Efficacy and crop tolerance of Stamina (pyraclostrobin) and Flite (triticonazole) seed treatment formulations against *Fusarium*, *Pythium* and *Rhizoctonia* soilborne diseases of maize

BY

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Declaration

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

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Alinke Heste Labuschagne

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Chapter 1
Preface

1.1 Background and motivation for the study

Maize (*Zea mays* L.) has gained in popularity. Today it is produced across most of South Africa and constitutes an important part of people's staple diet (Department of Agriculture, Forestry and Fisheries, 2013). Due to poor climatic and agricultural conditions in the past that had a negative impact on the national agricultural economy, a large section of the population faced food shortages. This situation was alleviated by imports of maize (Du Plessis, 2003). In terms of food security this is not a healthy situation because not all the maize produced is made available for human consumption. Livestock also consume a portion of this yield, especially in times of drought (Du Plessis, 2003). With erratic environmental conditions in parts of South Africa,food security cannot be taken for granted and thus the need for improved methods of production arises, especially for maize as the staple food crop.

Maize as a crop is fairly genetically uniform,and has also been cultivated in monoculture for centuries. It is thus prone to many diseases. *Rhizoctonia solani* (Kuhn), *Pythium* spp. (such as *Pythium irregular* (Buism) and *Pythium ultimum* (Trow)) and *Fusarium* spp. (such as *Fusarium oxysporum* (Schlecht) and *Fusarium graminearum* (Schwabe)) cause seedling blights and damping-off diseases that have some of the most devastating effects on crops (Garrett, 1970). Currently, the only effective means of controlling these diseases is the planting of seeds that are pre-treated with fungicides.

Young seedlings have a particularly low level of resistance to infection and survival of the seedlings of most plant species is mostly due to escape of seedling blights and damping-off diseases (Garrett, 1970) caused by pathogens such as *Pythium, Fusarium* and *Rhizoctonia* species. These pathogens are mainly unspecialized and have a wide distribution as saprophytes in the soil.

1.2 Objectives of the study

The primary aims of the study were to determine the efficacy of specific fungicide seed treatments for control of soil- and seed-borne pathogens of maize and to test forpossible

phytotoxic effects of the seed treatments, including any effect on the seed germination/seed vigour of maize.

The specific objectives of the study were to:

a) Determine the efficacy of the fungicides Stamina (pyraclostrobin, BAS 500 12 F) and Flite (triticonazole, BAS 595 05 F), a combination of Stamina and Flite, and the industry standard, Celest® XL (fludioxonil & mefenoxam) as seed treatments of maize against pathogenic strains of *Pythium, Fusarium* and *Rhizoctonia* spp. *in vitro*. For simplicity's sake and because combinations of fungicides are often used in treatments, the fungicide names (Stamina, Flite and Celest® XL) are used instead of the product codes or active ingredients throughout this dissertation.
b) Determine the effects that the fungicide treatments have on germination, seed vigour and emergence of maize by testing treated and untreated seed according to the International Seed Testing Association (ISTA) standards by means of a standard germination test, a cold soil test, rate of germination, moisture contentdetermination and an accelerated ageing test.
c) Assess any possible long-term effects of the seed treatments on seed germination and vigour by conducting accelerated ageing and long-term storage tests.
d) Determine the efficacy of the fungicides Stamina (pyraclostrobin), Flite (triticonazole), a combination of Stamina and Flite, and Celest® XL (fludioxonil & mefenoxam) as seed treatments of maize against pathogenic strains of *Pythium ultimum, Fusarium* spp. and *Rhizoctonia solani* in greenhouse trials. The effect of the treatments on the germination and vigour of the seedlings were also determined.

1.3 Structure of the dissertation

Chapter Two: This chapter contains a literature review of the history of maize production globally and in South Africa, as well as background information on the soil-borne pathogens *Pythium*, *Fusarium* and *Rhizoctonia* spp. Different chemical control measures for the damping-off diseases caused by these fungi are discussed. The literature on maize germination and vigour tests such as the standard germination test, the accelerated ageing test and the cold soil test is also reviewed.

Chapter Three: This chapter reports on the isolation, pathogenicity testing and *in vitro* inhibition of *Pythium ultimum*, *Fusarium* spp. and *Rhizoctonia solani* by Stamina (pyraclostrobin), Flite (triticonazole)and Celest® XL (fludioxonil & mefenoxam). Furthermore, the effect of seed treatment with the various test fungicides on seed-borne fungal contamination was assessed by plating the variously treated seeds onto potato dextrose agar and recording the resultant fungal growth after incubation.

Chapter Four: The germination vigour of two maize cultivars was evaluated by means of the standard germination test, rate of germination test, moisture content, 2-day and 4-day accelerated ageing as well as 3-month and 6-month long-term storage and cold soil test. These tests were conducted on treated seeds with the respective test fungicides and compared to untreated control seeds in order to establish whether the treatments had any adverse effects on germination and vigour.

Chapter Five: The efficacy of the test fungicides on the disease incidence due to *Pythium*, *Fusarium* and *Rhizoctonia* spp. inoculation was assessed in seedling trays in the greenhouse. Emergence, plant height and dry root and shoot mass of the plants were also recorded.

Chapter Six: In this chapter the overall findings of all the experiments are discussed and summarized and conclusions are drawn.

1.4 Literature cited

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DuPlessis, J., 2003. Maize production. Directorate of Agricultural Information Services, Department of Agriculture, Republic of South Africa pp 1-34.

Garrett, S.D., 1970. Pathogenic root-infecting fungi.Cambridge University Press pp 6-45.

Chapter 2
Literature Review

Introduction

Maize (*Zea mays* L.) is the most important global grain crop with 830 million tons being produced around the world in the 2010-2011 season (International Grains Council, 2013). Since the maize plant is genetically fairly uniform and has been cultivated in monoculture for many centuries, it is prone to many plant diseases. Some of the most devastating diseases are the seedling blights and damping-off caused by pathogenic fungi such as *Rhizoctonia solani* (Kuhn), *Pythium* and *Fusarium* species (Carroll, 2004). Currently, control of these diseases entails only planting seeds that are pre-treated with fungicides. Chemical treatments for control of maize damping-off diseases include pyraclostrobin, which is part of the strobilurin group of fungicides, triticonazole, which groups with the triazoles, fludioxonil, a member of the phenyl pyrrole group, and metalaxyl, which groups with the phenylamides (Pesticide Properties Database, University of Hertfordshire, 2010). When attempting to overcome damping-off diseases, it is also essential that only seeds of the best quality and with a high vigour are planted. Seed quality can be determined by testing the moisture content of the seed since seeds with too high a moisture content will have a very short shelflife (ISTA, 2012). A standard germination test should also be done in order to assess the germination potential of the seed. Germination vigour tests, such as the rate of germination test, the cold soil test and the accelerated ageing test, give an indication of how germination is affected by adverse environmental conditions (ISTA, 1995). These tests are invaluable when trying to estimate the actual field germination potential of the seed, since field conditions are rarely, if ever, ideal. These topics will be discussed in this literature review.

2.1 Maize production in South Africa

Maize as a crop plant is native to North America where its cultivation was developed by nativeAmericantribes many centuries ago. Maize was introduced into South Africa from the West Indies by the Portuguese shortly after the arrival of the first Dutch colonists (Saunders, 1930). Since then, the crop has gained popularity and today it is produced across most of the country and constitutes an important part of the South African staple diet **(**Department of Agriculture, Forestry and Fisheries, 2013).

In South Africa, maize is the most important grain crop.It is produced in many varying environments countrywide (DuPlessis, 2003) and is both a major feed grain and the staple food for the majority of the population. The gross value of maize production annually is around 15billion rand (Figure 1). About 60% of the maize produced in South Africa is white maize and about 40% yellow maize (Department of Agriculture, Forestry and Fisheries, 2013). Maize is mostly considered to be a breakfast cereal; however, in a processed form it is also used as fuel (ethanol) and starch (DuPlessis, 2003). Starch in turn can be enzymatically converted into products such as sorbitol, dextrin, sorbic and lactic acid, and appears in common household items such as beer, ice cream, syrup, shoe polish, glue, fireworks, ink, batteries, mustard, cosmetics, aspirin and paint (DuPlessis, 2003).

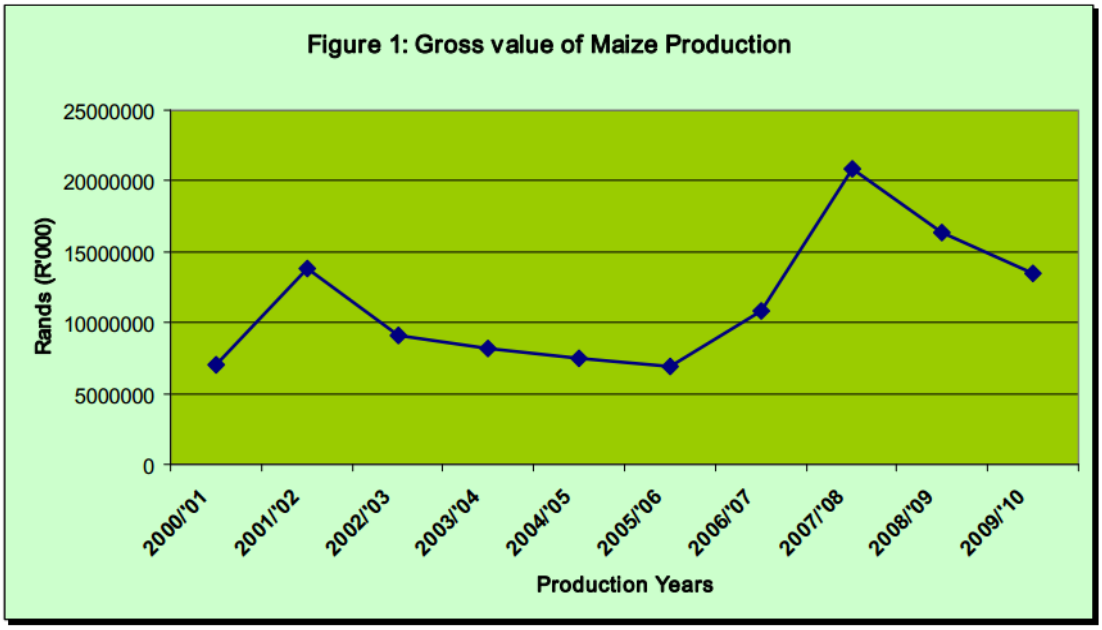

Figure 1: Gross value of maize production in South Africa (Department of Agriculture, Forestry and Fisheries, 2013).

2.2 Seedling diseases of maize

Seed and seedling diseases of maize can reduce plant survival, growth and yield. These diseases are most common under conditions of environmental stress and when seed quality has been compromised (Agarwal & Sinclair, 1996). Symptoms of these diseases include pre-emergence and post-emergence damping-off, low seedling vigour, stunting and discolouration and decomposition of the roots and mesocotyl (Dodd & White, 1999). Symptoms can result from

infection by many different fungal pathogens including *Pythium* spp., *Fusarium* spp. and *Rhizoctonia* spp. (Solorzano & Malvick, 2011).

