

**Effect of fungicide seed treatments on germination and vigour of  
maize seed**

**By**

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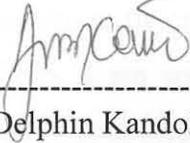
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I hereby declare that this dissertation herewith submitted for the degree of M. Inst. Agrar (Plant Protection) at the University of Pretoria is the result of my own work and has never been previously submitted by me for a degree at any other University or institution of higher education.



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## SUMMARY

Fungicides have been developed to protect plants against diseases and pests, which cause serious problems such as the loss of germination and vigour. The aim of this study was to test the germination and vigour of maize (*Zea mays* L.) seeds treated with several fungicides Apron<sup>®</sup> Star 42 WS (difenoconazole, thiamethoxam, and metalaxyl-m), Apron<sup>®</sup> XL (mefenoxam), Celest<sup>®</sup> XL (fludioxonil, mefenoxam) and thiram in the laboratory. In the greenhouse, the efficacy of fungicide treatment was evaluated in soil inoculated with *Fusarium graminearum*. The control consisted of untreated seeds. Germination and vigour were evaluated according to the International Seed Testing Association (ISTA) rules. The results from the standard germination tests showed that all the fungicide treated seeds did not differ to the untreated control. The conductivity of solute leakage was read following slow and fast imbibition. Maize seeds treated with Apron<sup>®</sup> Star 42 WS, Celest<sup>®</sup> XL, Apron<sup>®</sup> XL and thiram improved or maintain vigour, which was indicated by a reduced or equivalent solute leakage following fast imbibition when compared with the untreated control. The good performance of fungicide treated seed expressed during conductivity test after fast imbibition correlated with the tetrazolium. All the fungicide treated seeds maintained the same viability as the untreated control following fast imbibition. After 6 h after fast imbibition, Apron<sup>®</sup> Star 42 WS, Celest<sup>®</sup> XL and Apron<sup>®</sup> XL treated seeds maintained similar germination percentages when compared to the untreated control with the exception of thiram treated seeds that exhibited a decline in seed viability. There was reduction in vigour in all the fungicide treated seeds following 24 and 40 h fast imbibition as illustrated by the reduction in germination percentage below the acceptable level (70%) when compared with the untreated control. The greenhouse study showed that all the fungicide treated seeds maintained the same emergence percentage in both inoculated and uninoculated soil with the exception of thiram treated seeds, where emergence improved in inoculated soil when compared to the untreated control. Apron<sup>®</sup> Star 42 WS and Celest<sup>®</sup> XL reduced the disease caused by *F. graminearum* in the inoculated soil. This study also revealed that the application of Apron<sup>®</sup> Star 42 WS, Celest<sup>®</sup> XL and thiram to seeds improved both the shoot and root dry mass of plants in the inoculated soil.

## CHAPTER 1

### GENERAL INTRODUCTION

#### 1.1 Motivation of the study

*Zea mays* (L.), commonly referred to as maize, belongs to the Poaceae family (Inglett, 1970; Farnham *et al.*, 2003). It is thought to have originated in Mexico (Farnham *et al.*, 2003) but other evidence suggests that it may have originated from Africa or Asia (Inglett, 1970). The plant is referred to as Indian corn in the United States of America (Inglett, 1970; Farnham *et al.*, 2003). It is cultivated in a wide range of climatic conditions ranging from warm temperate areas in the humid sub-tropical regions as well as in the tropics (Berger, 1962).

Messing and Dooner (2006) revealed that until the past decade, maize was the most studied plant species having considerable economic value and served as the main staple food for millions of people in Africa and the Americas. Of the major grain crops, maize has the largest total annual grain production in the world (590.5 million metric tons (mmt) followed by wheat (*Triticum aestivum* L.) (567.7 mmt) and rice (*Oryza sativa* L.) (380.3 mmt) and its average yield per hectare (4.3t) represents more than that of either wheat or rice (Fapri, 2003). According to White (1999), 40% of the world's production is attributed to the United States of America. The other major producers are the Republic of China, the European Union, Mexico, Argentina, India, Romania and South Africa. The plant is cultivated for the production of grain and fodder, which constitute the basis for foods, feed, pharmaceutical and industrial products (Randhir & Shetty, 2005).

According to Finch-Savage (1995), for all agricultural crop species the quality of seed has a great impact on the economic production. In maize high productivity requires the establishment of an adequate plant population, which in turn is dependent on the quality (germination and vigour) of seeds, sowing depth and environmental factors such as temperature, water and oxygen levels, presence of pathogenic microorganisms and soil structure (Finch-Savage, 1995).

Shurtleff (1980) reported that maize seed is vulnerable to a wide range of diseases that reduce yield and quality (vigour and germination). Consequently, numerous fungicides are produced into the market in order to control the causal pathogens. Taylor and Harman (1990) revealed that fungicide seed treatment is considered as adequate means to improve the quality of the seed considerably and to increase plant growth and productivity. In addition, fungicides can also mitigate the internal and external seed or soilborne pathogens (Scot, 1989). Balie and Elward (1980) reported that besides helping to improve the physical properties of the seed, the chemical treatment of the seeds can reduce stress conditions and act as an efficient carrier of nutrients, fungicides and insecticides.

According to Knight *et al.* (1997), there are numerous effective fungicides on the market but the need for new chemicals that will improve yield and quality is of importance. For example Apron<sup>®</sup> Star 42 WS and Apron<sup>®</sup> XL are new products of Syngenta, Switzerland. Since Brocklehurst *et al.* (1987) reported that the application of fungicides to seed resulted in increased yield and vigour there is a need to evaluate the efficacy of these new chemicals on germination and vigour of maize seed. In addition, maize is susceptible to a wide range of pathogens, one of them being *Fusarium graminearum* Schw. (Shurtleff, 1980) and the effectiveness of these chemicals against this pathogen needs to be tested.

## **1.2 Aim and objectives of the study**

The aim of this study was to evaluate the effect of four fungicides, namely Apron<sup>®</sup> Star 42 WS, Apron<sup>®</sup> XL, Celest<sup>®</sup> XL and thiram, on the germination and vigour of maize seeds. The effect of these fungicides on emergence and their efficacy against *F. graminearum* in the greenhouse was also evaluated.



### 1.2.1 Objectives

The specific objectives of this study were to:

- Examine the influence of Apron<sup>®</sup> Star 42 WS, Apron<sup>®</sup> XL, Celest<sup>®</sup> XL and thiram on the germination and vigour of maize seeds *in vitro*.
- Test the efficacy of the above fungicides against *F. graminearum in vivo* by evaluating different parameters such as emergence, diseased plants and dry mass of shoots and roots.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Introduction

Although widely distributed, the botanical origin of maize has been a source of controversy (Farnham *et al.*, 2003). Various hypotheses have been formulated over the years trying to indicate the botanical origin of the crop. Of the different evidences the most reliable indicates that teosinte (*Zea mays* L. spp. *mexicana*) is probably the wild ancestor of maize (Galinat, 1977).

The crop was introduced to Africa from South America in the 16<sup>th</sup> century (FAO, 1996). The exact limit of climatic conditions for the growth of maize cannot be defined since it is cultivated in a wide range of environmental conditions, but preferably in warmer regions. It is grown in tropical, subtropical and temperate regions, where the bulk of maize is produced between 30°C and 55°C (Shaw, 1988).

The cereal serves as the basis of the diet of millions of African people due its high yield per hectare, its easy cultivation and adaptability to various zones, versatile food uses and storage characteristics (Asiedu, 1989). Since the life of populations in the rural areas is dependent mainly on basic grains such as maize, the increase of the availability of this commodity becomes a real necessity (Moreno-Martinez *et al.*, 1998).

Maize is subject to a wide range of pathogens, which include viruses, bacteria, nematodes, fungi, mycoplasmas and parasitic seed plants. Of all these organisms, fungi are the main cause of the majority of diseases on maize (Shurtleff, 1980). According to Agrios (2005), the use of chemicals is considered as one of the best options for managing these diseases in the field, in the greenhouse and in storage. However, there is a need for continuous search for new chemicals, which can provide good control of the pathogens and lead to the improvement of yield and quality.

## 2.2 The pathogen, *Fusarium graminearum*

The study of the hyphomycetes genus *Fusarium* has been given more attention than any other group of fungi. The Fusaria are a diverse, cosmopolitan group, which cause plant diseases, storage rots and human and animal toxicoses and mycoses (Booth, 1971)

*Fusarium graminearum* (teleomorph *Gibberella zae* [Schw.] Petch.) attacks a wide range of crops including maize, rice and wheat. Various diseases are caused by this pathogen among which, are ear blight, also known as scab or head blight, stem and root infections of wheat (Parry *et al.*, 1995), ear blight of rice (Ou, 1985) and ear and stalk rot of maize. The other parasitic strains are referred to as canker-causing organisms of many hardwood trees (Nelson *et al.*, 1981).

According to Joffe (1986) the infection by *F. graminearum* results in yield losses and the contamination of grain with trichothecene and estrogenic mycotoxins (zearalenone), making it unsuitable for human and animal consumption. In addition, Salas *et al.* (1997), quoted by O'Donnell (2000), reported a significant reduction in seed quality caused by discoloured and shrivelled kernels.

### 2.2.1 Origin, taxonomy and characteristics

According to Fry (2004), *Fusarium* belongs to the Kingdom: Fungi, Order: Hypocreales, Family: Hypocreaceae and the Genus: *Fusarium*.

Fusaria are found in soil, on subterranean and aerial plant parts, and debris. It was reported that the population of *Fusarium* spp. in agricultural fields exceed 100,000 propagules per gram of soil or more (Nelson *et al.*, 1981). Those authors revealed that humans also play a major role in the dissemination of *Fusarium* pathogens through the distribution of infected or infested seeds or other plant materials. The fungi prefer tropical and temperate regions and exist as common soilborne fungi, which include both saprophytes and parasites (McGovern *et al.*, 2001).

