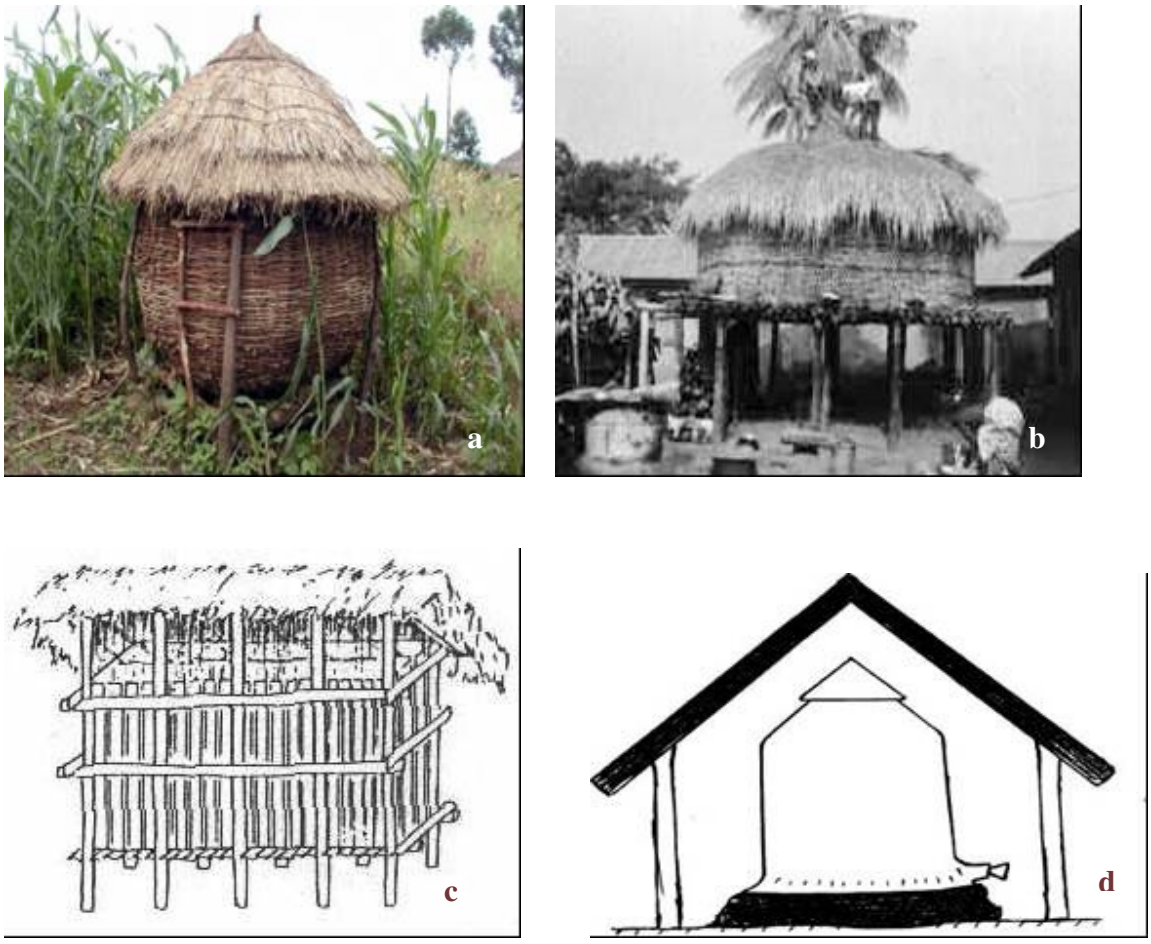

Figure 2.1: Storage structures used in South Africa, a woven wall granary (a), a high granary that is designed to keep moisture out (b), a rectangular crib, with woven roof (c) and a ferrumbu (d). (Pictures obtained from http://www.solutions-site.org and http://www.fao.org)

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.

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CHAPTER THREE

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

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

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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.
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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).

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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

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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

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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.

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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.

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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.

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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.

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