2.2.1 *Rhizoctonia*

Rhizoctonia solani (Kuhn) has been isolated from maize seed in a number of countries, including the USA, Canada, Thailand, India, Australia, France, Nepal, the United Kingdom, Hungary, Pakistan and South Africa (Naiz & Dawar, 2009).The survival of *R. solani* in soil is highly dependent on the presence of plant tissue, as can be seen in the disease cycle (Figure 2). The pathogen grows rapidly from this energy source, but as soon as it is depleted the pathogen survives poorly due to competition (Papavizas *et al*., 1975). *R. solani* survives best in soils at temperatures above 5°C and below 25°C. The activity of the pathogen is limited to the top 10cm of the soil (Papavizas *et al*., 1975). The genus *Rhizoctonia* causes diseases worldwide, leading to losses in almost all flower and vegetable crops, several turfgrasses and field crops and even shrubs, trees and perennial ornamentals (Adam, 1988). The most common symptoms of disease include damping-off of seedlings as well as root rot, stem rot and stem canker. *Rhizoctonia* can also cause foliage blights and spots, particularly on plant parts near the soil line (Guttierez *et al.*, 1997). Damping-off, the most common and severe symptom of *Rhizoctonia* infection, occurs mainly in cold, wet soils, and young seedlings are either killed pre-emergence or soon after they emerge (Dodd & White, 1999). Post-emergence, the fungus attacks the stem, causing water-soaked lesions with the stem eventually becoming soft and unable to support the weight of the growing seedling, thus the young plant collapses and decays (Guttierez *et al.*, 1997). In older seedlings, the fungus invades the outer cortical tissue, forming reddish-brown lesions, which may increase in size until they eventually girdle the plant, causing its death (Guttierez *et al.*, 1997). Root lesions may also form, both in young seedlings and mature plants. These lesions are reddish-brown and mainly occur below the soil line but under favourable conditions (cool, wet weather) the lesions will increase in size to cover the entire base of the plant and most of the roots, thus leading to weakening, chlorosis and even death of the plant (Peltier *et al*., 2010). Rhizoctonia diseases are difficult to control; however, disease can be avoided by avoiding poorly drained areas. Only disease-free seed should be planted under conditions that promote the rapid development of the seedlings. There should be wide spacings between the plants in order to promote good aeration (Dodd & White, 1999).

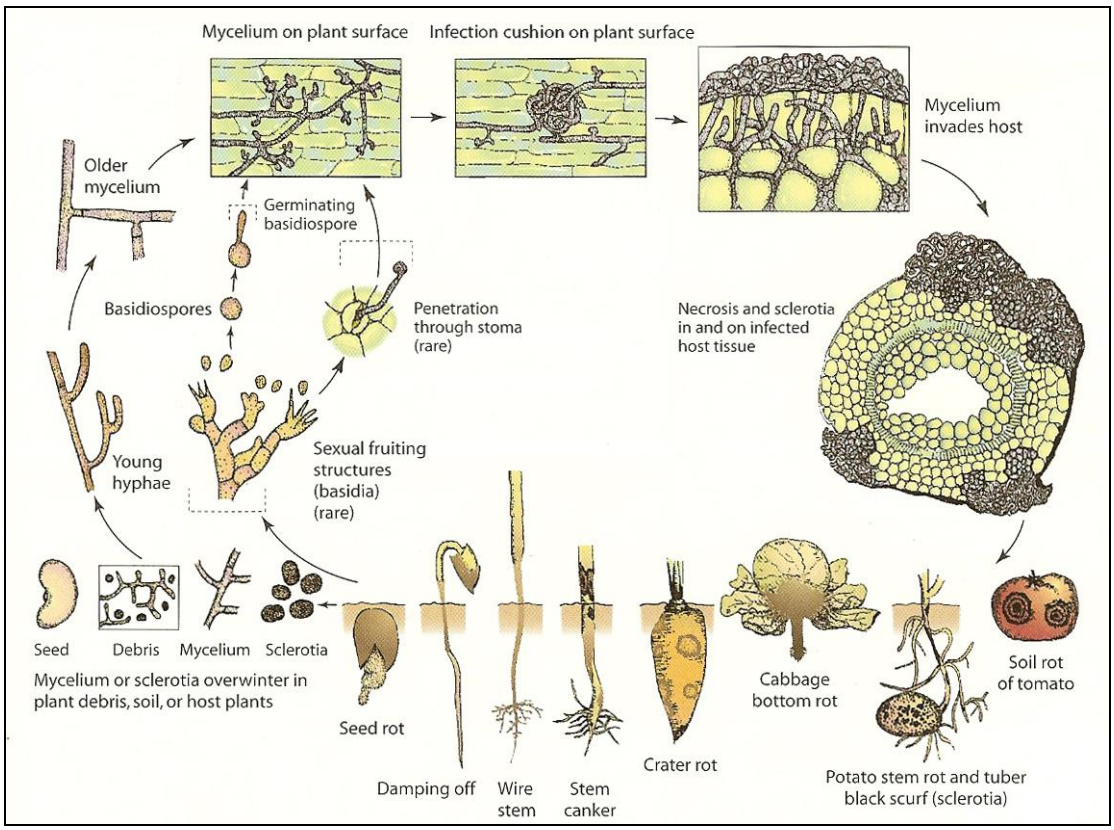

Figure 2: Disease cycle of *Rhizoctonia solani* (Agrios, 2005).

The genus *Rhizoctonia* is a very large group of complex and diverse fungi consisting of three genera of Basidiomycota, namely *Thanatephorus* (Frank)(anamorph *R. solani* Kuhn), *Ceratobasidium* (Rogers)(anamorph binucleate *Rhizoctonia*) and *Wairea* (Warcup and Talbot)(anamorph *R. zeae* Voorhees, *R. orzae* Ryker and Gooch) (Singleton *et al.*, 1992). *R. solani* (*T. cucumeris* (Frank)) can be divided into 13 numbered anastomosis or mating groups (AGs) which are characterized by hyphal fusion between paired strains from the same group.The AGs can be further divided into more than 20 subgroups, all of which are genetically distinct as shown by molecular analysis (Roberts, 2000). All *Rhizoctonia* fungi occur mostly as sterile mycelium and sometimes as small sclerotia that show no internal tissue differentiation. Some species of *Rhizoctonia* are multinucleate while others are binucleate. The mycelium is colourless when young and turns yellowish or light brown with age. It consists of long cells which branch at approximately right angles to the main hyphal strand (Agrios, 2005).At the junction, a slight constriction forms and there are no cross-walls (Figure 3). The morphology of the mycelium can be used in identifying this fungus.

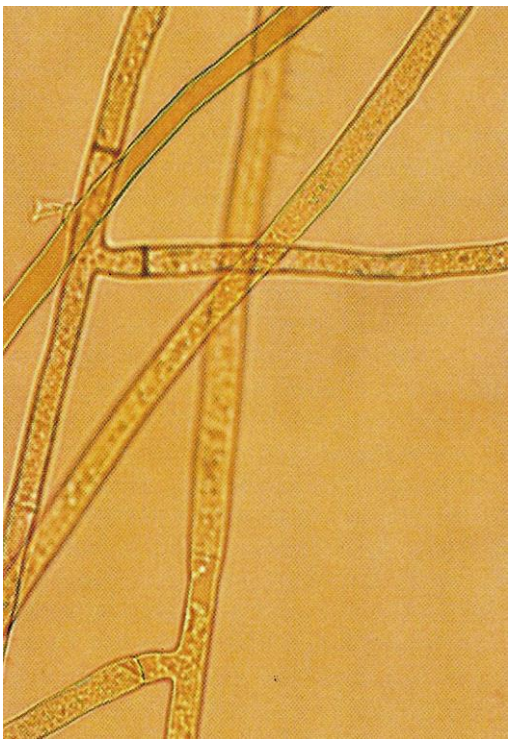

Figure 3: Typical *Rhizoctonia* mycelium, showing right-angle branching, constriction and septa close to the branching point (Photograph by Plant Pathology Department, University of Florida, as cited in Agrios, 2005).

2.2.2 *Pythium*

Pythium spp. are frequently associated with seed and seedling diseases and have been commonly isolated from maize early in the season when the soils are moist and cool (Rao *et al*., 1978). In studies done in Ohio, *P. arrhenomanes* Drechs., *P .dissotocum* Drechs., *P. graminicola* Subramanian, and *P. ultimum* Trow were isolated from maize seedlings (Rao *et al*., 1978; Lipps & Deep, 1991). Other commonly isolated *Pythium* species are *P. irregular* Buis and *P. debaryanum* Hesse (McGee, 1988; Mao *et al*., 1998). As maize kernels germinate, seed-borne species of *Pythium* grow and attack the plumule, causing death of the seedling pre-emergence (Kommendahl & Windels, 1986).

The genus *Pythium* was created by Pringsheim in 1858. It falls within the family Pythiaceae, order Peronosporales and class Oomycetes and consists of over 120 species (Singleton *et al.*, 1992). The Oomycetes are not true fungi (Kingdom Fungi, Eumycota), but are closely related to algae and free-living photosynthetic groups and are classified in their own kingdom, Straminipila (previously Chromista). The name Oomycota means ‘egg fungi’ and refers to the round oogonia containing the spherical oospores (Kamoun, 2009). *Pythium* also produces zoospores, which are biflagellate with an anterior tinsel and a posterior whiplash flagellum. The hyphae are coarse and

coenocytic with few cross-walls. Chlymydospores may also be produced with appressoria (Kamoun, 2009).

Pythium causes the greatest damage to seed and seedling roots during germination, either pre-emergence or post-emergence. The extent of the losses caused varies depending mostly on the soil moisture and temperature. Older plants are seldom killed when infected with this damping-off pathogen, but they develop root and stem lesions and root rots. Their growth is also drastically retarded and their yield considerably reduced (Kamoun, 2009). Seeds that are infected by the pathogen often fail to germinate and become mushy and then turn brown and shrivel, finally disintegrating (Dodd & White, 1999). Young seedlings can be infected pre-emergence at any point on the developing plant and the infection spreads rapidly from this point. The invaded cells collapse and the seedling dies (pre-emergence damping-off)(Broders *et al*., 2007a). Once the seedlings have emerged they can still be infected at the roots or stem below the soil line. The infected areas soon become water-soaked and discoloured and collapse. The basal part of the seedling stem becomes softer and much thinner than the rest of the stem, and as a result the seedling falls over (Broders *et al*., 2007a). The fungus continues to invade the fallen seedling which then withers and dies (post-emergence damping-off). In older plants, *Pythium* may kill rootlets or cause lesions on the roots and stem, which leads to the plants becoming stunted, resulting in yield loss (Broders *et al*., 2007a).

Pythium produces a white, rapidly growing mycelium. The mycelium produces sporangia, which germinate directly by producing one or many germ tubes or producing a short hypha at the end of which forms a balloon-like secondary sporangium called a vesicle (Figure 4). Each vesicle releases 100 or more zoospores, which form cysts and then germinate, forming a germ tube. This germ tube directly penetrates the host tissue (Agrios, 2005). The mycelium of *Pythium* also gives rise to antheridia (club shaped) and oogonia (spherical). The antheridium fertilizes the oogonium to form a thick-walled survival spore called an oospore (Kamoun, 2009). Oospores are resistant to adverse temperature and moisture conditions. Temperatures above 18°C favour germination by means of germ tubes while temperatures between 10°C and 18°C induce germination by means of zoospores (Botha, undated).

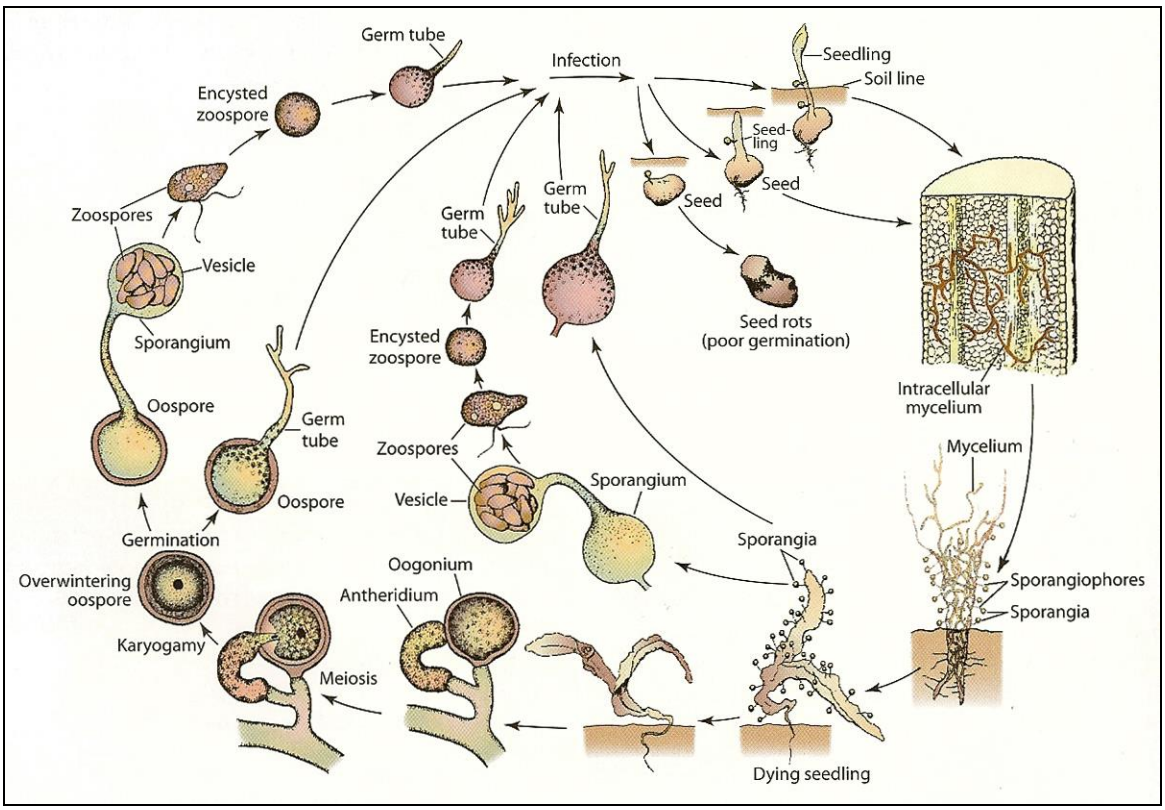

Figure 4: Disease cycle of *Pythium* damping-off (Agrios, 2005).