All *Fusarium* species have a unique taxonomic feature in common, that is the production of distinctly foot shaped macroconidia (Nelson *et al.*, 1981). During its asexual reproduction, *F. graminearum* produces filamentous hyphae, having multiseptate, fusiform macroconidia. The homothallic sexual phase, *G. zeae*, has a fruiting body containing asci where ascospores develop. Cook (1981) revealed that both ascospores and macroconidia have infectious capacity.

### **2.2.2 Ecology, epidemiology and environmental conditions**

*Fusarium* species can be found in noncultivated land with *F. oxysporum* Schlecht emend Snyder, Hans., *F. solani* (Mart.) Sacc. (emend Snyder, Hans.) and *F. roseum* (Lk.) emend Snyder and Hans., being the most widespread and predominant species. All species are closely associated with roots and organic matter such as debris and occur in all climates (Nelson *et al.*, 1981).

*Fusarium* species are predominant in the upper layer of the soil (Nelson *et al.*, 1981). This zone represents the part most affected by agricultural practices such as tillage, fertilization, liming, herbicide application and irrigation (Rupe *et al.*, 1999). According to Toussoun and Nelson (1976), a high microbial activity occurs in the upper 15 cm zone.

The distribution and predominance of this pathogen depends essentially on climatic conditions, particularly temperature and moisture. According to Xu (2003), the warmer conditions favour *F. graminearum*. The fungus persists and multiplies on infected crop residues of small grains and reaches its highest potential during continued moist weather (Ramirez & Chulze, 2004). When spores land on maize silks during this unfavourable condition, mycelia grow down the silk channel toward the developing seeds, which become infected (Reid *et al.*, 1992).

### 2.2.3 Symptoms

*Fusarium graminearum* may cause diseases such as *Gibberella* stalk rot, *Gibberella* ear rot, Red rot and Pink rot (McGee, 1988) and *Fusarium* head blight. The pathogen also causes root rot and seedling blight. On early-infected plants, the leaves suddenly turn a dull greyish-green while the basal internodes soften and become tan to dark brown (Shurtleff, 1980). Black perithecia may occur near the lower nodes.

The characteristic of *F. graminearum* is a pinkish to reddish coloured mould on the kernels. Colonisation can occur over eight weeks or more in the field and growth may continue in storage under certain conditions (Harris, 1999). The fungus is one of the most devastating pathogens that cause maize stalk rots. A red mould can be noticed at the tip and spreads to the entire ear. Black perithecia can develop on the seeds and husk (McGee, 1988).

### 2.2.4 Control

The pathogen can effectively be controlled by general fumigation of the soil and the greenhouse (Cerkauskas, 2001). Chemical seed treatment can give a good control of *Fusarium* since the fungus can be found on diseased seedlings (Koenning, 2004). In addition, treating seeds with chemicals has also proven to be excellent in controlling decay and other diseases caused by seedborne pathogens (Loria, 1993). Haidukowski *et al.* (2004) reported that treatments with cyproconazole plus prochloraz, and a mixture of tebuconazole plus azoxystrobin significantly decreased *Fusarium* head blight caused by an artificial inoculation of *F. graminearum* and *F. culmorum* (W.G.Sm.) Sacc. According to Menniti *et al.* (2003), the chemicals prochloraz, tebuconazole, epoxiconazole or bromuconazole applied at the recommended rate provided control of the fungus when disease pressure in the field was low to medium. Essential oils such as oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) have been used for the inhibition of *Fusarium* spp. (Daferera *et al.*, 2002).

The pathogen can also be controlled biologically. The results found by Khan *et al.* (1999) suggests that the bacteria AS 43.4 (*Bacillus* sp.) isolated from wheat anthers

reduced the disease severity of *Fusarium* under glasshouse conditions by 67-95%. Bujold *et al.* (2001) reported in their studies on wheat and maize residues (straw/stalk and grain) that the inoculation of the residues with a *Microsphaeropsis* sp. isolate (P 130 A) reduced *G. zaeae* ascospores by 73%.

Other practices can contribute to a certain extent to the development or reduction of the pathogen. The type and application rate of fertilizers, especially nitrogen (N) can affect disease incidence and severity. For example an increase in nitrogen application from 70 to 170 kg N ha<sup>-1</sup> increased the incidence of the pathogen (Martin *et al.*, 1991). However, Reid *et al.* (2001) revealed that the severity of the fungus decreased with 100 Kg N ha<sup>-1</sup> fertilizer. The use of transgenic maize hybrid plants can consistently decrease *F. graminearum* infection. Munkvold *et al.* (1997) found that maize hybrids with cryIA (b) genes that are expressed in kernels reduced *Fusarium* ear rot and *Fusarium* infection of the kernels, when compared to their nontransgenic counterparts.

### **2.3 Fungicide seed treatment**

Seed treatment refers to biological, chemical, mechanical or physical substances applied to seeds or seedlings (www.syngenta.com, 2006) for the management of internal or external seed or soilborne pathogens (Scot, 1989). Among these are *Fusarium*, *Pythium* and *Rhizoctonia* that cause seed rot, pre- and post- emergence root and stem diseases of young seedlings resulting in poor establishment (Hagedorn, 1984).

Seed companies routinely treat maize seeds with fungicides (Marchi & Cicero, 2003) to protect them against fungal infection and to improve their emergence (Munkvold & O'Mara, 2002). According to Taylor and Harman (1990) this application has become a common practice that improves the value of seed and its productivity. Similarly coating with chemicals such as Apron<sup>®</sup> XL (mefenoxam), Apron<sup>®</sup> Star 42 WS (difenoconazole, metalaxyl-m, and thiamethoxam), Celest<sup>®</sup> XL (mefenoxam and fludioxonil), and thiram can protect seeds and assure optimum emergence of the crop (www.syngenta.com, 2006). Nuninger *et al.* (1996) reported that metalaxyl and

mefenoxam are both anilide fungicides. Metalaxyl is marketed under various formulations and trade names including Ridomyl, Fongamil Neu, and Apron. According to Falloon *et al.* (2000), the Apron compound includes Apron<sup>®</sup> 35 SD (35% metalaxyl), Apron<sup>®</sup> 45 (45% metalaxyl), Apron<sup>®</sup> C and Apron<sup>®</sup> 70 SD (both 35% metalaxyl + 35% captan), Apron<sup>®</sup> TZ (45% metalaxyl + 24% thiabendazole), and Apron<sup>®</sup> XL (35% metalaxyl).

Apron was developed and introduced because of the growing demand (Knight *et al.*, 1997) for crop protection agents having a reduced rate and a safe environmental profile (Monkiedje & Spiteller, 2002; [www.syngenta.com](http://www.syngenta.com), 2006).

### **2.3.1 Apron<sup>®</sup> XL**

Mefenoxam, the active ingredient of Apron<sup>®</sup> XL, was introduced in 1996. It is sold under various formulations and the trade names include Ridomil Gold, Apron<sup>®</sup> XL, Subdue, and Maxx (Nuninger *et al.*, 1996). According to the International Standard Organization (ISO), the chemical is referred to as metalaxyl-m. In France mefenoxam has replaced metalaxyl since 2003. The manufacturer Syngenta has changed metalaxyl to mefenoxam in all its formulations since it provides the same level of efficacy but at half the application rate (Monkiedje *et al.*, 2002; Benigni & Bompeix, 2006). Mefenoxam is classified as a reduced risk agent due to its acceptable environmental profile and low use rate ([www.syngenta.com](http://www.syngenta.com), 2006).

Maize seeds treated with Apron<sup>®</sup> XL have increased yield and vigour and accordingly fewer seeds are destroyed by pathogens ([www.syngenta.com](http://www.syngenta.com), 2006). Luz (2003) revealed that the mixture of mefenoxam (metalaxyl-m), fludioxonil and a biological agent significantly increased the germination and grain yield of maize. 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 in the presence of the compound.

Mefenoxam, also called R-metalaxyl, can provide control of plant diseases. Tsrer *et al.* (2005) reported that Ridomyl Gold (mefenoxam) reduced the disease incidence of root rot and wilt on kangaroo paw (*Anigozanthos* spp.). According to Wada (2003), the combination of metalaxyl with carboxin and furathocarb at 9.9 g a.i. gave excellent control of smut (*Ustilago scitaminea* Syd.). Brantner and Windels (1998) revealed that maize seeds treated with mefenoxam combined with metalaxyl at the standard rate or half rate, sown in soil infested with *Pythium* resulted in a significant reduction of disease.

### **2.3.2 Apron<sup>®</sup> Star 42 WS**

Apron<sup>®</sup> Star 42 WS is a new fungicide-insecticide that combines three active ingredients, namely thiamethoxam, metalaxyl-m (mefenoxam) and difenoconazole (www.syngenta.com, 2006). The trade names include Cruiser and Actara (Horii *et al.*, 2007).

#### **2.3.2.1 Thiamethoxam**

The active ingredient thiamethoxam is a new chemical having a wide spectrum insecticidal effect (Maienfisch *et al.*, 2001; www.syngenta.com, 2006). It belongs to the neonicotinoid group and can be applied to soil, leaves or directly to seeds. Its systemic properties allow it to be distributed in the young and growing plant (Elbert & Nauen, 2000).

Seedlings treated with thiamethoxam acquire good vigour, which result in rapid, healthy stand establishment, robust early season plant growth, early flowering and high yield (Doyle *et al.*, 2001). Horii *et al.* (2007) revealed that maize, pea (*Pisum sativum* L.) and soybean (*Glycine max* (L.) Merr.) treated with the combination of thiamethoxam and fish protein hydrolysates slightly improved vigour of seedlings during germination. According to Wu *et al.* (2006), the use of thiamethoxam as a seed treatment on *Brassica oleracea* var. *botrytis* did not significantly affect seed germination.

Thiamethoxam can be used also for the management of pests and diseases. When testing thiamethoxam and imidacloprid on maize seeds, Pataky *et al.* (2000) concluded that the insecticides controlled Stewart's wilt during the very early growth of maize. Mullin *et al.* (2005) reported that maize seeds treated with neonicotinoid insecticides (thiamethoxam, imidacloprid or clothianidin) resulted in direct mortality of *Carabidae* species.

### ***2.3.2.2 Difenconazole***

Difenconazole 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). It belongs to a triazole group; their common characteristic refers to the presence of the 1,2,4-triazole ring connected to the hydrophobic backbone through position 1 (Wu *et al.*, 2001).

According to Ward *et al.* (1997), the treatment of maize seed with difenconazole fungicide gave the highest grain yield. The authors attributed this fact to the growth regulating properties that is characteristic of triazole fungicides. Gopinath *et al.* (2006) revealed that the application of difenconazole on chilli (*Capsicum annum* L.) plants improved the quality of fruit and increased fruit yield up to 63%. Munkvold and O'Mara (2002) reported that the application of difenconazole to maize seed significantly improved emergence. In addition, when compared to the untreated control, the fungicide improved dry weight of shoots and roots.

Difenconazole can be used for the management of plant diseases. Cook *et al.* (2002) revealed that wheat inoculated with *Pythium* spp, *Gaeumannomyces graminis* (Sacc.) Arx & Olivier var. *tritici* Walker, *Rhizoctonia solani* Kühn AG 8, and *R. oryzae* Ryker & Gooch and treated with the combination of difenconazole, rhizobacteria and mefenoxam gave a significantly high yield. Milus and Chalkley (1997) found that the combination of crop rotation, tillage and seed treatment with difenconazole is important for the reduction of losses due to *Stagonospora* blotch of wheat caused by *Stagonospora nodurum* (Berk.). Jones (1999) reported that difenconazole was less effective for the control of *F. graminearum* group 2.

### 2.3.2.3 *Metalaxyl-m or mefenoxam*

Nuninger *et al.* (1996) pointed out that the systemic fungicide metalaxyl was introduced in 1977 for the control of Oomycete fungi. Ridomyl-25ws was the first form of this fungicide whereas metalaxyl-m is the latest form of this chemical.

### 2.3.3 Thiram

The commercial names of this fungicide include Arasan, Aules, Falitiram and Fernacole (Sharma *et al.*, 2005). Thiram is an organic sulphur fungicide, classified under the dithiocarbamates. It is an excellent protectant compound registered for a large number of important crops (Agrios, 2005). Thiram is sold as dust, flowable, wettable powder, water dispersible granules, and water suspension formulations and in mixtures with other fungicides (Sharma *et al.*, 2005).

The fungicide helps to improve the quality of many crops. Southwell *et al.* (2003) found that the combination of thiram and carboxim gave the highest percentage germination and emergence of maize seed. During his study, Xue (2003) revealed that the fungicide thiram increased the germination and emergence of pea by 33 and 29%. However, conflicting reports concerning the efficiency of this fungicide for the control of pathogens have been revealed. Marchi and Cicero (2003) reported that maize seeds treated with thiram did not affect the electrical conductivity of soaking solutions. Aamil *et al.* (2004) reported that chickpea (*Cicer arietinum* L.) seed treated with thiram applied at a high rate dramatically affected plant vitality and seed germination. They concluded that at normal dose rates, these effects were also observed but to a lesser extent.

The fungicide is also used as a seed treatment for the protection of seeds of fruit, vegetable, ornamental and turf crops. According to Southwell *et al.* (2003), the combination of thiram and carboxim effectively reduced *Gaeumannomyces zeae* carryover in the seed. Lamprecht *et al.* (1990) revealed that the fungicide thiram significantly lowered pre- and post-emergence damping-off of cultivars artificially inoculated with *F. graminearum* Group 1.

### 2.3.4 Celest<sup>®</sup> XL

Celest<sup>®</sup> XL is a water-based odourless chemical (www.syngenta.com, 2006) that includes two active ingredients, namely fludioxonil and mefenoxam (metalaxyl-m). It is marketed in some countries under the name Maxim. Fludioxonil, one of the active ingredients belongs to the unique and new class of phenylpyrroles and is derived from the antibiotic pyrrolnitrin, which is produced by many *Pseudomonas* spp. (Errampelli, 2004).

The fungicide fludioxonil is used as a seed treatment providing protection during germination and early growth stages of plant development, resulting in improved emergence and germination (www.syngenta.com, 2006). During the evaluation of fludioxonil on maize seeds, it was found that the fungicide increased the shoot and root length of the plant (Munkvold & O'Mara, 2002) and this was manifested as a significant improvement in emergence. Luz (2003) reported that combining fludioxonil with metalaxyl-m improved the germination and yield of wheat.

According to Solorzano *et al.* (2005), maize plants grown from seeds of hybrid W-A treated with fludioxonil or the combination of fludioxonil and mefenoxam or fludioxonil, mefenoxam, and azoxystrobin gave high yield when compared to the untreated control. Moraes *et al.* (2003) found that the application of fludioxonil and metalaxyl-m to maize seeds controlled *F. verticillioides* (Sacc.).

## 2.4 Seed quality

According to Albuquerque and de Carvalho (2003), the quality of seed is dependent on germination and vigour. Hampton (2002) reported that it is important to ensure the information regarding the quality of a seed lot before it is sold. Consequently, a farmer must have the knowledge of the performance of the seed lot before it is planted (Taylor & Harman, 1990; Freitas *et al.*, 2002). Muliokela and Kaliangile (1995) reported that most farmers have recognised the importance of good seed quality, which results in seedlings that grow fast and perform well in a wide range of field conditions.

However, the information related to seed quality has been placed under the seed industries, official seed testing laboratories and many privately operated commercial laboratories (Anon, 1952; Copeland & McDonald, 2001). In the evaluation of seed quality, various tests can be used. These include the standard germination, seedling vigour classification, early primary root emission, cool germination, electrical conductivity, field emergence and accelerated ageing (Freitas *et al.*, 2002).

## **2.5 Moisture content**

Cabrera and Mourad (1995) revealed that seed moisture content might be the single most important component for the determination of seed quality. Dale and Wilson (1995) reported that the moisture content of seed could be kept at acceptable levels if the relative humidity of the warehouse atmosphere is well monitored. However, Jianfang *et al.* (1998) revealed that although the seed might be stored at ambient temperature the strict control of its water content leads to a maximum viability. According to Bern *et al.* (2003), the loss or gain in moisture of maize seed is related to the quantity of water vapour pressure that surrounds the seeds.

Dale and Wilson (1995) revealed that seeds, although conveniently dried and handled in bulk, absorb water from the outside environment. Moisture basically moves in small amounts by diffusion between air spaces in the seed. Taylor (2003) concluded that in the seed, water is subject to passive movement since it moves from high to low concentrations. However, even if seeds are dried to appropriately low moisture content for storage following harvest they can be subject to mechanical damage such as physical damage of the seed coat and internal tissues, especially during handling and conditioning, which can hamper seed quality (Peterson *et al.*, 1995).

## **2.6 Seed viability**

### **2.6.1 Germination test**

The germination test, being the most used and accepted indication of seed quality worldwide, provides the farmer with information related to the quality and the storage capacity of the seed lot (Copeland & McDonald, 2001). According to Hilhorst (1995),

the process of germination begins with the imbibition of water by dry seed, followed by the embryo expansion growth and usually culminates in rupture of the covering layers and emergence of the radicle, which is considered to be the end of germination.

Egli and Tekrony (1995) revealed that the germination test precisely predicts field emergence only when seedbed conditions are most suitable for germination and emergence. In unfavourable seedbed conditions the results issued by the germination test overestimate field emergence. According to Priestly (1986), during the germination test, seeds are germinated in controlled laboratory conditions.

The essential parts taken into account during the germination evaluation are roots and shoots, axes, cotyledons, terminal buds and for the Poaceae, the coleoptiles. The seeds are evaluated according to healthy, dead and abnormal seedlings (Justice & Bass, 1979). According to Gooding *et al.* (2000), certain requirements must be met when conducting the germination test. These include: a representative sample of the seed must be provided; during the test, dormancy must be avoided at all costs; the testing environmental conditions must be optimal in order to enable germination; and during the evaluation, the abnormal seedlings should be excluded since they are unable to produce plants which would survive in the field.

However, Hampton (1995) revealed certain failures in the definition of the germination test described both by the International Seed Testing Association (ISTA) and the Association of Official Seed Analysts (AOSA). The author concluded that the germination test results do not reflect the seed lot performance in the field or during storage and it is not completely standardised. For instance, ISTA rules do not give the exact amount of water, which is needed in the growing media. In addition, the AOSA rules suppress the dead and more diseased seedlings but the weak, semi-lame and robust are placed in the same category. During the statistical analysis the percentage of viable seeds at high levels might be minimised and this leads to the under-detection of an important quality factor (Hampton, 1995).

## 2.7 Seed vigour tests

It has been established that favourable conditions, which are encountered in the laboratory when estimating the germination test, are rare in the field. This results in the overestimation of the planting value of a given seed lot by the germination results (Copeland & McDonald, 2001). Halmer (2000) attributed this fact to a phenomenon known as vigour, which reveals that high germination seedlot can perform poorly in the field. Because good conditions are scarce in the field, attention is given to vigour tests, which are important parameters to be measured in the evaluation of seed quality (Byrum & Copeland, 1995). Therefore, seed vigour tests have been developed for providing the ability of a seedlot to perform under field conditions (Sako *et al.*, 2001).