2.2.3 *Fusarium*

Many *Fusarium* spp. have been isolated from maize seed, including *Fusarium crockwellense* Burgess Nelson & Toussoun, *F. cladosporium* Wollen & Reinking, *F. culmorum* (W.G.Smith) Sacc., *F. equiseti* (Corda) Sacc., *F. graminearum*, Schwave., *F. moniliforme* Sheld., *F. nivale* (Frs) Cess., *F. oxysporum* Schlecht., emend.synd & Hans., *F. proliferatum* (Matsushimal Nirenverg), *F. semitectum* Berk & Rev., *F. solani* Mart., *F. subglutinans* Wr. & Reinle (Naiz & Dawar, 2009).

The genus *Fusarium* belongs to the class Sordariomycetes, order Hypocreales (Kistler, 2001) and produces macroconidia, microconidia and chlamydospores (Singleton *et al.*, 1992). All macroconidia have a foot-shaped basal cell when produced in conidiophores (although this may not be distinct in all species). The shape of the macroconidium is the key criterium used in taxonomic classification (Singleton *et al.*, 1992). The teleomorph stage, if formed, is the Hypocreales. *Fusarium* species are very variable when observed in culture because they readily undergo phenotypic changes in response to different environments (Singleton *et al.*, 1992). The fungus can survive in the soil as either mycelium or spores even in the absence of host plants.

When a host is present, mycelium from germinating spores penetrates the host and enters the xylem, where it causes wilting symptoms (Broders *et al.*, 2007b).

One of the major problems caused by *Fusarium* infection, other than low germination rate and damping-off, is the production of several mycotoxins, most importantly fumonisins (Nayaka *et al.*, 2008). Fumonisins, which were discovered in 1988, are produced by *F. verticillioides* (Sacc.), *F. proliferatum* (Masushima) and several otherfusaria. These fumonisins contaminate food and feed and are implicated in many side-effects such as oesophageal cancer (Nayaka *et al.*, 2008). *Fusarium solani* cause corneal ulcers while *F. oxysporum* produce zeralenone α and β, which cause haemorrhage and necrosis in bone marrow. *F. proliferatum* and *F. verticillioides* cause epidemiologically human oesophageal cancer (Desjardins *et al*., 2006). Anne *et al*. (2000), Curtui *et al*. (1998) and Susan *et al*. (2005) isolated several *Fusarium* species from maize seed, viz. *Fusarium verticillioides, F. graminearum, F. proliferatum, F. acuminatum, F. avenaceum, F. clamydosporium, F. equiseti, F. oxysporum, F. semitectum* and *F. torulosum* which produce mycotoxins, viz. Toxins deoxyninalenol (DON), 3-acetyl DON,15-acetyl DON, fusarenon X(FX),T-2 toxin (T2), diacetoxyscir phenol (DAS), zearalenone (ZEA), fumonisin, aflatoxin B1,ochratoxin A (OA) and citrinum(CT) respectively. DON and acetyl DON were the major mycotoxin in *Fusarium* species. Proper storage of maize seed is needed to minimize the fungalinfestation and mycotoxin production during storage and provide disease-free seeds for human consumption (Naiz & Dawar, 2009).

Fusarium is one of the major pathogen species on many agricultural crops including maize (DuPlessis, 2003). Several *Fusarium* spp. are pathogenic on maize, causing a number of diseases including seedling disease, stalk rot and ear rot. *Gibberella* stalk rot is caused by *Gibberella zeae* (Schwein), (anamorph: *Fusarium graminearum*), while *Fusarium* stalk rot is caused by *Fusarium verticillioides* (teleomorph: *Gibberella fujikuroi* (Sawada)) (Bell *et al*., 2009). Both *Gibberella* and *Fusarium* stalk rot occur worldwide (CAB international, 1998). *Gibberella* stalk rot usually occurs in cooler climates, while *Fusarium* is more widespread in warmer, dry climates (Bell *et al*., 2009). Factors contributing to disease severity include among others planting density, prevailing climatic conditions and rate of fertilization. Under favourable conditions (dry and warm), severecrop damage is known to occur (Dodd, 1980).

In the case of *Fusarium*, stalk rot caused by *Fusarium verticillioides* (a seed-borne pathogen (Bell *et al*., 2009)), damage is due to pre-mature plant death, lodging and obstruction of water and nutrient translocation during grain formation (Michealson, 1957). Damage is most severe

early in the season when the disease causes early death of the plant and inhibition of kernel filling. Lodging occurs when the plants are affected later in the season (Michealson, 1957). *Fusarium verticillioides* as a seed-borne pathogen can affect the following year's yields severely when contaminated seed is used as planting material. Precise yield loss data for most stalk rots are difficult to obtain (Bell *et al*., 2009).

2.3 Chemical seed treatments to control maize diseases

Different chemicals can control the various maize damping-off diseases when applied as seed treatments as can be seen in Table 1.

Table 1: Summary of current maize seed treatment active ingredients, examples of trade names and the pathogens they control (Peltier *et al*., 2010; European Commission: Health and Consumer Protection Directorate-General, 2007).

Active ingredient	Trade name examples	*Rhizoctonia solani*	*Fusarium* spp.	*Pythium* spp.
Pyraclostrobin	Stamina, Acceleron DX-109	x	x	x
Metalaxyl Mefenoxam	Celest® XL, Apron Maxx, Apron XL, Maxim XL			x
Fludioxonil	Celest® XL, Maxim, Maxim XL	x	x	
Triticonazole	Flite®, Tilt®, Bumper®	x	x	x

2.3.1 Pyraclostrobin

Strobilurin fungicides (such as pyraclostrobin) have a structure that is derived from a series of natural compounds such as strobilurin, oudemansin and myxothiazole. These compounds are found in several Basidiomycetes and Oomycetes (Steffens *et al.*, 1996). These fungicides act by binding to the Qo centre of the cytochrome bc_1 complex in the mitochondria, thus inhibiting electron transfer. This in turn interferes with ATP synthesis (Jin *et al.*, 2009). Pyraclostrobin is non-systemic and has a broad spectrum of activity, showing some control of each of the four major groups of plant pathogens, namely Ascomycetes, Basidiomycetes, Deuteromycetes and Oomycetes. Pathogens that are controlled include amongst others *Phytophthora infestans* (Mont.) de Bary and *Alternaria solani* Sorauer on tomatoes as well as *Rhizoctonia solani* and *Pythium aphanidermatum* (Edson) Fitzp. on turfgrass (Tomlin, 2009). Pyraclostrobin is mainly

used as a preventive fungicide but has been shown to have curative properties in some cases (Bartlett *et al*., 2002).

Pyraclostrobin-carbamate was discovered by BASF scientists in 2000 and announced in the same year, with the first sales taking place in 2002. The structure of the product is characterized by its nature as a derivative of carbamate (N-methoxycarbamate) as the toxophore group (Balba, 2007). The chemical formula of pyraclostrobin is $C_{19}H_{18}ClN_3O_4$, as isillustrated in Figure 5 (Pesticide Properties Database, University of Hertfordshire, 2010).

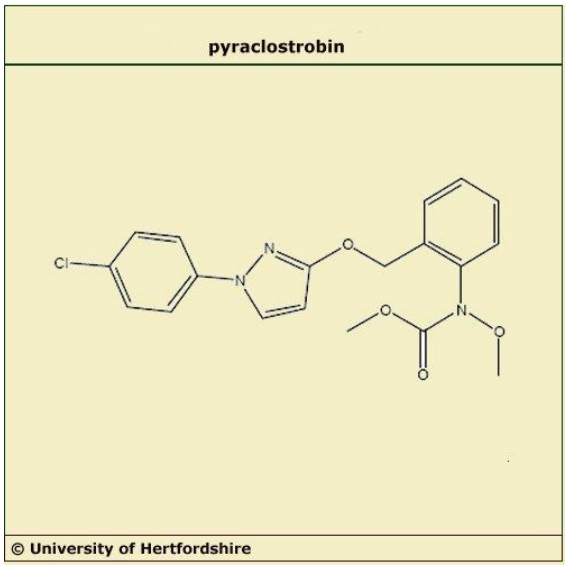

Figure 5: Structural formula of pyraclostrobin (Pesticide Properties Database, University of Hertfordshire, 2010).

2.3.2 Triticonazole

Triticonazole is part of the triazole group of fungicides. This is a large and fast-growing group of excellent systemic fungicides. This group of compounds shows long-term prophylactic and curative activity against a wide spectrum of leaf, root and seedling diseases which include leaf spots, blights, powdery mildews, rusts, bunts, etc. caused by Ascomycetes, Oomycetes and Basidiomycetes (European Commission: Health and Consumer Protection Directorate-General, 2007). They are applied as foliar sprays and as seed and soil treatments. The triazole group of fungicides are all ergosterol biosynthesis inhibitors (Schwin, 1984). Triticonazole was first registered in 1993 in France (European Commission: Health and Consumer Protection Directorate-General, 2010). Its chemical formula is $C_{17}H_{20}ClN_3O$ and is illustrated in Figure 6 (Pesticide Properties Database, University of Hertfordshire, 2010).

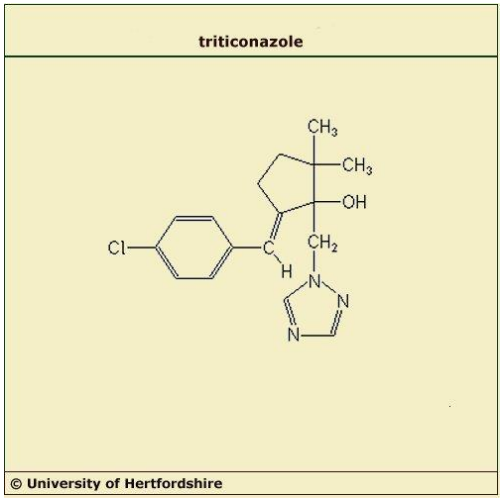

Figure 6: Structural formula of triticonazole (Pesticide Properties Database, University of Hertfordshire, 2010).

2.3.3 Fludioxonil

Fludioxonil is registered as a fungicide seed treatment for control of a range of diseases including those caused by *Fusarium, Rhizoctonia* and *Alternaria* spp. (Julia & Senn, 2005). It was first reported in 1990 and the first sales took place in 1993 in France. Fludioxonil belongs to the phenyl pyrrole group of synthetic fungicides and acts non-systemically but with a long residual activity (Julia & Senn, 2005). The mode of action of the fungicide is to inhibit transport-associated phosphorylation of glucose, thus reducing mycelial growth (European Commission: Health and Consumer Protection Directorate-General, 2010). The chemical formula of fludioxonil is $C_{12}H_6F_2N_2O_2$, as illustrated in Figure 7 (Pesticide Properties Database, University of Hertfordshire, 2010).