The definition of vigour proposed by several authors has been a source of controversy. Although all of them pointed out the several performance characteristics, the number of causative factors involved in the definitions was not the same (Perry, 1980). Accordingly, the ISTA committee decided to give a broad definition of seed vigour. The concept has been proven to be very complicated as they spent 27 years to provide a satisfactory definition (Copeland & McDonald, 2005). Seed vigour is defined as the properties that determine the behaviour of seed or seedlot in the laboratory or under field conditions (ISTA, 2007).

According to Perry (1980), several factors are cited to be the cause of change in vigour. These are the genetic constitution of the seed, environmental conditions and nutrition of the mother plant; level of maturity at harvest; seed size, weight or specific gravity; mechanical integrity; deterioration and ageing; and pathogens. Despite these differences Byrum and Copeland (1995) have suggested that ideally vigour tests should be repeatable and provide a good indication of the performance of seed under a wide range of field conditions reported to be less than optimum.

Vigour tests may be classified according to each author. For instance Isley in 1957 divided vigour tests into direct and indirect tests (Copeland & McDonald, 2005). The direct tests such as the cold test simulate the adverse field conditions in the laboratory (Bruggink *et al.*, 1991) and the indirect tests such as the conductivity test assess the

specific physiological component. Despite the presence of all these tests for the measurement of vigour, there is no reliable test, which evaluates the different species (Fernandez & Johnston, 1995).

It is noted that despite the effort to develop several tests for the measurement of seed vigour, few of them have been approved by seed analysts and testing associations (Perry, 1980). These include biochemical tests and stress tests, which are discussed below.

## **2.7.1 Biochemical tests**

### ***2.7.1.1 Conductivity test***

Waller (1901), as cited by Barton (1961) initiated the term conductivity when attempting to evaluate the viability of seeds by means of an electrical “after-current”. In his work on fresh bean (*Phaseolus vulgaris* L.) seeds, Barton (1961) confirmed the correlation between the electrical reaction and the germinative capacity. Fernandez and Johnston (1995) reported that the conductivity of seed leachate is a good indication of seed deterioration. The assessment of vigour by conductivity is a method which allows one to identify and therefore to phase out seed lots having reduced vigour and therefore avoid their delivery to the market (Powell, 1998). It was in 2001 that conductivity became accepted as a seed vigour test by ISTA (ISTA, 2001) because the basic requirements for vigour tests were met. These included repeatability of the results, correlation with emergence, simple procedures, reduced cost and quick results (Marchi & Cicero, 2003).

Conductivity tests are based on the fact that the progression of seed deterioration results in loss of rigidity and water permeability, which leads the cell contents to escape into solution and increase its electrical conductivity (Coolbear, 1995). Chloride, phosphate, potassium and magnesium are among the ions that leak from seeds (Gallagher & Fuerst, 2006). 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 decreasing leakage from the embryo (Hampton *et al.*, 1992).

An apparatus called a conductivity meter is used to monitor the electrolyte leakage from each seed (Copeland & McDonald, 2001).

### ***2.7.1.2 Tetrazolium test***

Lakon (1928), as quoted by Copeland and Mc Donald (2005), is considered to be the initiator for the development of this test, when he was trying to differentiate between the dead and live tissues by treating the seeds with selenium salts. His attempts with tetrazolium salts were more effective. According to Tulo (1987), as cited by Kolasinska *et al.* (2000), this test can be used to determine the loss of germination ability of a seed lot.

The principle of the tetrazolium test is based in the fact that the seed imbibes the colourless triphenyl tetrazolium chloride or bromide. By means of enzymes, the compound is reduced to the red triphenyl formazan, which is then precipitated within live cells (Roberts, 1972). In the absence of active enzymes, the dead seeds remain colourless while the live parts of the embryo are stained red. The seeds will be then classified according to the staining pattern of the embryo and the intensity of the colouration (Copeland & McDonald, 2001). The staining pattern can be also detected using a machine having vision algorithm with which Xie and Paulsen (2001) were able to predict the viability loss in maize seed.

The tetrazolium test presents many advantages over other biochemical tests since it has a low toxicity both for the seed and humans and can stain the seeds permanently (Barton, 1961). However, MacKay (1972) reported that this test cannot detect the phytotoxicity due to seed dressings, since seed lots of little value for planting may be ranked as being of high viability. According to the author, the heat injury caused by high temperatures during artificial drying is also readily missed.

## **2.7.2 Stress tests**

### ***2.7.2.1 Cold test or soil cold test***

The soil cold test, one of the oldest and most accepted seed vigour tests by seed industries, is used to evaluate vigour and to predict emergence of various crops (Hampton, 1995). In this method, seeds are placed in towels lined with moist medium containing soil and kept at a low temperature (Heydecker, 1972). The low temperature encourages the growth of soilborne pathogens for a specific period after which the seeds are placed under favourable conditions for germination to determine their capacity for survival and possibly their residual growth potential (Heydecker, 1972).

Different methods have been developed for the evaluation of the cold test. These include the deep box method, rolled towel method and tray method (ISTA, 2007). The greatest disadvantage of the cold test is that the result cannot be regarded as absolute because soils differ in pH, moisture, particle composition, and the level of pathogens, all of which contribute to divergent results (Copeland & McDonald, 2005).

### ***2.7.2.2 Accelerated ageing test***

According to Gallagher and Fuerst (2006), the accelerated ageing test has been designed to artificially hasten deterioration of seeds by exposure to high temperatures (35-45°C) and high humidity levels (85-100%). Marcos Filho (1999), cited by Torres and Marcos Filho (2003), reported these two conditions to be the most detrimental factors in connection to the intensity and velocity of deterioration. In this environment seedlots having a low quality will deteriorate more rapidly than the vigorous seedlots, presenting a differentiated decrease in viability (Gallagher & Fuerst, 2006).

## **2.8 Seed performance enhancement**

Different techniques referred to as seed enhancements have been developed and evolved aiming to increase the performance of various seed in the field (Taylor, 2003). Taylor *et al.* (1998) defined seed enhancement as all the postharvest

techniques, which result in improved germination or seedling growth or facilitate the delivery of seed and other material that are needed at the time of sowing. According to Copeland and McDonald (2005), seed enhancements cover many aspects among them being seed hydration, biological seed treatments and seed coating. Hydration includes three main approaches, which are prehydration, priming and solid matrix. Only two topics will be discussed further in this literature review.

### **2.8.1 Prehydration**

According to Lima *et al.* (2003), the seed can be imbibed in pure water without any solute. This is called prehydration. This process initiates hydration of the seed before it encounters the unfavourable soil environment (Harris *et al.*, 2001). The technique is used especially for tropical crops, which are sown in hot and dry conditions (Harris *et al.*, 2001).

During prehydration, seeds take up water and move from an anhydrous state to a fully hydrated organism having the ability of developing and responding to environmental stimuli (Vertucci, 1989). This is an important stage for germination and is the primary phase in the sequence of metabolic phenomena (Lima *et al.*, 2003). Pollock (1972) revealed that when a dry seed is imbibed in a moist environment, the water is taken up in three phases: an initial stage during which water is taken up quickly; a lag period during which the absorption is reduced; a second uptake stage is in correlation with embryo growth. However, Legesse and Powell (1992) revealed that differences in water uptake between seed of different groups could be due to the restriction of water to penetrate the testa (seed coat).

According to Woodstock (1988) the early impact of seed quality on performance can be noticed in the initial phase of water uptake after sowing, which has been said to be a period of peril. The capability of the seed to overcome this period successfully and to give an autotrophic self-sustaining plant is related to the inherent soundness and vigour of the seed (Finch-Savage, 1995).

The imbibition of seeds in water has been proven to improve seed quality. Tesfaye (1992) reported that pre-treatment of onset seed by soaking seed in water promoted germination, when compared to the control (unsoaked seed) that had poor percentage germination. During their study, Caseiro *et al.* (2004) revealed that hydropriming, defined as the soaking of seed in water, promoted the speed of germination in onion (*Allium cepa* L.) seed, especially after 96 h moistening. According to the authors, this can be due to the huge amount of water imbibed by the seed during the process. Harris *et al.* (2001) found that the positive result of hydropriming of maize and chickpea seeds was observed after 24 h and 10 h imbibition, respectively. The benefits included faster emergence, better stand and a lower incidence of re-sowing, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest and higher grain yield. However, Wang *et al.* (2002) reported that although soaking seed in water can improve germination for several crops, the real reason for this improvement is not well understood.

### **2.8.2 Seed priming**

According to Harris (1996), seed priming is a concept used synonymously with prehydration, but in reality it refers to the soaking of seeds in a concentrated solution (Finch-Savage *et al.*, 2004). Seed priming is an old well-known practice aiming to enhance seedling establishment by increasing uniform seed germination (McDonald, 1998; Artola *et al.*, 2003; Horii *et al.*, 2007) especially in bad conditions (Parera & Cantliffe, 1994). The additional advantage of this technique, as stated by Parera and Cantliffe (1994), is the reduction of time between seed sowing and seedling emergence and the synchronization of emergence.

Priming can be applied independently or in combination with other seed treatments and sowing techniques (Brocklehurst *et al.*, 1987). Bray (1995) reported that osmotic solutions are used in priming but under conditions that prevent germination and allowing certain metabolic processes to be expressed. In addition, other substances such as polyethylene glycol (PEG) might be used (Foti *et al.*, 2002).

Finch-Savage *et al.* (2004) stipulated that priming in maize kernels gives variable results because it can lead to positive, neutral or negative effects. However, Subedi and Ma (2005) revealed that, despite the benefits of seed priming on seedling vigour and stand establishment, none of the seed priming treatments used in their study improved maize yield under temperate humid conditions.

According to Parera and Cantliffe (1994), the success or the failure of priming treatments depends on a number of elements including plant species, osmoticum, water potential of the priming agent, time required for the process, temperature, vigour, dehydration, and storage conditions following priming. However, in the conduct of both prehydration and seed priming, Taylor *et al.* (1998) revealed that an advanced level of priming could not be reached because subsequent seed dehydration causes the irreversible death of the embryo. Consequently, the incomplete hydration is suitable and triggers some metabolic activity and repair mechanisms. This is considered to be the base of priming (Parera & Cantliffe, 1994)

## 2.9 Conclusion

Worldwide maize, wheat and rice represent the largest source of income, when compared to other crops (Farnham *et al.*, 2003), with maize having the highest production (Fapri, 2003). In Africa, maize plays a significant role in human consumption especially in rural areas for the sustainability of the population (Messing & Dooner, 2006).

According to Shurtleff (1980), diseases are of great concern in maize production since they represent the major constraints for the crop. *Fusarium graminearum* causes various diseases including *Gibberella* stalk rot, *Gibberella* ear rot, Red rot and Pink ear rot, which result in the reduction of yield and quality of the maize crop (McGee, 1988). This fungus can be controlled in various ways. However, the use of fungicides represents one of the most promising methods for the eradication of the pathogen. Of the different chemical treatment methods, fungicide seed treatment provides an effective control of the pathogen and therefore improves seed quality.

However, some constraints that face the use of chemicals are the controversial reports concerning their efficacy against the diseases and the pressing need by populations demanding fungicides to be environmentally safe (Knight *et al.*, 1997). Consequently, there is a need for new chemical compounds, which can provide effective control of *F. graminearum* and improve maize quality yet be environmentally safe. It must be pointed out that not only the environmental safety is of major concern in the fungicide industry; the effective protection of health and safety of people who work and use the products is also taken into account as stipulated by Syngenta policy (www.syngenta.com, 2008).

## CHAPTER 3

### EFFECT OF FUNGICIDE SEED TREATMENTS ON GERMINATION AND VIGOUR OF MAIZE SEEDS *IN VITRO*

#### 3.1 Introduction

Initially, fungicides were developed for the protection of crops against diseases and pests (Falloon *et al.*, 2000). However, new fungicides are needed on the market to overcome resistant pathogens. Besides this, the population demands are becoming focussed on crop protection agents having reduced toxicity for both humans and wildlife (Knight *et al.*, 1997). When applied to the seeds, these chemicals have proven to be a form of crop insurance that protects investment of growers (Bierman *et al.*, 2006) since it has been reported that attack of seeds by fungi can result in a reduced seed quality (Shurtleff, 1980). Accordingly, different germination and vigour tests (standard germination test, electrical conductivity, ageing test, tetrazolium test, etc.) are routinely performed in order to evaluate the value of the seeds before they are planted. Consequently, seeds of different crops are sold to farmers as processed and treated. This is performed by seed companies devoted to ensure that seed of high quality is delivered. The fungicides Celest<sup>®</sup> XL (fludioxonil and mefenoxam), Apron<sup>®</sup> XL (mefenoxam) and Apron<sup>®</sup> Star 42 WS (thiamethoxam, metalaxyl-m and difenoconazole) were produced by Syngenta for their low risk to the environment (www.syngenta.com, 2006).

The effect of these fungicides on the germination and vigour of maize seeds is largely unknown. Csinos (2004) revealed that tobacco (*Nicotiana tabacum* L.) blackshank seeds treated with Ultra Flourish 2E (mefenoxam) gave the highest vigour, whereas FAC 321 2E (metalaxyl) treated seeds had the lowest vigour. Cahill (2000) reported that the treatment of seed in general with fludioxonil accelerated germination. When conducting the cold test on maize (*Zea mays* L.) seeds, Pinto (1997) reported that thiram promoted their viability and emergence. According to Luz (2003), wheat (*Triticum aestivum* L.) seeds chemically treated with difenoconazole improved plant stand and grain yield.

The present study was undertaken to test the effects of Apron<sup>®</sup> XL, Celest<sup>®</sup> XL, thiram, and Apron<sup>®</sup> Star 42 WS on germination and vigour of maize seeds *in vitro*.

## **3.2 Materials and methods**

### **3.2.1 Origin of maize seeds and fungicides**

Maize seeds, of the cultivar Maverik (batch number D 14 H) were supplied by Agricol (Pty) Ltd, Silverton, Pretoria. The chemicals Apron<sup>®</sup> Star 42 WS, thiram, Apron<sup>®</sup> XL and Celest<sup>®</sup> XL were supplied by Syngenta South Africa (Pty) Ltd.

### **3.2.2 Fungicide seed treatments**

The experiments were performed in the laboratory of the Department of Microbiology and Plant Pathology at the University of Pretoria, Pretoria. Prior to chemical seed treatment, seeds were rinsed with tap water in a clean plastic bowl to remove impurities. Fungicides were applied to the seeds at their recommended rates of application: thiram at 200 g/100 kg, Celest<sup>®</sup> XL at 100 ml/100 kg, Apron<sup>®</sup> XL at 20 ml/100 kg, and Apron<sup>®</sup> Star 42 WS at 250 g/100 kg. The control consisted of untreated seeds. After treatment, the seeds were allowed to dry in a laminar flow cabinet.

### **3.2.3 Moisture content**

The moisture content of the seeds was determined using the high constant temperature oven method (ISTA, 2007) at the Department of Agriculture, Roodeplaat. Before the process, seeds were air-dried on a laminar flow cabinet to remove excess water and ground using a grinding-mill. Two replicates of 10 g per treatment were weighed and placed into metallic containers that were initially weighed. The container and the seed powder were weighed prior to being incubated in a drying oven at 130°C for 4 h. After incubation, the seed powder was cooled down in a desiccator for 30 min. The

container and the seed powder were reweighed. The percentage of moisture content was calculated using the formula:

$$(M_2 - M_3) \times 100 / (M_2 - M_1)$$

Where:

$M_1$  is the weight in grams of the container and its cover,

$M_2$  is the weight in grams of the container, its cover and its contents before drying,  
and

$M_3$  is the weight in grams of the container, cover and contents after drying.

### 3.2.4 Standard germination test

The standard germination test was performed according to the procedure of the International Seed Testing Association (ISTA) using rolled paper towels. Four replicates of 50 seeds per treatment were used. The sheets were moistened with 150 ml of distilled water. Each roll consisted of two sheets of germination paper, followed by a layer of white paper towel and a third sheet of germination paper on which 25 seeds were placed in a line, embryo side down and the fourth sheet of germination paper was used to cover the seeds. They were then rolled and sealed within plastic bags (Figure 3.1) and incubated under a 12 h light/dark regime at  $25 \pm 1^\circ\text{C}$ .

The first rating was done seven days after incubation, recording the germinated and ungerminated seeds. The second rating after nine days involved the counting of normal and abnormal seedlings (Figure 3.2) and these being characterised using ISTA (2007) rules as follows: a) Normal seedlings, referring to seedlings, which have the following features: -root: at least one long main root; -shoot: no damaged coleoptile and emerging leaf; -seedling: free of pathogen. b) Abnormal seedlings, referring to those unable to develop into normal plantlets because one or more essential characteristics have irreplaceable deficiencies: -root: absence of main root, short or stump root; -shoot: lack of coleoptile or leaf; -seedling: small, deformed or swollen coleoptile (Van Waes, 1995).

### **3.2.5 Fast and slow imbibition tests**

Seeds were weighed individually prior to imbibition. Four replicates of 24 seeds per treatment were used for both tests. The tests were conducted according to the ISTA (2007) rules. The seeds subjected to fast imbibition were individually soaked in 4 ml of distilled water contained in ice cube trays for 6, 24 and 40 h. They were removed, air-dried and reweighed after which they were submitted to the germination test at  $25\pm 1^\circ\text{C}$ . The counting of germinated and ungerminated seeds was performed after 7 d. For the slow imbibition, seeds were placed directly onto rolled paper towels moistened with 150 ml of distilled water, for 6, 24 and 40 h after which they were removed and air-dried. They were then reweighed and subjected to the germination test at  $25\pm 1^\circ\text{C}$  for further rating of germinated and ungerminated seeds as described above.

### **3.2.6 Conductivity test**

The electrical conductivity of the imbibition solution from the seeds that were submitted to slow and fast imbibition was determined using a conductivity meter (EC 215, Hanna instruments, South Africa) and expressed as  $\mu\text{S}$ . For fast imbibition, seeds were placed in an ice tray, each individual seed imbibed in 4 ml of distilled water. They were then kept in the dark for 24 h as stipulated by ISTA (2007) after which the electrical conductivity of each individual seed was measured using a conductivity meter. For slow imbibition, seeds were placed in rolled paper towels moistened with distilled water for 40 h at  $25^\circ\text{C}$ , after which they were removed and placed in 4 ml of distilled water for 6 h in the dark as stipulated in the ISTA (2006) rules. The conductivity was measured as stipulated above. Four replicates of 24 seeds were used per treatment and incubated at  $25\pm 1^\circ\text{C}$ .

### **3.2.7 Tetrazolium test**

The seeds from the conductivity test were submitted to the tetrazolium viability test. The seed coat was removed from the imbibed seed using a scalpel and a longitudinal section was cut through the embryo. The seeds were placed in an ice tray in which

each individual seed was imbibed in 4 ml of 1% 2,3,5-triphenyl-tetrazolium chloride solution (Research Organic, Johannesburg) and incubated in oven at  $30\pm 1^\circ\text{C}$  for 2 h to permit embryo colouration. The staining pattern was examined with a stereomicroscope (Nikon/SMZ-1) and seeds were evaluated and classified into three categories, namely totally stained (viable seeds), partially stained and unstained (ISTA, 2007).