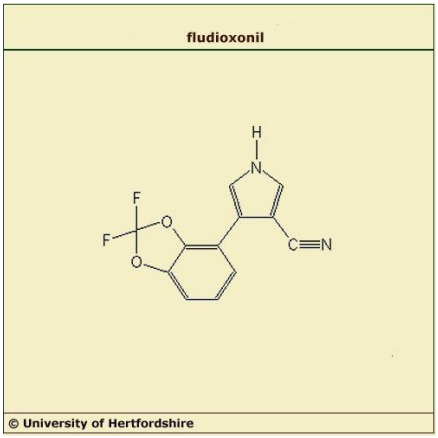

Figure 7: Structural formula of fludioxonil (Pesticide Properties Database, University of Hertfordshire, 2010).

2.3.4 Metalaxyl (mefenoxam)

Metalaxyl is a fungicide registered for the control of diseases caused by air and soil-borne Peronosporales (O'Neil, 2001). It was first reported and introduced in the USA in 1996. It falls into the phenylamide group of synthetic fungicides and it has a curative as well as preventive action (O'Neil, 2001). The fungicide works by inhibiting protein synthesis in fungi by interfering with the synthesis of ribosomal RNA (Tomlin, 2009). The chemical formula for metalaxyl is $C_{15}H_{21}NO_4$ as illustrated in Figure 8 (Pesticide Properties Database, University of Hertfordshire, 2010).

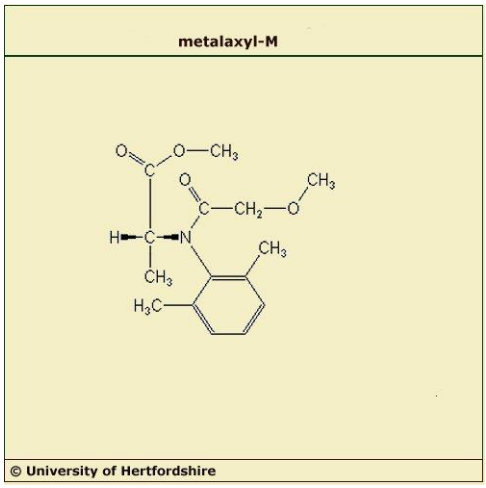

Figure 8: Structural formula of metalaxyl (Pesticide Properties Database, University of Hertfordshire, 2010).

2.4 Maize seed quality and vigour

Testing the moisture content and standard germination of seed lots gives a good indication of the quality of the seed (ISTA, 2012). If seed lots consistently perform worse than other seed lots of the same species when field conditions deteriorate, they are by definition of lower vigour (Beckendam *et al.*, 1987). In order to assess the vigour of the seed lots, tests such as the accelerated ageing test and the cold soil test should be carried out.

For many crops the results of vigour tests have been related to field performance (the cold soil test, accelerated ageing, rate of germination), seed storability (accelerated ageing) and as a measure of physical injury (conductivity, tetrazolium) (Association of Official Seed Analysts, 1983; ISTA, 1995).

2.4.1 Moisture content

Seeds can be classified into two typesin terms of moisture content, namely recalcitrant and orthodox seeds (Chin & Krishnapillay, 1989). Most seeds, including maize, fall into the orthodox category. Orthodox seeds are seeds that can be dried to a low moisture content (e.g. 0.05g H_2Og^{-1} fresh weight) and will tolerate freezing temperatures, whereas recalcitrant seeds cannot be dried and will not tolerate freezing temperatures (Chin & Krishnapillay, 1989). Harrington's rule of thumb (Beweley *et al.*, 2006) states that between 0.05 and 0.15g H_2Og^{-1}fresh weight, if the seed moisture content is increased by 1%, the lifespan of orthodox seeds will be reduced by 50%. Orthodox seeds require low (e.g. 0.05g H_2Og^{-1} fresh weight) moisture content for successful long-term storage. At physiological maturity, the moisture content of orthodox seeds is between 0.3 and 0.5g H_2Og^{-1} fresh weight, after which they undergo drying in order to reach a harvest moisture content of 0.15-0.2g H_2Og^{-1} fresh weight (Chin & Krishnapillay, 1989). Most maize is harvested at 22–30% moisture and then further dried to 15.5% moisture for safe storage, preventing microbial growth (McDonough *et al*., 2004).

2.4.2 Standard germination

The standard germination test is used to effectively determine the germination potential of a particular seed lot under ideal conditions (ISTA, 2012). In the case of maize this is done by planting seeds in rolls of germination paper and incubating them at 25°C for 7 days (ISTA, 2012) (Figure 9). Seedlings are then categorized in terms of normal, abnormal, hard, fresh and dead according to the seedling definitions established by the International Seed Testing Association (ISTA, 2012). Although a standard germination test provides invaluable information, additional tests such as accelerated ageing and the cold soil test can also determine the vigour of the seed lot (ISTA, 2012).

Figure 9: Four sheets of germination paper rolled up and placed in a plastic bag in an upright position for the between-paper germination method.

2.4.3 Rate of germination

Matthews and Khajeh-Hosseini (2006) demonstrated that the faster the seedling emergence of a maize seed lot (lower mean emergence time, MET), the greater its final emergence and seedling growth. Therefore, measurement of mean germination time, the reciprocal of which is the rate of germination, is a possible alternative to the cold soil test for the assessment of the germination vigour of a maize seedlot (Matthews & Khajeh-Hosseini, 2006).

2.4.4 Cold soil test

The cold soil test is one of the most widely used and most reliable vigour tests for maize (Isley, 1950; Crosier, 1958; Burris & Navratil, 1979). Estimating field emergence under adverse planting conditions (vigour) is essential when attempting to manage crops effectively (Burris & Navratil, 1979). Laboratory cold soil tests attempt to mimic stress factors that are assumed to be present at the early stages of planting (Hooks & Zuber, 1963). Three stress factors that are incorporated into most cold soil test methods are temperature, excessive moisture leading to only slightly aerobic conditions and pathogen stress. This is achieved by using the between-paper germination method but placing the seeds on un-sterilized soil obtained from a maize field (Figure 10) and incubating the seed at 5°C for 7 days (ISTA, 2012). Of the three stress factors, the most success has been achieved in relating seed-bed temperature and emergence, both in the field and in the laboratory (Alessi & Power, 1971). It has also been reported that low

imbibitional temperature can cause a significant reduction in the subsequent seedling vigour of maize (Obendorf, 1972).

Figure 10: Maize seedlings after undergoing the cold soil germination test.

2.4.5 Accelerated ageing

Accelerated ageing is a reliable vigour test for seed of various crops including maize (Association of Official Seed Analysts, 1983; ISTA, 2012). The test has been used to predict seed storability (Delouche and Baskin, 1973) and has performed well in predicting the field emergence of maize (Medina & Filho, 1991). During an accelerated ageing test, seeds are exposed to high humidity (95–100%) and temperature (35°C) in order to simulate seed degradation and stress during long-term storage (Figure 11)(ISTA, 2012).

Cereal grains such as maize can be successfully stored for long periods of time without microbial spoilage occurring; however, as the seeds age, biochemical changes do occur. The grain respires, which means that dry matter is lost and that functional and nutritional aspects of the grain are altered (Reed, 1992).

Figure 11: Maize seeds placed on a wire mesh in a container used for the accelerated ageing test.

2.5 Conclusion

Pythium, *Fusarium* and *Rhizoctonia* are three pathogens that cause major economic losses due to damping-off diseases in maize (Garrett, 1970). Fungicide seed treatments for control of these pathogens need to be thoroughly tested to ensure that they control the diseases effectively and that they do not have any negative effects on the germination or vigour of the maize seed (Anaso *et al*., 1989; Rane & Ruhl, 2002). The germination and vigour of maize seed can be reliably determined by tests such as the standard germination test, the rate of germination test, the cold soil test and the accelerated ageing test (ISTA, 2012; Matthews & Khajeh-Hosseini**,** 2006). The efficacy of the fungicides can be tested *in vitro*, but this in isolation is not sufficient to give an accurate representation of what the field performance of the fungicide treated seeds will be, and thus greenhouse and field trials should be conducted as well (Allen *et al*., 2004).

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Chapter 3
Isolation of *Pythium, Fusarium* and *Rhizoctonia* from Soil and *in vitro* Efficacy of Stamina, Flite and Celest® XL against these Pathogens

Abstract

The genera *Pythium, Fusarium* and *Rhizoctonia*are three pathogens that cause severe losses due to damping-off diseases in maize. The aim of the following experiments was to isolate these three pathogens, test their pathogenicity and then obtain pathogenic cultures in order to test the efficacy of Stamina, Flite and Celest® XL for controlling the pathogens *in vitro*. Fungi were isolated from various plant and soil samples and subjected to pathogenicity testing both in Petri plates and using agar plug inoculations in a between-paper germination test method. Two pathogenic isolates were obtained, one *Fusarium* sp. and one *Rhizoctonia* sp. Monsanto DKC78-15B seeds were treated with Stamina, Flite, a combination of Stamina and Flite, and Celest® XL in the form of a slurry. Two experiments were conducted using these isolated fungi as well as fungal isolates obtained from the University of Pretoria's culture collection and the Agricultural Research Council. Firstly, PDA was amended with fungicides at a rate of 1, 2 or 3ppm. The three different fungi were plated onto these amended PDA plates and the diameter of the fungal growth was measured. It was found that Flite, as well as Stamina plus Flite, inhibited *Fusarium* at all concentrations, while Stamina on its own did not exhibit a significant level of control. *Pythium* was perfectly controlled by the 3ppm concentration of Flite as well as all combination treatments of Stamina and Flite. *Rhizoctonia* was significantly controlled by all Stamina and combination treatments as well as by the 3ppm treatment of Flite. This experiment was repeated a second time with different fungal isolates and similar results were obtained. It was also found that Celest® XL achieved perfect control of all pathogens at all concentrations. Secondly, treated seeds were plated onto PDA. In this case the fungi isolated from the seeds were found to be common saprophytic fungi, mainly *Acremonium* sp. and *Trichoderma* sp. Overall, from the above experiments it can be concluded that Celest® XL and a combination of Stamina and Flite provided the highest possible level of control of *Pythium, Fusarium* and *Rhizoctonia* spp. *in vitro*.

3.1 Introduction

Diseases that affect seeds prior to or shortly after germination are termed "seed rots" and are caused by various fungi, including *Pythium, Fusarium* and *Rhizoctonia* (Harman & Stasz, 1986).

Seedling blights or "damping-off" diseases can be divided into two groups, namely pre-emergence and post-emergence damping-off. In the case of post-emergence damping-off, the lower stems and roots of the seedlings are affected from emergence until the second or third leaf stage of the plant (Suryanarayana, 1978). Damping-off of maize caused by *Pythium* and *Fusarium* species are extremely destructive diseases affecting seedling stands and therefore yield (Mao *et al*., 1998). *Fusarium verticillioides* Sheld, *F. subglutinans* Tossoun and Marasas and *F. graminearum* Shwabe are the species most frequently isolated from maize seeds and are also most frequently causal agents of seedling blights (Bell *et al*., 2009). *Fusarium* species are also the causal agents of stalk and ear rot in maize (Bell *et al*., 2009). The most common and severe symptom of *Rhizoctonia* infection on maize is damping-off, which mainly occurs in cold, wet soils. Young seedlings are either killed pre-emergence or soon after they emerge (Dodd & White, 1999).

Pythium is an Oomycete, which infects the root tips and mesocotyls of germinating seeds and is found in the soil in all the areas where maize is produced (Botha, undated). Some commonly isolated *Pythium* species are *P. irregular* Buis, *P. debaryanum* Hesse and *P. ultimum* Trow (McGee, 1988; Mao *et al*., 1998). When maize kernels germinate, seed-borne species of *Pythium* grow and attack the plumule, causing death of the seedling pre-emergence (Kommendahl & Windels, 1986). *Fusarium* belongs to the class Sordariomycetes, order Hypocreales (Kistler, 2001). Several species of *Fusarium* have been associated with diseases in maize at all growth stages, causing seed, root, stalk and ear rot. In many cases these are either soil- or seed-borne pathogens (Dodd & White, 1999). *Rhizoctonia* forms part of a very large and complex group of diverse fungi consisting of three genera of Basidiomycota (Singleton *et al.*, 1992). *Rhizoctonia* causes severe losses in maize yield due to pre-emergence damping-off (Dodd & White, 1999).