### **3.2.8 Statistical analysis**

The data were analysed using analysis of variance, however, those expressed in term of percentage (standard germination, tetrazolium test, imbibition tests) were subjected to arcsin transformation. The means were separated using the Student t-test ( $P\leq 0.05$ ).

## **3.3 Results**

### **3.3.1 Moisture content**

All the seeds treated with the chemicals had expressed the recommendable rate of moisture content of below 14%. The mean of each sample did not exceed 0.02%, which is the recommended tolerance for maize seeds.

### **3.3.2 Standard germination test**

The present study revealed that there were no significant effects of the chemicals on the percentage germination in the standard germination test. The percentage germination of all the treated seeds was initially more than 78.5% (Table 3.1).

### **3.3.3 Seed vigour tests**

#### ***3.3.3.1 Conductivity test***

The conductivity of all the treatments that were rapidly imbibed (fast imbibition) was significantly decreased when compared to the untreated control, indicating a decrease in solute leakage from the seeds, with the exception of thiram (Table 3.1). All the

treatments from the slow imbibition treatment did not significantly differ from the untreated control. However, the interaction within each treatment between slow and fast imbibition revealed that only thiram had significantly increased the conductivity in fast imbibition indicating an increase in solute leakage (Table 3.1).

### ***3.3.3.2 Tetrazolium test***

The tetrazolium test after fast imbibition indicated that there was no significant difference in seed viability for all the treatments when compared to the untreated control (Table 3.1). The seeds from the slow imbibition proved that only Apron<sup>®</sup> XL and Celest<sup>®</sup> XL significantly improved seed viability when compared to the untreated control whereas the fungicides Apron<sup>®</sup> Star 42 WS and thiram significantly decreased seed viability. When comparing between the fungicide-treated seed from the fast and the slow imbibition during the tetrazolium test, Apron<sup>®</sup> Star 42 WS treated seed was statistically the same as the untreated control, seed viability was significantly improved by thiram and decreased by Celest<sup>®</sup> XL and Apron<sup>®</sup> XL following fast imbibition (Table 3.1).

### ***3.3.3.3 Fast and slow imbibition germination tests***

The results of the effects of Apron<sup>®</sup> Star 42 WS, Apron<sup>®</sup> XL, thiram and Celest<sup>®</sup> XL on the germination and vigour of maize seeds after being imbibed are presented in Table 3.2. After 6 h fast imbibition, germination percentages of all the treatments were statistically the same as the untreated control, with the exception of thiram treated seeds, which was significantly lower. In the 6 h slow imbibition none of the treatments differed from the untreated control. However, when comparing between the fast and slow imbibition of each treatment after 6 h, Celest<sup>®</sup> XL and Apron<sup>®</sup> XL significantly decreased the germination in the fast imbibition test.

Fast imbibition for 24 h showed that Apron<sup>®</sup> Star 42 WS did not statistically differ from the untreated control whereas Celest<sup>®</sup> XL, Apron<sup>®</sup> XL and thiram had a significantly reduced percentage germination. After 24 h slow imbibition, all the treatments did not differ significantly from the untreated control. However, when

comparing 24 h fast and slow imbibition for each fungicide, it was found that the control and thiram significantly decreased germination following fast imbibition. After 40 h fast and slow imbibition, all the fungicide treatments did not differ significantly in percentage germination from the untreated control with the exception of Apron<sup>®</sup> Star 42 WS which had a significantly lower percentage germination after slow imbibition. However, when comparing the percentage germination between treated seeds subjected to fast or slow imbibition for 40 h, this was significantly decreased in the Apron<sup>®</sup> Star 42 WS, thiram and Apron<sup>®</sup> XL treatments in the fast imbibition test (Table 3.2).



Figure 3.1 Maize seeds placed in an upright position in rolled paper towels within plastic bags for the germination test.



Figure 3.2 Normal maize seedlings (a), and abnormal maize seedlings showing few adventitious roots (b).

Table 3.1 Percentage standard germination, electrical conductivity and percentage of viable seeds of maize seeds treated with fungicides.

Treat-ments	Std. Germ. (%)	Conductivity (μS)		Tetrazolium test	
				Viable seed (%)	Viable seed (%)
		Fast imb.	Slow imb.	Fast imb.	Slow imb.
Un.cont	78.5 <sup>#</sup> a	100.2 <sup>*c**z</sup>	51.38az	34.4ay	36.5 cz
Apr-St.	82 a	68.45abz	52.97az	24 az	9.4 az
Cel-XL	79a	47.25az	51.72az	34.4 ay	46.9dz
Thiram	82.5 a	88.6bcz	42.17ay	22.9 az	21.9 by
Ap-XL	83 a	50.6az	43.78az	26.1 az	62.5 ez
Lsd	6.07	25.39	17.98	14.45	9.45

Un.cont= untreated control, Std. Ger.=Standard germination test, Fast Imb.=Fast imbibition, Slow Imb.=Slow imbibition, Apr-St.= Apron<sup>®</sup> Star 42 WS, Cel-XL=Celest<sup>®</sup> XL, Apr-XL=Apron<sup>®</sup> XL.

<sup>#</sup>Each value is a mean of four replicates of 50 seeds. Means within column not followed by the same letter are significantly different.

\* Each value is a mean of four replicates of 24 seeds. Means within a COLUMN not followed by the same letter are significantly different (P≤0.05).

\*\* Means within a ROW per test not followed by the same letter are significantly different (P≤0.05).



Table 3.2 Percentage germination of maize seeds treated with fungicides exposed to fast and slow imbibition.

Treatments	Germination (%)					
	6 h F.	6 h Sl.	24 h F.	24 h Sl.	40 h F.	40 h Sl.
Un.cont	80.2 <sup>*b**z</sup>	79.2 <sup>az</sup>	75 <sup>dy</sup>	81.3 <sup>az</sup>	60.4 <sup>ay</sup>	83.4 <sup>bz</sup>
Apr-St.	70.8 <sup>abz</sup>	85.4 <sup>az</sup>	67.7 <sup>dcz</sup>	74 <sup>az</sup>	56.2 <sup>ay</sup>	63.6 <sup>az</sup>
Cel-XL	75 <sup>by</sup>	77.1 <sup>az</sup>	50 <sup>az</sup>	82.3 <sup>az</sup>	66.7 <sup>az</sup>	86.5 <sup>bz</sup>
Thiram	64.6 <sup>az</sup>	82 <sup>az</sup>	61.5 <sup>bcy</sup>	62.5 <sup>az</sup>	52.1 <sup>ay</sup>	77.1 <sup>abz</sup>
Ap-XL	70.9 <sup>aby</sup>	80.2 <sup>az</sup>	56.3 <sup>abz</sup>	82.3 <sup>az</sup>	59.4 <sup>ay</sup>	80.2 <sup>bz</sup>
Lsd	9.68	10.2	11.2	20.11	15.9	14.81

Un.cont=untreated control, h F.=number of hours after fast imbibition, h Sl.=number of hours after slow imbibition, Apr-St=Apron<sup>®</sup> Star 42 WS, Cel-XL=Celest<sup>®</sup> XL, Apr-XL=Apron<sup>®</sup> XL.

\* Each value is a mean percentage of four replicates of 24 seeds. Means within a COLUMN not followed by the same letter are significantly different ( $P \leq 0.05$ ).

\*\* Each value is mean of four replicates of 24 seeds. Means within a ROW not followed by the same letter are significantly different ( $P \leq 0.05$ ) within a specific time of interval.

### 3.4 Discussion

From this experiment it was found that there was no reduction in the standard germination of all the fungicide treated seeds possibly because of the favourable conditions that are encountered within the laboratory (Copeland and McDonald, 2001). However, we assumed that the storage fungus, *Rhizopus*, which developed in all the treatments, hampered the germination of some seeds.

The results of the conductivity test showed the effectiveness of fungicides maintaining seed vigour after maize seeds were subjected to stress conditions. Apron<sup>®</sup> Star 42 WS (thiamethoxam, metalaxyl-m and difenoconazole), Celest<sup>®</sup> XL (fludioxonil and mefenoxam) and Apron<sup>®</sup> XL (mefenoxam) treated seeds had improved vigour although subjected to fast imbibition. This is indicated by the reduced seed leachate when compared to the untreated control. This finding agrees with Doyle *et al.* (2001) who proved that seedlings treated with thiamethoxam had a particular advantage of improved vigour. Csinos (2004) revealed that mefenoxam improved vigour of tobacco (*Nicotiana tabacum* L.). In the present study, thiram treated seeds maintained the same vigour as the untreated control. This agrees with Zhang and Hampton (1999) who found that at the recommended application rates, the conductivity of thiram-fosetyl-aluminium-thiabendazole treated pea (*Pisum sativum* L.) seed did not differ from the untreated control. The comparison between the fast and slow imbibition for conductivity test revealed a significant increase of seed leachate for thiram treated seeds following fast imbibition. This is an indication of decreased vigour (Hampton *et al.*, 1992). The good performance of the fungicide treated seed expressed during conductivity test after fast imbibition was reflected in the tetrazolium test when compared to the untreated control. Surprisingly, under standard germination conditions (slow imbibition) Apron<sup>®</sup> Star 42 WS and thiram treated seeds had decreased viability when compared to the untreated control. This may have been due to the mechanical damage that occurred in the seedlot, which then allowed the access of chemicals to the embryo, resulting in the damage of the physiological process (Scott *et al.*, 1985) and viability of seeds would therefore be likely to decrease. Anonymous (1996), quoted by Zhang and Hampton (1999), reported that any chemical application to the seed at the recommended dosage should

not produce any detrimental effect on seed performance and seedling development. We can therefore confirm that results of conductivity and tetrazolium tests adhere to this statement. The same result was achieved 6 h after treated seeds were submitted to fast imbibition with the exception of thiram treated seeds. Seeds treated with Apron<sup>®</sup> Star 42 WS, Celest<sup>®</sup> XL and Apron<sup>®</sup> XL and subjected to 6 h fast imbibition maintained a similar germination percentage to the untreated control. However, when compared to the standard germination percentage there was a decrease of germination percentage 6 h following fast imbibition for Apron<sup>®</sup> Star 42 WS, Celest<sup>®</sup> XL and Apron<sup>®</sup> XL treated seeds but this was still above the acceptable germination percentage for maize, which is 70% (Plant Protection, 1976). Following 6 h fast imbibition, thiram treated seeds failed to maintain the high germination percentage expressed during the standard germination test. This may have been due to mechanical damage that occurs to some of the seeds during handling allowing access of this fungicide to the embryo thus damaging the physiological process. However, this was not determined. After 24 and 40 h fast imbibition, all the fungicide treated seeds had reduced vigour, which was illustrated by the reduction in germination percentage below the acceptable level (70%).