One of the most economical and easiest methods of protecting seeds from such fungal infections is by pre-treating the seeds with fungicides (Anaso *et al*., 1989; Rane & Ruhl, 2002). Prior to sale, the vast majority of commercially produced maize seed is treated with a fungicide to protect the seed from fungal infection (Kommendahl & Windels, 1986; Munkvold & O'Mara, 2002). Pyraclostrobin (the active ingredient of Stamina) is a strobilurin fungicide which was derived from natural strobilurins found in several Basidiomycetes and Oomycetes (Steffens *et al.*, 1996). It is non-systemic and acts by interfering with ATPsynthesis, thus it has a broad spectrum of activity (Jin *et al.*, 2009; Tomlin, 2009). Triticonazole (the active ingredient of Flite) is a triazole fungicide. It has excellent systemic properties and long-term prophylactic and curative properties against many leaf, root and seedling diseases caused by Ascomycetes, Basidiomycetes and

Oomycetes (European Commission: Health and Consumer Protection Directorate-General, 2007). The active ingredients of Celest® XL are fludioxonil and mefenoxam. Fludioxonil is a seed treatment for the control of *Fusarium*, *Rhizoctonia* and *Alternaria* spp. and is a phenyl pyrrole fungicide which is non-systemic but has a long residual activity (Julia &Senn, 2005). Metalaxyl (mefenoxam) controls air and soil-borne Peronosporales (O'Neil, 2001). It has both curative and prophylactic action and groups with the phenylamides. Its mode of action is to interfere with ribosomal RNA synthesis, thus inhibiting protein synthesis (Tomlin, 2009).

The aim of this chapter was to isolate *Pythium*, *Fusarium* and *Rhizoctonia* spp.from soil and plant material and then determine the efficacy of three different fungicides (Stamina, Flite and Celest® XL) against these pathogens *in vitro*. In the following chapters, the effect of the three fungicides on the germination and vigour of maize seed are tested and the efficacy of the fungicides for controlling *Pythium*, *Fusarium* and *Rhizoctonia* spp.are evaluated in the greenhouse.

3.2 Materials and methods

Pythium spp., *Fusarium* spp. and *Rhizoctonia* spp. were isolated from different soil and plant samples of maize obtained from BASF – Delmas CoSar experimental farm and PANNAR in Greytown as well as the University of Pretoria's long-term maize trial on the L.C. de Villiers experimental farm. After isolation, all fungi were identified by light microscopy using Leslie & Summerell (2006) as a reference for *Fusarium*, Van der Plaats-Niterink (1981) for *Pythium* and Sneh *et al*. (1996) for *Rhizoctonia*.

3.2.1 Isolation of *Pythium*

Plant material was surface sterilized by rinsing in 70% ethanol, then placing in 0.5% sodium hypochlorite for 5 minutes and rinsing in sterile, distilled water. The plant material was then plated onto PARP (pimaricin + ampicillin + rifampicin + pentachloronitrobenzene (PCNB) agar) (Jeffers & Martin, 1986). After incubation at 25°C for 48h, the plates were observed under acompound microscope and cultures that were identified were plated ontopotato dextrose agar (PDA, Biolab). Soil samples were processed as follows:10g of soil was placed in a 236ml plastic cup and covered with 90ml of distilled water, and 20 citrus leaf disks (5mm diameter) were floated on top of the water in each cup and incubated for 48h at 25°C(12h day/night light cycle). The citrus leaf disks were then plated onto the PARP medium and incubated for 7 days at 25°C on a 12h day/12h dark/ light cycle. Two *Pythium ultimum* var. *ultimum* isolateswere also

obtained from Dr Wilhelm Botha of the Agricultural Research Council (ARC), Roodeplaat, Pretoria**.** These isolates were obtained from maize soil.The isolate codes were Py495 and Py496.

3.2.2 Isolation of *Fusarium*

Plant material was cut into squares of approximately 2.5mm^2 and surface sterilized by rinsing in 70% ethanol, then placing in 0.5% sodium hypochlorite for 5 minutes and rinsing in sterile, distilled water. The squares were plated onto Rose Bengal Glyceraldehyde Urea (RBGU) (Van Wyk *et al*., 1986) selective medium. After incubation at 25°C for 7 days (12h day/ 12h night light cycle), the plates were observed under a compound microscope and the cultures that were identified were plated onto PDA (Biolab). Soil samples, also obtained from the above-mentioned sources, were processed by placing 10g in 90ml of distilled water and vortexing the samples. A dilution series was then made by placing 10ml of this suspension into 90ml of water. This process was repeated until 10^{-4} and 10^{-5} dilutionswereobtained. These dilutions were then plated onto RBGU medium and incubated at 25°C for 7 days on a 12h day/ 12h dark/ light cycle. Pure cultures that were obtained were plated onto PDA. More *Fusarium* sp. isolates that had been isolated from maize roots were obtained from the University of Pretoria culture collection (*Fusarium oxysporum*, UPGH 107, *Fusarium solani*, UPGH121).

3.2.3 Isolation of *Rhizoctonia*

Soil samples were processed as follows: 1g of autoclaved table beet seed was added to 100g of soil in a 9cm glass Petri plate which was moistened and this was incubated at 25°C for 48h. The seeds were then removed and rinsed under running tap water before being plated onto water agar containing chlorotetracycline hydrochloride added to be selective for *Rhizoctonia* (Papavizas & Davey, 1962). A *Rhizoctonia solani* isolate (RPPR-110376) was also purchased from the Plant Protection Research Institute (PPRI), Pretoria.

3.2.4 Pathogenicity tests

Pathogenicity tests were done with the fungal isolates, firstly in Petri plates by placing 8 seeds in a circle equidistant from a plug of the fungal culture (2.5mm^2), allowing the seeds to germinate.The amount of infection of the seedlings was recorded. Five replicates were used.The Petri plates were incubated at 25°C for between 2 and 7 days depending on the rate of growth of the fungus. Pathogenicity was assessed as percentage discolouration of roots and shoots. Secondly, isolates that looked promising in these tests were then further tested by inoculating germinated seeds using the between-paper germination method. Four sheets of standard germination paper, with dimensions 30 x 55mm, were placed on top of each other. A sheet of

absorbent paper towel was placed between the second and third layer of the germination paper and the sheets were soaked in distilled water. Maize seeds were placed just below the top layer of the germination paper. Two agar plugs (5mm in diameter) of the suspected pathogen were placed one above and one below each of the seeds. The sheets were rolled into a cylinder, placed in a plastic bag, sealed with a rubber band and incubated upright at 25°C in a 12h day/night light cycle for 7 days. Pathogenicity was again assessed in terms of percentage discolouration of roots and shoots. Other isolates from the UP culture collection (UPGH 107, UPGH121) and PPRI (RPPR-110376)) were also revived and tested in Petri plates and between paper. In this way one*Fusarium* sp. which caused a high disease incidence on Monsanto DKC78-15B seedlings, in both the Petri plates and in the between-paper germination method,was obtained. These tests were repeated on PANNAR 6Q308B seed and a pathogenic isolate of each of the three pathogens was obtained.

3.2.5 Seed treatment

Maize seeds of the two cultivars were treated using the following application rates:

Table 2: Treatments applied to maize seed throughout trials to determine efficacy and crop tolerance of Stamina, Flite and Celest®XL formulations against *Pythium Fusarium* and *Rhizoctonia* spp. soilborne diseases of maize.

Treatment	Name of Product	Formulation Concentration (g/l)	Type	Dose rate (Product ml/1000 seed) (=300g)
1.	Negative control: Uninoculated	-	-	-
2.	Positive control: Inoculated	-	-	-
3.	Stamina(BAS 500 12 F - pyraclostrobin)(Target rate)	200	FS	0.075
4.	Stamina (BAS 500 12 F - pyraclostrobin)	200	FS	0.15
5.	Flite (BAS 595 05 F - triticonazole) + Stamina (BAS 500 12 F - pyraclostrobin)	200 200	FS FS	0.075 0.075
6.	Celest® XL (fludioxonil and mefenoxam)	25 and 10	FS	0.33

For simplicity's sake the fungicide names Stamina and Flite are used instead of BAS 500 12 F and BAS 595 05 F, respectively, throughout this dissertation. The application rates were applied

in slurry form. Each treatment was pipetted into the bottom of a plastic bag and the bag was rubbed together to ensure even spread of the fungicide. The maize seed was wetted thoroughly with distilled water, the excess water was drained off and the seed was then added to the bag. Air was blown into the bag, which was then shaken gently until there was almost no more moisture on the sides of the plastic bag and the seeds were covered evenly with the treatment (Figure12). This process was repeated for each application rate. After treatment, the seeds were placed on paper towels and allowed to air-dry overnight in a laminar flow bench. The seeds weresealed in brown paper bags for storage.

Figure 12: Left =Monsanto DKC78-15B maize seed being treated with a fungicide slurry, Right = Seed treated with Stamina+Flite.

3.2.6 Media amended with fungicides

In order to test the efficacy of Stamina in controlling each of the pathogens, in 2010 PDA (Biolab) was prepared with Stamina added at a concentration of 1, 2 or 3ppm active ingredient. Five replicates were used, and each of the three pathogens (*Pythium* sp., *Fusarium* sp. and *Rhizoctonia* sp.) were plated onto this media and onto plain PDA as a control. The diameter of the fungal growth was measured every second day and compared. These tests were repeated. Stamina and Flite were each added to the PDA at a rate of 1, 2 or 3ppm active ingredient, and the two were also combined at a rate of 1, 2 or 3ppm of each of the active ingredients. Celest® XL was also amended to PDA at a concentration of 1, 2 or 3ppm mefenoxam and 2.5, 3 and 7.5ppm fludioxonil. The trial was repeated a third time and this time six replicates per treatment were used. In total there were 13 treatments: Stamina at a rate of 1, 2 and 3ppm active ingredient, Flite at a rate of 1, 2 and 3ppm active ingredient, the combination of Stamina and Flite at a rate of 1, 2 and 3ppm of each of the active ingredients and unamended PDA (Biolab) as a control. The diameters of the fungal growth were measured daily for up to seven days depending on the rate of growth of the fungus,which was compared to the rate of growth of the fungus on the unamended PDA control.

3.2.7 Treated seeds plated onto PDA

Monsanto DKC78-15Bseeds treated with the respective fungicides as described above were plated onto PDA (Biolab) as follows. Five seeds were placed equidistantly per Petri plate and five replicates were used. The number of seeds/seedlings with visible fungal growth was recorded. Two controls (untreated, uninoculated) were used to increase the diversity of the fungi possibly present on untreated seeds. This experiment was not repeated because only qualitative information could be obtained and did not add significant value to the overall objective of the study.

3.2.8 Statistical analysis

Linear mixed model analysis, also known as REML analysis (Payne *et al*., 2009b), was applied to the means over two diameters measured per plate to model the correlation over the time period in a repeated measurements analysis (Payne *et al*., 2009a). The fixed effects were specified as day, treatment and the day-by-treatment interaction, while the random effects were specified as the plate-by-day interaction. An antedependence model of order 1 was found to best model the correlation over days.

3.3 Results

3.3.1 Pathogenicity tests

Pathogenicity tests were conducted as described in the materials and methods above and it was found that *Rhizoctonia solani* RPPR-11036, *Fusarium solani* UPGH 121, *Fusarium oxysporum* UPGH 107 and *Pythium ultimum* var *ultimum* Py495 and Py496 had a high level of pathogenicityon both Monsanto DKC78-15B and PANNAR 6Q308B maize seed.

3.3.2 Media amended with fungicides

As can be seen in Table 3, growth of *Fusarium oxysporum* UPGH 107was completely inhibited by Flite at all concentrations as well as the combination of Stamina and Flite at all concentrations up to and including 10 days after inoculation.Stamina showed a much lower level of control of *Fusarium oxysporum* UPGH 107and was not significantly different from the diameter of growth on unamended PDA in most cases. It should, however, be noted that the appearance of the fungus did differ significantly from that on the PDA. On the PDA the fungus appeared much more fluffy and vigorous, whereas on Stamina at all concentrations the growth was spindly.

Table 3: Growth of *Fusarium oxysporum* UPGH 107on PDA media amended with Stamina and Flite at a concentration of 1, 2, and 3ppm active ingredient and 4-10 days after inoculation (dai).

Dai *	Treatment	Mean Diameter (mm)
4	1ppmF	0[1]a**
6	1ppmF	0a
8	1ppmF	0a
10	1ppmF	0a
4	1ppmF+S	0a
6	1ppmF+S	0a
8	1ppmF+S	0a
10	1ppmF+S	0a
4	1ppmS	13bc
6	1ppmS	14.6bcde
8	1ppmS	22efg
10	1ppmS	24.6h
4	2ppmF	0a
6	2ppmF	0a
8	2ppmF	0a
10	2ppmF	0a
4	2ppmF+S	0a
6	2ppmF+S	0a
8	2ppmF+S	0a
10	2ppmF+S	0a
4	2ppmS	13.6bcd
6	2ppmS	19defg
8	2ppmS	22.5fgh
10	2ppmS	26h
4	3ppmF	0a
6	3ppmF	0a
8	3ppmF	0a
10	3ppmF	0a
4	3ppmF+S	0a
6	3ppmF+S	0a
8	3ppmF+S	0a
10	3ppmF+S	0a
4	3ppmS	11.2b
6	3ppmS	17.2cdef
8	3ppmS	20.5defg
10	3ppmS	25.8h
4	PDA	14.8bcde
6	PDA	18.2cdef
8	PDA	22.8fgh
10	PDA	26.3h

[1]Each value is a mean diameter of five replicates
*dai = days after inoculation
**Means within a COLUMN per pathogen not followed by the same letter are significantly different ($P \leq 0.05$)

In Table 4 data is presented of the growth of *Pythium ultimum* var *ultimum* Py 495 recorded 24 and 48 hours after inoculation on the different media. This specific isolate of *Pythium* sp. was particularly fast growing, and so by the end of the 48-hour period the control plates were already overgrown while there was no growth present on the high concentration of Flite or any of the combination of Stamina and Flite plates. Stamina showed no control of the *Pythium ultimum* var *ultimum* Py 495 at concentrations of 1 and 2ppm, but did show a significant level of control at the 3ppm concentration, reducing the growth of the fungus.

Table 4: Growth of *Pythium ultimum* var *ultimum* Py 495 on PDA media amended with Stamina and Flite at a concentration of 1, 2, and 3ppm active ingredient and 24 to 48 hours after inoculation.

Hours	Treatment	Mean Diameter (mm)
24	1 ppmF	56.2[1]e**
48	1 ppmF	90f
24	1 ppmS	90f
48	1 ppmS	90f
24	1 ppmF+S	0a
48	1ppmF+S	0a
24	2 ppmF	44d
48	2 ppmF	90f
24	2 ppmS	90f
48	2 ppmS	90f
24	2 ppmF+S	0a
48	2 ppmF+S	0a
24	3ppmF	0a
48	3 ppmF	0a
24	3 ppmS	2.2b
48	3 ppmS	31.5c
24	3 ppmF+S	0a
48	3 ppmF+S	0a
24	PDA	90f
48	PDA	90f

[1]Each value is a mean diameter of three replicates
**Means within a COLUMN per pathogen not followed by the same letter are significantly different ($P \leq 0.05$)

When Rhizoctonia solani RPPR-11036 was plated onto the different media for a period of eight days it was found thatgrowth was significantly inhibited by all the treatments at all of the concentrations except for 1 and 2ppm Flite after 8 days (Table 5). Interestingly, it was also found that the rate of growth of the fungus is significantly greater in the Stamina 2ppm treatment than in the 1ppm or 3ppm treatment.This trend can also be seen in the combination of Stamina and Flite treatments, which suggests a possible interaction between the fungus and Stamina. Furthermore, Stamina at a rate of 1ppm showed no significant growth for days 2-4 but a

significant growth after 8 days thus suggesting that the fungus built up some level of resistance to the fungicide.

Table 5: Growth of *Rhizoctonia solani* RPPR-11036 on PDA media amended with Stamina(S) and Flite (F) at a concentration of 1, 2, 3, 4 or 6ppm active ingredient and 2-8 days after inoculation (dai).

Dai*	Treatment	Mean Diameter (mm)
2	1ppmF	16.8[1]cde**
4	1ppmF	39.3jkl
6	1ppmF	68.8op
8	1ppmF	86q
2	1ppmF+S	10.8abcd
4	1ppmF+S	13.2bcd
6	1ppmF+S	21.3defgh
8	1ppmF+S	26.5efgh
2	1ppmS	0a
4	1ppmS	0a
6	1ppmS	13.2bcd
8	1ppmS	17cde
2	2ppmF	18.2cdef
4	2ppmF	37.8ijkl
6	2ppmF	61no
8	2ppmF	90q
2	2ppmF+S	10.7abcd
4	2ppmF+S	18.3cdef
6	2ppmF+S	29.2fghijk
8	2ppmF+S	32.5hijk
2	2ppmS	8.5abc
4	2ppmS	15.5bcde
6	2ppmS	40.3kl
8	2ppmS	52.2mn
2	3ppmF	15bcd
4	3ppmF	28.7fghij
6	3ppmF	48.5lm
8	3ppmF	62.8nop
2	3ppmF+S	17.2cde
4	3ppmF+S	34.7ijk
6	3ppmF+S	57.2mn
8	3ppmF+S	72.7p
2	3ppmS	4.8ab
4	3ppmS	14.5bcd
6	3ppmS	18.7cdefg
8	3ppmS	29.8ghijk
2	PDA	40.2kl
4	PDA	90q

Dai*	Treatment	Mean Diameter (mm)
6	PDA	90q
8	PDA	90q

[1]Each value is a mean diameter of three replicates and two diameters per replicate
[*]dai = days after inoculation

**Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

Celest®XL entirely inhibited growth of all pathogens at all concentrations for up to four days as can be seen in Table 6.When the experiment was repeated with the same treatments but different isolates and twice the number of replicates for a period of 7 days, the following results were obtained: Celest® XL again entirely inhibited growth of all pathogens at all concentrations up to 7 days. The growth of *Fusarium solani* UPGH121 was entirely inhibited by all concentrations of Flite as well as all combination treatments of Stamina and Flite. This isolate of *Fusarium* sp. was significantly inhibited by Stamina at all concentrations as well, but to a lesser extent than the other treatments (Table 7).

As can be seen in Table 8, *Pythium ultimum* var *ultimum* Py 496 was again entirely inhibited by Celest® XL. It was also inhibited by all concentrations of the combination Stamina and Flite treatment. All concentrations of Flite showed a lower level of inhibition although it was still significant up to 3dai, and all Stamina treatments also showed some level of inhibition up to 3 dai. After 7 days it can be seen that Celest® XL and Stamina + Flite still showed total inhibition, while there was no longer any difference between the control and the Stamina or Flite treatments alone, except for Stamina at a rate of 3ppm, which still showed total inhibition of *Pythium* sp.

As can be seen in Table 9, *Rhizoctonia solani* RPPR-11036was entirely inhibited by Celest® XL and was inhibited to a lesser extent albeit still significantly by all other treatments. This result is similar to that obtained in the previous experiment with *Rhizoctonia solani* RPPR-11036, except that in that case 1 and 2ppm Flite did not significantly inhibit the fungal growth.

Table 6: Growth of *Pythium ultimum* var *ultimum* Py 496, *Fusarium solani* UPGH121 and *Rhizoctonia solani* RPPR-11036 on PDA media amended with Celest® XL at a concentration of 1, 2, and 3ppm active ingredient and 1-4 days after inoculation.

Pythium

Dai*	Treatment	Mean Diameter (mm)
1	1ppmCelest	0a
2	1ppmCelest	0a
3	1ppmCelest	0a
4	1ppmCelest	0a
5	1ppmCelest	15b
1	2ppmCelest	0a
2	2ppmCelest	0a
3	2ppmCelest	0a
4	2ppmCelest	0a
5	2ppmCelest	0a
1	3ppmCelest	0a
2	3ppmCelest	0a
3	3ppmCelest	0a
4	3ppmCelest	0a
5	3ppmCelest	0a
1	PDA	38c
2	PDA	90d
3	PDA	90d
4	PDA	90d
5	PDA	90d

[1]Each value is a mean diameter of three replicates
*dai = days after inoculation
**Means within a COLUMN per pathogen not followed by the same letter are significantly different ($P \leq 0.05$)

Table 6 continued

Fusarium

Dai*	Treatment	Mean Diameter (mm)
1	1ppmCelest	0[1]a**
2	1ppmCelest	0a
3	1ppmCelest	0a
4	1ppmCelest	0a
5	1ppmCelest	1.7a
1	2ppmCelest	0a
2	2ppmCelest	0a
3	2ppmCelest	0a
4	2ppmCelest	0a
5	2ppmCelest	1a
1	3ppmCelest	0a
2	3ppmCelest	0a
3	3ppmCelest	0a
4	3ppmCelest	0a
5	3ppmCelest	0.7a
1	PDA	14b
2	PDA	29.3c
3	PDA	40d
4	PDA	50.3e
5	PDA	61.2f

[1]Each value is a mean diameter of three replicates
**Means within a COLUMN per pathogen not followed by the same letter are significantly different ($P \leq 0.05$)

Table 6 continued

Rhizoctonia

Dai*	Treatment	Mean Diameter (mm)
1	1ppmCelest	0[1]a**
2	1ppmCelest	0a
3	1ppmCelest	0a
4	1ppmCelest	0a
5	1ppmCelest	0a
1	2ppmCelest	0a
2	2ppmCelest	0a
3	2ppmCelest	0a
4	2ppmCelest	0a
5	2ppmCelest	0a
1	3ppmCelest	0a
2	3ppmCelest	0a
3	3ppmCelest	0a
4	3ppmCelest	0a
5	3ppmCelest	0a
1	PDA	19.2b
2	PDA	37.3c
3	PDA	54.3d
4	PDA	69.8e
5	PDA	83.5f

[1]Each value is a mean diameter of three replicates
*dai = days after inoculation
**Means within a COLUMN per pathogen not followed by the same letter are significantly different ($P \leq 0.05$)

Table 7: Growth of *Fusarium solani* UPGH121 on PDA media amended with Celest® XL, Stamina and Flite at a concentration of 1, 2, and 3ppm active ingredient and 1-7 days after inoculation (dai).

Dai*	Treatment	Mean Diameter (mm)
1	1ppmCelest	0[1]a**
2	1ppmCelest	0a
3	1ppmCelest	0a
4	1ppmCelest	0a
5	1ppmCelest	0a
6	1ppmCelest	0a
7	1ppmCelest	0a
1	1ppmF+S	0a
2	1ppmF+S	0a
3	1ppmF+S	0a
4	1ppmF+S	0a
5	1ppmF+S	0a
6	1ppmF+S	0a
7	1ppmF+S	0a
1	1ppmF	0a
2	1ppmF	0a
3	1ppmF	0a
4	1ppmF	0a
5	1ppmF	0a
6	1ppmF	0a
7	1ppmF	0a
1	1ppmS	0a
2	1ppmS	9.5c
3	1ppmS	13.7e
4	1ppmS	19.4g
5	1ppmS	24.1h
6	1ppmS	30.7k
7	1ppmS	36.8l
1	2ppmCelest	0a
2	2ppmCelest	0a
3	2ppmCelest	0a
4	2ppmCelest	0a
5	2ppmCelest	0a
6	2ppmCelest	0a
7	2ppmCelest	0a
1	2ppmF+S	0a
2	2ppmF+S	0a
3	2ppmF+S	0a
4	2ppmF+S	0a
5	2ppmF+S	0a
6	2ppmF+S	0a
7	2ppmF+S	0a

Dai*	Treatment	Mean Diameter (mm)
1	2ppmF	0a
2	2ppmF	0a
3	2ppmF	0a
4	2ppmF	0a
5	2ppmF	0a
6	2ppmF	0a
7	2ppmF	0a
1	2ppmS	0a
2	2ppmS	8.7c
3	2ppmS	12.5de
4	2ppmS	17.7fg
5	2ppmS	24.1h
6	2ppmS	29.7jk
7	2ppmS	35.7l
1	3ppmCelest	0a
2	3ppmCelest	0a
3	3ppmCelest	0a
4	3ppmCelest	0a
5	3ppmCelest	0a
6	3ppmCelest	0a
7	3ppmCelest	0a
1	3ppmF+S	0a
2	3ppmF+S	0a
3	3ppmF+S	0a
4	3ppmF+S	0a
5	3ppmF+S	0a
6	3ppmF+S	0a
7	3ppmF+S	0a
1	3ppmF	0a
2	3ppmF	0a
3	3ppmF	0a
4	3ppmF	0a
5	3ppmF	0a
6	3ppmF	0a
7	3ppmF	0a
1	3ppmS	0a
2	3ppmS	5.7b
3	3ppmS	11.4d
4	3ppmS	17.3f
5	3ppmS	22.9h
6	3ppmS	27.1ij
7	3ppmS	32.2k
1	PDA	11.4d
2	PDA	24.8hi
3	PDA	37l

Dai*	Treatment	Mean Diameter (mm)
4	PDA	51.1m
5	PDA	62.7n
6	PDA	78.8o
7	PDA	90p

[1]Each value is a mean diameter of three replicates
*dai = days after inoculation
**Means within a COLUMN per pathogen not followed by the same letter are significantly different ($P \leq 0.05$)

Table 8: Growth of *Pythium ultimum* var *ultimum* Py 496 on PDA media amended with Celest® XL, Stamina and Flite at a concentration of 1, 2, and 3ppm active ingredient and 1-7 days after inoculation (dai).