Based on the findings of this study it can be recommended to apply Apron<sup>®</sup> Star 42 WS, Celest<sup>®</sup> XL, thiram and Apron<sup>®</sup> XL on the seeds before planting in order to maintain the vigour of maize as reflected by the results of the conductivity and tetrazolium tests. Follow-up research should include planting of the treated seed in the field and correlation with the vigour test results should be conducted.

## CHAPTER 4

### EFFICACY OF FUNGICIDE SEED TREATMENTS AGAINST *FUSARIUM GRAMINEARUM* IN A GREENHOUSE TRIAL

#### 4.1 Introduction

*Fusarium graminearum* Schw. is a pathogen that causes diseases in a wide range of plants. The fungus is a serious threat to maize (*Zea mays* L.) causing seed rot, seedling blight, root rot, stalk rot and ear rot (Shurtleff, 1980). According to Salas *et al.* (1997), cited in O'Donnell *et al.* (2000), the damage caused by *F. graminearum* results in reduction of yield and seed quality caused by the presence of discoloured seeds, which in turn can be contaminated by mycotoxins.

Several methods have been developed for the management of *F. graminearum*. Martin *et al.* (1991) reported that removing or burying crop residues lead to a reduced source of inoculum. The use of transgenic plants resistant to the fungus can consistently decrease the disease caused by this pathogen. According to Munkvold *et al.* (1997), maize hybrids with Cry IA (b) genes that are expressed in kernels reduced *Fusarium* ear rot and *Fusarium* infection of kernels, when compared to their non-transgenic counterparts. Luz (2001) used plant growth-promoting rhizobacteria (PGPBR) to decrease the pathogens *F. graminearum*, *Drechslera tritici-repentis* (Died), *Stagonospora nodorum* (Berk) and *Bipolaris sorokiniana* Sacc. in Sorok. (Shoem).

The application of chemicals as soil drenches, sprays or in irrigation water results in the reduction of losses, however, the better economic option is to treat seeds (Jensen *et al.*, 1998). Normally, a compound applied to seed may provide protection during germination, emergence and the early establishment stage of the plant (Jensen *et al.*, 1998). Jones (2000) concluded that the fungicides fludioxonil and mancozeb inhibited *Fusarium* species. McGovern *et al.* (2001) reported that mefenoxam (metalaxyl-m) is effective in reducing diseases caused by *Fusarium* species. Song *et al.* (2004) found the combination of thiram and procloraz effective in inhibiting the mycelial growth of

some *Fusarium* species. According to Brian *et al.* (2004), thiamethoxam provided long and more consistent protection of the crop from leafhoppers [*Empoasca fabae* (Harris)]. Allen *et al.* (2004) indicated that difenoconazole exhibited a limited efficacy on longleaf pine (*Pinus palustris* Mill.) infected with four species of *Fusarium*.

The aim of this study was to evaluate the effect of four chemicals namely Celest<sup>®</sup> XL (fludioxonil and mefenoxam), Apron<sup>®</sup> XL (mefenoxam), thiram and Apron<sup>®</sup> Star 42 WS (thiamethoxam, metalaxyl-m and difenoconazole) used as seed treatments against *F. graminearum* in the greenhouse. In addition, their effects on emergence and dry shoot mass and dry root mass were investigated.

## 4.2 Materials and methods

### 4.2.1 Preparation of the pathogen

*Fusarium graminearum* (CAMS 1256) isolated from maize was obtained from the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, Pretoria. The pathogen was cultured on potato dextrose agar (PDA) and incubated at 25°C under normal light and dark for 12 h periods, respectively, for 7 d.

### 4.2.2 Inoculation of pasteurised soil

Polystyrene seedling trays having 128 cells each were filled with pasteurised soil (Braaks, Pretoria) and drenched with tap water until run-off a day before inoculation. Using a 5 mm cork borer, mycelial plugs were taken from the growing fungal cultures and inoculated into the soil (two mycelial plugs in each cell of the seedling tray equally distanced). A day after inoculation, maize seed was sown in the space between the two fungal plugs. Each treatment had 4 replicates of 25 seeds per replicate. The replicates were placed on two different tables, one with seedling trays containing the soil inoculated with *F. graminearum* and the other table having the seedling trays with uninoculated soil, serving as the uninoculated control. The

seedling trays were arranged in a randomised block design in a greenhouse and watered daily with tap water until harvesting. Plants were maintained at temperatures between 22-25°C. The trial was repeated twice.

#### **4.2.3 Data collection**

Maize seedlings were harvested 21 days after sowing. The percentage of emerged seedlings per replicate and per treatment was recorded. The seedlings were removed from the trays and washed with tap water to remove soil attached to the roots for further observation recording the percentage of diseased and healthy seedlings. The roots were separated from the shoots and placed separately per replicate in labelled paper bags. They were then dried for 48 h at 65°C at the Department of Plant Science, University of Pretoria, Pretoria. After drying, the dry mass of roots and shoots per replicate were recorded.

#### **4.2.4 Statistical analysis**

The data were analysed using analysis of variance, however, those expressed in term of percentage (emergence and diseased plants) were subjected to arcsin transformation. The means were separated using the Student t-test ( $P \leq 0.05$ ).

### **4.3 Results**

The percentage emergence, the shoot and root dry mass and the effects of Apron<sup>®</sup> Star 42 WS, Apron<sup>®</sup> XL, Celest<sup>®</sup> XL and thiram on maize seed grown in soil infected with *F. graminearum* and in uninoculated soil are presented in Table 4.1. The percentage emergence in the inoculated soil showed that all the treatments were not significantly different from the untreated control, with the exception of thiram that gave a significantly higher emergence. In the uninoculated soil the percentage emergence of all the treatments was statistically the same to the untreated control. When comparing percentage emergence between the inoculated and the uninoculated soil for each fungicide, all the treatments did not significantly differ from each other.

Apron<sup>®</sup> Star 42 WS and Celest<sup>®</sup> XL controlled *F. graminearum*. As was expected, the results from the uninoculated soil showed that all the treatments did not statistically differ from the untreated control. With each of the fungicide treatments, the percentage of diseased plants was significantly higher in all the treatments in inoculated soil when compared to the uninoculated soil. The symptoms included brown lesions on stems, root rot (Figure 4.1) and necrosis and yellowing of leaves. All the fungicides significantly increased the shoot dry mass of plants in the inoculated soil when compared to those of the untreated control, with the exception of Apron<sup>®</sup> XL. The results obtained from the uninoculated soil suggested that none of the fungicides significantly increased the shoot dry mass when compared to the untreated control. However, comparing each treatment between the inoculated and the uninoculated soil, showed that the Apron<sup>®</sup> XL treatment significantly decreased the dry shoot mass of plants in the inoculated soil. The results obtained from the inoculated soil suggested that all the fungicides significantly increased the root dry mass with the exception of Apron<sup>®</sup> XL when compared to the untreated control. Only Apron<sup>®</sup> Star 42 WS and thiram significantly improved the dry root mass of plants in uninoculated soil when compared to the untreated control. Celest<sup>®</sup> XL and Apron<sup>®</sup> XL treatments were statistically similar to the untreated control. There were no differences within a fungicide treatment in dry root mass when comparing plants grown in inoculated or uninoculated soil.

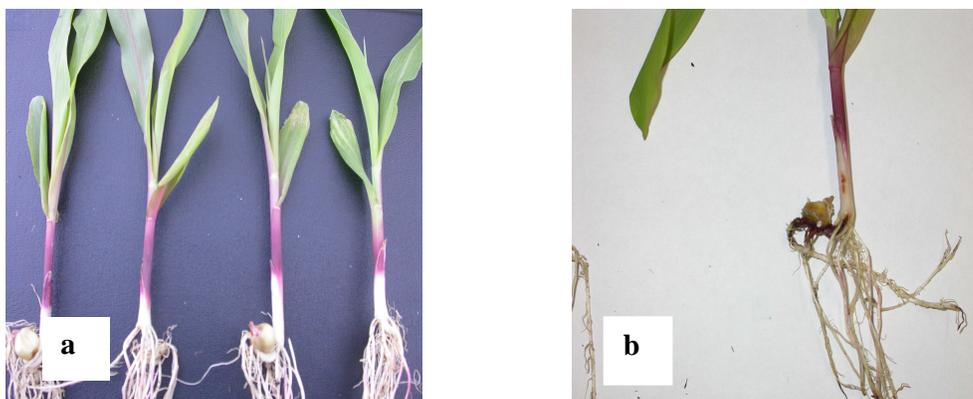


Figure 4.1 Healthy maize seedlings (a), and root rot symptoms caused by *F. graminearum* on a maize seedling (b).