Dai*	Treatment	Mean Diameter (mm)
1	1ppmCelest	0[1]a**
2	1ppmCelest	0a
3	1ppmCelest	0a
4	1ppmCelest	0a
5	1ppmCelest	0a
6	1ppmCelest	0a
7	1ppmCelest	0a
1	1ppmF+S	0a
2	1ppmF+S	0a
3	1ppmF+S	0a
4	1ppmF+S	0a
5	1ppmF+S	0a
6	1ppmF+S	0a
7	1ppmF+S	0a
1	1ppmF	34.3bc
2	1ppmF	57.8h
3	1ppmF	90k
4	1ppmF	90k
5	1ppmF	90k
6	1ppmF	90k
7	1ppmF	90k
1	1ppmS	0a
2	1ppmS	42.9de
3	1ppmS	90k
4	1ppmS	90k
5	1ppmS	90k
6	1ppmS	90k
7	1ppmS	90k
1	2ppmCelest	0a
2	2ppmCelest	0a
3	2ppmCelest	0a
4	2ppmCelest	0a
5	2ppmCelest	0a

Dai*	Treatment	Mean Diameter (mm)
6	2ppmCelest	0a
7	2ppmCelest	0a
1	2ppmF+S	0a
2	2ppmF+S	0a
3	2ppmF+S	0a
4	2ppmF+S	0a
5	2ppmF+S	0a
6	2ppmF+S	0a
7	2ppmF+S	0a
1	2ppmF	32.4b
2	2ppmF	45.7ef
3	2ppmF	76.8j
4	2ppmF	90k
5	2ppmF	90k
6	2ppmF	90k
7	2ppmF	90k
1	2ppmS	0a
2	2ppmS	38.4cd
3	2ppmS	71.2i
4	2ppmS	90k
5	2ppmS	90k
6	2ppmS	90k
7	2ppmS	90k
1	3ppmCelest	0a
2	3ppmCelest	0a
3	3ppmCelest	0a
4	3ppmCelest	0a
5	3ppmCelest	0a
6	3ppmCelest	0a
7	3ppmCelest	0a
1	3ppmF+S	0a
2	3ppmF+S	0a
3	3ppmF+S	0a
4	3ppmF+S	0a
5	3ppmF+S	0a
6	3ppmF+S	0a
7	3ppmF+S	0a
1	3ppmF	31.8b
2	3ppmF	51.5g
3	3ppmF	79.3j
4	3ppmF	90k
5	3ppmF	90k
6	3ppmF	90k
7	3ppmF	90k
1	3ppmS	0a

Dai*	Treatment	Mean Diameter (mm)
2	3ppmS	0a
3	3ppmS	0a
4	3ppmS	0a
5	3ppmS	0a
6	3ppmS	0a
7	3ppmS	0a
1	PDA	49.7fg
2	PDA	90k
3	PDA	90k
4	PDA	90k
5	PDA	90k
6	PDA	90k
7	PDA	90k

[1]Each value is a mean diameter of six replicates
*dai = days after inoculation
**Means within a COLUMN per pathogen not followed by the same letter are significantly different ($P \leq 0.05$)

Table 9: Growth of *Rhizoctonia solani* RPPR-11036 on PDA media amended with Celest® XL, Stamina and Flite at a concentration of 1, 2, and 3ppm active ingredient and 1-7 days after inoculation (dai).

Dai*	Treatment	Mean Diameter (mm)
1	1ppmCelest	0a
2	1ppmCelest	0a
3	1ppmCelest	0a
4	1ppmCelest	0a
5	1ppmCelest	0a
6	1ppmCelest	0a
7	1ppmCelest	0a
1	1ppmF+S	0a
2	1ppmF+S	9.9b
3	1ppmF+S	19.1d
4	1ppmF+S	26.7ef
5	1ppmF+S	36.8g
6	1ppmF+S	47h
7	1ppmF+S	55.7i
1	1ppmF	0a
2	1ppmF	22.7d
3	1ppmF	36.8g
4	1ppmF	53.3i
5	1ppmF	75.1l
6	1ppmF	90m
7	1ppmF	90m
1	1ppmS	0a
2	1ppmS	14.2bc

Dai*	Treatment	Mean Diameter (mm)
3	1ppmS	22.5d
4	1ppmS	31.1ef
5	1ppmS	38.3g
6	1ppmS	45.6h
7	1ppmS	53.3i
1	2ppmCelest	0a
2	2ppmCelest	0a
3	2ppmCelest	0a
4	2ppmCelest	0a
5	2ppmCelest	0a
6	2ppmCelest	0a
7	2ppmCelest	0a
1	2ppmF+S	0a
2	2ppmF+S	11.1b
3	2ppmF+S	20d
4	2ppmF+S	28.4ef
5	2ppmF+S	37.8g
6	2ppmF+S	45.2h
7	2ppmF+S	53.2i
1	2ppmF	0a
2	2ppmF	16.2bcd
3	2ppmF	24d
4	2ppmF	33.3f
5	2ppmF	40.9gh
6	2ppmF	47.2h
7	2ppmF	51.8i
1	2ppmS	0a
2	2ppmS	13b
3	2ppmS	23.7d
4	2ppmS	32.7f
5	2ppmS	40.6g
6	2ppmS	50hi
7	2ppmS	60.5j
1	3ppmCelest	0a
2	3ppmCelest	0a
3	3ppmCelest	0a
4	3ppmCelest	0a
5	3ppmCelest	0a
6	3ppmCelest	0a
7	3ppmCelest	0a
1	3ppmF+S	0a
2	3ppmF+S	13.7bc
3	3ppmF+S	25.1de
4	3ppmF+S	35g
5	3ppmF+S	46.7h

Dai*	Treatment	Mean Diameter (mm)
6	3ppmF+S	58.2i
7	3ppmF+S	71.5kl
1	3ppmF	0a
2	3ppmF	14.7bcd
3	3ppmF	25.6def
4	3ppmF	36.4g
5	3ppmF	47.7h
6	3ppmF	58.3i
7	3ppmF	70.3k
1	3ppmS	0a
2	3ppmS	17.9bcd
3	3ppmS	24.3d
4	3ppmS	34.8fg
5	3ppmS	46.5h
6	3ppmS	53.5i
7	3ppmS	62.7jk
1	PDA	24.7d
2	PDA	59.8ij
3	PDA	90m
4	PDA	90m
5	PDA	90m
6	PDA	90m
7	PDA	90m

[1]Each value is a mean diameter of five replicates
*dai = days after inoculation
**Means within a COLUMN per pathogen not followed by the same letter are significantly different ($P \leq 0.05$)

3.3.3 Treated seed plated onto PDA

The fungi isolated from all the seeds were found to be common saprophytic fungi found in soil and on seed. *Acremonium* sp. was most prevalent in both controls, Stamina, Flite and the combination of Stamina and Flite treatments. In the Celest® XL treatment, *Rhizopus* and *Trichoderma* spp. were the most prevalent (Table 10).

Table 10: Percentage infection of fungicide-treated Monsanto DKC78-15B maize seeds plated on PDA.

Treatment	*Incidence (%)*							
	Acremonium	***Alternaria***	***Aspergillus***	***Bacterium***	***Fusarium***	***Penicillium***	***Rhizopus***	***Trichoderma***
Control 1 (untreated, uninoculated)	48	4			4	4	8	8
Control 2 (untreated, uninoculated)	52		4				4	4
Stamina (0.075ml/1000 seed)	76				8			
Stamina (0.15ml/1000 seed)	72				8			
Stamina + Flite (0.075ml each/1000 seed)	48			16			8	
Celest® XL (0.33ml/1000 seed)							32	68

3.4 Discussion and conclusion

In the experiments where the different fungicides were added to PDA and the diameters of the fungal growth measured, fairly consistent results were obtained. The combination of Stamina and Flitewas clearly very effective in inhibiting all three pathogens. When treated seeds were plated onto Petri plates overgrown with *Rhizoctonia solani* RPPR-11034, *Fusarium oxysporum* UPGH 107, *Fusarium solani* UPGH121 and *Pythium ultimum* var *ultimum* Py495 and Py496 the combination of Stamina and Flite showed the highest level of control of *Pythium ultimum* and *Rhizoctonia solani*. Celest® XL and the Stamina and Flite combination treatment showed equally good control of *Fusarium* spp. A possible interaction between *Rhizoctonia solani* RPPR-11036 and Stamina was observed since the 1 and 3ppm concentrations of the fungicide controlled the groth of the fungus but at a rate of 2ppm the fungal growth was still strong. This same trend was observed with the combination of Stamina and Flite treatment. It was also observed that Stamina inhibited *Rhizoctonia solani* RPPR-11036 up to 2 days after inoculation but after that the fungus appeared to develop some level of resistance to the fungicide and grow strongly again.

It is claimed by BASF on their website that Stamina provides robust disease control and increased emergence of seedlings under certain cold conditions (BASF, 2008). No specific results in terms of which pathogens the fungicide was tested against could be found; however,

the website further states that on maize, Stamina controls seed and seedling damping-off diseases caused by *Rhizoctonia solani*, *Pythium* and *Fusarium* species (BASF, 2008). Research by Jin *et al.* (2009) showed that pyraclostrobin (the active ingredient in Stamina) is a strobilurin, which acts by binding to the Qo centre of the cytochrome complex of the mitochondria of the fungus and thus inhibits respiration. It was also found that pyraclostrobin has a broad spectrum of activity showing some level of control of each of the four major groups of plant pathogens (Ascomycetes, Basidiomycetes, Deuteromycetes and Oomycetes) (Tomlin, 2009)The current study showed, however, that Stamina on its own did not provide a consistently acceptable level of control by reducing growth of *Pythium, Fusarium* and *Rhizoctonia* spp. *in vitro*, especially when compared to Celest® XL.