Table 4.1 Percentage emergence, diseased seedlings and dry shoot and root mass of uninoculated and *Fusarium graminearum* inoculated soil planted with maize seed treated with Apron® Star 42 WS, Apron® XL, Celest® XL and thiram.

Treatment	Emergence (%)		Diseased plants (%)		Dry shoot mass (g)		Dry root mass (g)	
	Inocul.	Uninoc.	Inocul.	Uninoc.	Inocul.	Uninoc.	Inocul.	Uninoc.
Un.cont	66*a**z	63az	57bz	1ay	2.69az	5az	1.94ay	2.97abz
Ap.® St	64az	70az	25az	2ay	4.05bz	5.87az	2.85bz	3.65cz
Cel.® XL	69az	62az	23az	5ay	4.34bz	5.09az	2.84bz	2.88az
Thiram	82bz	74az	42abz	1ay	5.23cz	5.97az	3.44bz	3.75cz
Ap.® XL	68az	76az	46bz	2ay	3.18ay	5.69az	2.04az	3.56bcz
Lsd	7	14.97	20.45	4.81	0.84	1.25	0.63	0.66

Un.cont=untreated control, Tream. = Treatment, Inocul. =Inoculated soil, Uninoc. =Uninoculated soil, Ap.®St =Apron® Star 42 WS, Ap.® XL =Apron® XL, Cel.® XL = Celest® XL.

\* Each value is means of four replicates of 25 seeds. Means within a COLUMN not followed by the same letter are significantly different ( $P \leq 0.05$ ).

\*\* Each value is mean of four replicates of 25 seeds. Means within a ROW not followed by the same letter are significantly different ( $P \leq 0.05$ ) within a specific time of interval.

#### 4.4 Discussion

All the fungicide treated seeds maintained the same emergence percentage in both inoculated and uninoculated soil with the exception of thiram treated seeds, which improved emergence percentage in the inoculated soil when compared to the untreated control. This fungicide has been reported to improve emergence (Whitehead, 1995). According to Pinto (1997), thiram increased emergence of maize seed infected with *Pythium* sp. In addition, Southwell *et al.* (2003) concluded that the combination of thiram-carboxim applied to wheat (*Triticum aestivum* L.) infected with *F. graminearum* improved the emergence. Results from inoculated and uninoculated soil proved that all the fungicides helped seeds to maintain a high emergence percentage in the presence of *F. graminearum*. This is an indication of the maintenance of seed quality by these fungicides.

Apron<sup>®</sup> Star 42 WS (thiamethoxam, metalaxy-m and difenoconazole) and Celest<sup>®</sup> XL (fludioxonil and mefenoxam) showed their effectiveness by significantly reducing the disease caused by *F. graminearum* in the inoculated soil. The reduced number of diseased plants in the Apron<sup>®</sup> Star treatment may be due to its broad spectrum activity against many pests and diseases including *Fusarium* spp. (www.syngenta.com, 2006). The findings in the current study concur with those of Pataky *et al.* (2000) who reported that maize seeds treated with the insecticide thiamethoxam decreased the incidence of Stewart's wilt. Moraes *et al.* (2003) revealed that metalaxyl-m provided good control of the disease caused by *F. verticillioides* (Sacc.). Furthermore, Wang *et al.* (2005) found that the combination of difenoconazole, metalaxyl and fludioxonil was effective in controlling *Fusarium* root rot. Celest<sup>®</sup> XL contains fludioxonil, which is one of the active ingredients registered for the control of *Fusarium* and *Rhizoctonia* species (www.syngenta.com, 2006). Kojima *et al.* (2004) found that various fungi including *F. solani* (Mart.) Sacc., were unable to infect the host in the presence of fludioxonil. Celest<sup>®</sup> XL also contains mefenoxam which according to McGovern (2001) was effective for the control of diseases caused by *Fusarium* spp. In the current study thiram and Apron<sup>®</sup> XL (mefenoxam) did not control the disease caused by *F. graminearum*. In contrast, Xue (2003) revealed a significant inhibition of *F. solani* by thiram. However, in the current study it cannot be absolutely concluded that thiram and Apron<sup>®</sup> XL failed to control the pathogen because the observations were made at an early seedling growth stage (21 d). In addition, *F. graminearum* is not a damping-off pathogen and can therefore still cause serious damage at later growth stages. Consequently, there is a need to examine the effect of thiram and Apron<sup>®</sup> XL treated seeds against *F. graminearum* at different plant growth stages. Apron<sup>®</sup> XL is a systemic fungicide, which is translocated into the plants and therefore can provide good control of the disease even at a later growth stage.

The application of Apron<sup>®</sup> Star 42 WS, Celest<sup>®</sup> XL and thiram to seeds improved both the shoot dry and root dry mass of plants in the inoculated soil when compared to the untreated control. The improved shoot dry and root dry mass by Apron<sup>®</sup> Star 42 WS, Celest<sup>®</sup> XL and thiram is due to their broad-spectrum activity. In addition, these fungicides have been reported to reduce storage fungi (Alabi & Emechebe, 1990; Smith *et al.*, 1999), which can hamper the growth of plants and reduce their performance. The results of this study concur with Xue (2003) who indicated that

thiram increased shoot length of pea (*Pisum sativum* L.) by 29% when compared to the control. Smith *et al.* (1999) concluded that fludioxonil and thiram improved shoot and root length in cowpeas (*Vigna unguiculata* (L.) Walp.). Thiram promoted growth of plants even in the presence of *F. graminearum* in the current study. Apron<sup>®</sup> XL did not improve both the dry shoot and dry root mass of plants grown in the inoculated soil possibly because it failed to control the pathogen and the seedlings that emerged from seeds treated with this fungicide were stunted. This however was not due to phytotoxicity as the dry shoot mass and dry root mass were statistically similar to the untreated control for all the fungicide treated seeds in the uninoculated soil.

The *in vivo* study showed that all the fungicide treated seeds maintained the same emergence percentage as the untreated control. Apron<sup>®</sup> Star 42 WS and Celest<sup>®</sup> XL could be applied to the seeds before planting in a field infected with *F. graminearum* as they controlled the disease caused by this pathogen under greenhouse conditions. It is suggested that the same fungicides be tested against this pathogen but under field conditions. There is a need for future studies to test the efficacy of thiram and Apron<sup>®</sup> XL on maize seedlings at later growth stages since the observations have been made on very young seedlings during the earlier stage of growth.

## CHAPTER 5

### GENERAL CONCLUSION

Shurtleff (1980) reported that maize is subjected to attack by several fungal pathogens including *Fusarium graminearum* Schw. This plant pathogen represents a serious threat in the maize field production, resulting in the reduction of seed quality (McGee, 1988). Numerous fungicides have been and are continually being developed in order to control this pathogen and therefore to improve seed quality.

The *in vitro* study revealed that all the fungicide treated seeds maintained a high germination percentage although they did not differ from each other with Apron<sup>®</sup> XL having the highest rate (83%) and the lowest being Celest<sup>®</sup> XL (79%) (Chapter 3). We assume however, that *Rhizopus*, which invaded most seedlings in the laboratory during the experiment, hampered the germination of the seed. Since it was established that the ISTA standard germination test for maize does not predict field emergence where this plant is sown there is a need to use vigour tests to determine the performance of a seedlot under stress conditions. The fact that standard germination often overestimates field capacity is reflected in the *in vivo* greenhouse study where all the fungicide treated seeds had reduced emergence percentage with the exception of thiram treated seeds, which were only slightly affected (Chapter 4). The fungicide treated seeds had higher germination percentages in the laboratory than in the greenhouse. This may have been due to favourable conditions that are encountered to the laboratory.

Three tests, namely the conductivity test, tetrazolium test and fast imbibition germination test, were used to determine vigour of treated or untreated maize seed. As expected vigour of fungicide treated seeds was maintained when the seeds were slowly imbibed. This is true since the slow imbibition mimics the normal conditions of seeds imbibing water. From this experiment it was found that under stress conditions (fast imbibition), Apron<sup>®</sup> Star 42 WS, Celest<sup>®</sup> XL, Apron<sup>®</sup> XL and thiram treated seeds increased or maintained vigour, which was reflected by a reduced or equivalent seed leachate when compared to the untreated control. These results were

mirrored in the tetrazolium test. Treating seeds with these fungicides could help to maintain the same level of viable seeds as the untreated control even under stressful conditions such as subjection to fast imbibition (Chapter 3).

Apron<sup>®</sup> Star 42 WS and Celest<sup>®</sup> XL reduced the disease caused by *F. graminearum* in the inoculated soil with Celest<sup>®</sup> XL being the best treatment when compared to the untreated control. Thiram and Apron<sup>®</sup> XL failed to provide good control of the pathogen. However, *F. graminearum* is not a damping-off pathogen and can cause disease at a later stage of plant development. Accordingly, thiram and Apron<sup>®</sup> XL and the other fungicides could still provide control at later stages. In addition, the systemic fungicide Apron<sup>®</sup> XL is translocated into the plant and can protect the growing plant. The efficacy of these fungicide treatments needs to be done in the field over an extended period. The increase of dry shoot and dry root mass in the inoculated soil suggests that Apron<sup>®</sup> Star 42 WS, Celest<sup>®</sup> XL and thiram could be applied to the seeds in order to promote growth of the plants even when the soil is infected with *F. graminearum*.

Based on the results of this study all the fungicides have the potential to improve or to maintain vigour under stress conditions. The use of Apron<sup>®</sup> Star 42 WS and Celest<sup>®</sup> XL is an encouraging option for the control of the disease caused by *F. graminearum* in the maize field, especially where this pathogen represents a serious threat. There is a need to assess the effect of these fungicides as seed treatments against *F. graminearum* under field conditions where conditions differ to those in the greenhouse.

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