The active ingredient of Flite is triticonazole, which is part of the triazole group of fungicides (Pesticide Properties Database, 2010). This group of fungicides has excellent systemic properties and has been shown to have long-term prophylactic and curative activity against a wide spectrum of diseases caused by Ascomycetes, Oomycetes and Basidiomycetes (Pesticide Properties Database, 2010). In the current study it was found that Flite, in combination with Stamina, reduced the growth of *Pythium, Fusarium* and *Rhizoctonia* spp. *in vitro*, but not to the same extentas that of Celest® XL

It was found that Celest® XL completely inhibited all three fungi (*Rhizoctonia solani* RPPR-11034, *Fusarium oxysporum* UPGH 107, *Fusarium solani* UPGH121 and *Pythium ultimum* var *ultimum* Py495 and Py496) at all concentrationsby inhibiting all fungal growth,and thus it was a good industry standard control. This result correlates well with the results of McGovern *et al.* (2002), who showed that the incidence of *Rhizoctonia* sp. was consistently decreased by fludioxonil. *Pythium ultimum* was shown to be insensitive to fludioxonil by Okada *et al.* (2005). This result was further confirmed when Martinez *et al.* (2005) evaluated different fungicides for the control of carrot (*Daucus carota* L.) cavity. Fludioxonil was also tested for effectiveness against *Fusarium graminearum* on maize by Broders *et al.* (2007) and was found to provide sufficient inhibition of mycelial growth *in vitro*. Jones (2000) further found that fludioxonil reduced *Fusarium graminearum* incidence and severity while increasing the percentage of plump kernels and yield in barley. In this study the same treated and untreated Monsanto DKC 78-15B maize seed was plated onto PDA and the fungi that grew were identified as common saprophytes, the most prevalent being *Acremonium, Rhizopus* and *Trichoderma* spp.

In the chapters that follow, the effect of these fungicides on the germination and vigour of two seed lots of maize is assessed and greenhouse tests are conducted with the treated seed in order to establish the efficacy of the fungicides *in vivo*.

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Chapter 4
Effect of Stamina, Flite and Celest® XL on Germination Vigour of Two *Zea mays* Cultivars

Abstract

The aim of this chapter is to test the effect of Stamina, Flite and Celest® XL on the germination and vigour of two *Zea mays* cultivars (Monsanto DKC78-15B and PANNAR 6Q308B).The moisture content of the two maize cultivars was tested according to standardized International Seed Testing Association (ISTA) methods and found to be within the acceptable range for good quality maize seed. The seeds were then treated with a low and a high dosage rate of Stamina, a combination of Stamina and Flite and lastly with Celest® XL. These treated seeds (along with an untreated control) were subjected to a standard germination test, rate of germination test, cold soil test, two- and four-day accelerated ageing as well as three and six month long-term storage. In the standard germination test it was found that there were no significant differences in germination percentage between any of the treatments on Monsanto seed in any of the tests. The untreated control did, however, have a slightly lower germination than both of the two Stamina treatments after four-day accelerated ageing. PANNAR 6Q308B showed no significant differences in percentage germination after standard germination or the cold soil test. After four-day accelerated ageing the combination treatment of Stamina and Flite had significantly higher germination than the untreated control. When all the germination tests were repeated on the PANNAR seed, it was found that there were no significant differences in germination percentages between any of the treatments in the standard germination test. After the cold soil test, only Celest® XL showed a significantly higher germination percentage than the untreated control. After two-day accelerated ageing, the low concentration of Stamina and the Celest® XL treatments unexpectedly showed a lower germination percentage than any of the other treatments, including the untreated control, which had the highest germination percentage in this experiment. Thus, overall none of the treatments appeared to have a significant impact on germination percentage in either of the cultivars and across all experiments since, even when significant differences were observed, these results were not consistent in repeated experiments.

4.1 Introduction

An essential aspect of plant disease control is treating seed with fungicides. Seed-borne pathogens are a major concern because they cause immense croplosses and can easily be spread from one area to another along with the seed (Mathre & Hansing, 1986). The vast majority of

commercial maize seed in South Africa is pre-treated with fungicides before planting (DuPlessis, 2003). Seed treatments have been shown to effectively control damping-off diseases such as those caused by *Pythium, Fusarium* and *Rhizoctonia* spp. (McGovern *et al.*, 2002; Okada *et al.*, 2005, Broders *et al.*, 2007). However, seed treatments can have possible phytotoxic effects or negative effects on seed germination and vigour, and thus need to be thoroughly tested (Mathre & Hansing, 1986).

ISTA has prescribed various sets of rules for accurately determining the moisture content of most major crop species produced globally (ISTA, 2012).The moisture content of seeds has a great effect on seed quality because it affects the physiological processes of the seed, both prior to and during germination (Vertucci, 1989). If seeds are not harvested at the correct time or maturity and the seed moisture is too high, this will have an adverse impact on seed longevity in storage and may also result in seed injury due to heat, frost, pathogens and mechanical damage, and it will also influence the seed weight (Grabe, 1989).If the seeds are too dry on the other hand, they may absorb moisture too slowly and not reach the critical moisture level necessary for germination to occur (Delouche, 2004 as cited in Govender, 2005).

It was hypothesized by Gange *et al.* (1992) that pesticides used on seeds could have either a phytotoxic or stimulatory effect on seed germination. Subjecting treated seed to vigour tests can also give an indication of how specific treatments can indirectly increase germination (Nijenstein & Kruse, 2000; Noli *et al*., 2008). Germination is defined as the formation of a seedling which possesses all the necessary structures for it to develop into a normal plant under favourable conditions (ISTA, 2012).

The standard germination test can, however, not be viewed in isolation. When attempting to estimate the field emergence of a seed lot, the germination vigour should also be assessed and this is achieved though tests such as the cold soil test and the rate of germination and accelerated ageing tests (ISTA, 2012; Matthews & Khajeh-Hosseini, 2006). Seed vigour is not a single, measurable property but a concept describing several characteristics associated with seed lot performance (Hampton, 1995; Copeland & McDonald, 2001). The rate of germination test was developed by Matthews & Khajeh-Hosseini (2006). They demonstrated that the faster the seedling emergence of a maize seed lot, the greater its final emergence and seedling growth. The cold soil test is one of the most reliable and widely used vigour tests for maize (Isley, 1950; Crosier, 1958; Burris & Navratil, 1979), and the accelerated ageing test is a reliable vigour test for seed of many crops, including maize (Association of Official Seed Analysts, 1983; ISTA,

2012). The test has performed well in predicting field emergence of maize (Medina & Filho, 1991).

The aim of this chapter was to determine whether any of the fungicides (Stamina, Flite or Celest® XL) had any effect on either the germination or vigour of the maize cultivars Monsanto DKC 78-15D and PANNAR 6Q308B.

4.2 Materials and methods

4.2.1 Seed treatment

Monsanto DKC78-15B and PANNAR 6Q308B seeds were treated with the following: Stamina at a rate of 0.075ml/1 000 seed, Stamina at a rate of 0.15ml/1 000 seed, a combination of Stamina and Flite at a rate of 0.075ml each/1 000 seed and Celest® XL at a rate of 0.33ml/1 000 seed. The treatments were applied by means of a slurry as described in Section 3.2.5.

4.2.2 Moisture content

The moisture content of Monsanto DKC 78-15B and PANNAR 6Q308B seed was determined according to ISTA standards as follows using two replicates: A 10g sample was drawn and spread evenly over the surface of a 90mm glass Petri dish and placed in an oven at a temperature of 130–133°C to dry for a period of 4 hours. The sample was then placed in a desiccator to cool for 20 minutes. The moisture content was calculated to one decimal place using the following formula (ISTA, 2012) and the test was repeated:

(Weight of container and seed before drying – weight of container and seed after drying) * (100/(weight of container and seed before drying – weight of container))

S1= moisture lost in pre-drying
S2= moisture lost in main drying

Original moisture content = (S1+S2) – (S1*S2)/100

4.2.3 Standard germination

Standard germination tests were carried out on the Monsanto DKC78-15B and PANNAR 6Q308B seed. This was done by placing 50 seeds between the third and fourth layer of four sheets of germination paper (25.4x38.1cm). The germination paper was watered with 50ml of water, rolled up and tied in the centre with an elastic band. Two rolls of germination paper were placed in a plastic bag to constitute one replicate of 100 seeds. Four replicates of 100 seeds were done for each of the five treatments.The treatments used were as follows: an untreated control,

Stamina at a rate of 0.075ml/1 000 seed, Stamina at a rate of 0.15ml/1 000 seed, a combination of Stamina and Flite at a rate of 0.075ml each/1 000 seed and Celest® XL at a rate of 0.33ml/1 000 seed.Plastic bags containing germinating seeds were incubated at 25°C in an upright position for 7 to 10 days, after which the percentage germination was determined using ISTA rules. These tests were carried out in 2010 and repeated in 2011.A completely randomized experimental design was used as opposed to a factorial design, since each of the two maize cultivars were evaluated separately.

4.2.4 Rate of germination

Two replicates of 50 Monsanto DKC78-15B maize seeds treated as above with a high and a low concentration of Stamina, a combination of Stamina and Flite, and Celest® XL were placed between paper (as described in Section 4.2.3)and incubated at 16°C. Counts were made of just germination time (JG), that is, when the radicle first appeared through the pericarp, and physiological germination (G), when a 2mm radicle had been produced. The following formula was used:

$MJGT = \sum (nt)/ \sum n$

where n = number of seeds newly germinated (just germinated criterion) at time t;

t = days from when set to germinate.

The MGT was calculated using the same formula, but using the physiological assessment of germination (2mm radicle)(Matthews & Khajeh-Hosseini, 2006).

4.2.5 Accelerated ageing and long-term storage

For short-term accelerated ageing (AA), the same treatments that were used in the standard germination test were used. The maize cultivars (Monsanto DKC78-15B and PANNAR 6Q308B) were placed on grids above water (the water was poured into the container so that it was about 2cm deep and did not touch the grid) in a sealed accelerated ageing container (Figure 13), which was incubated at 45°C. Sufficient seeds were used to cover the $10cm^2$ grid and two replicates were used. At the end of a 2-day or 4-day incubation period the seeds were removed and subjected to the standard germination test. The root and shoot length of the seedlings weremeasured at the end of the germination period. Four replicates of 50 seeds were used. Long-term accelerated ageing tests were carried out in the same way, but the seeds were placed over a saturated salt solution (75% relative humidity) at 30°C for 3 months and 6 months respectively,

after which they were subjected to the standard germination test. Accelerated ageing tests were carried out in 2010 and repeated in 2011.

Figure 13: Maize seed placed on a metal grid in an accelerated ageing container in preparation for undergoing an accelerated ageing test.

4.2.6 Cold soil test

Germination paper was prepared as for the standard germination test, but with one difference: 100g of soil from a cultivated maize field was placed in rows on the paper (Figure 14) and the seeds were placed equidistant from each other on the soil. Soil was obtained from fields at PANNAR in Greytown andBASF – Delmas CoSar experimental farm as well as from the University of Pretoria's long-term maize trial on the experimental farm in Hatfield, Pretoria. Four replicates of 50 seeds of each of the cultivars were used. Paper towels were rolled up and placed individually in plastic bags. They were incubated in an upright position at 5°C for seven days and then at 25°C for a further seven days. Percentage germination was then determined usingthe ISTA rules (ISTA, 2012). The results were presented as the number of normal seedlings that had germinated by the end of the test period.

Figure 14: Maize seedlings after undergoing the cold soil test.

4.2.7 Statistical analysis

A general analysis of variance (ANOVA) was performed on all the data and means were separated with Fischer's least significant difference test (LSD) at the 5% level of significance ($P \leq 0.05$), and standard error of differences (SEDs) were determined using GenStat® software (Payne, Welham & Harding, 2009).

4.3 Results

4.3.1 Moisture content

The moisture content of the Monsanto DKC78-15B seed wasfound to be 13.96% in the first replicate and 12.89% in the second. The moisture content of the PANNAR 6Q308B seed was found to be 13.4% in the first replicate and 12.3% in the second.

4.3.2 Rate of germination

The Monsanto DKC78-15B seed showed very high values for MJGT and MGT. The values reached between 0.99 and 1 and 0.97 and 0.99 respectively only four days after planting (Table 11). The values were calculated as follows:

$MJGT = \sum (nt) / \sum n$

where n = number of seeds newly germinated (just germinated criterion) at time t;

t = days from when set to germinate.

The MGT was calculated using the same formula, but using the physiological assessment of germination (2mm radicle) (Matthews & Khajeh-Hosseini 2006).

Table 11: Mean just germinated and meangerminated values of treated Monsanto DKC78-15B maize seeds in the rate of germination test.

Treatment	MJGT*2 dap**	MGT 2 dap	MJGT 4 dap	MGT 4 dap	MJGT 6 dap	MGT 6 dap	MJGT 8 dap	MGT 8 dap
Untreated control 1	0.03[1]	0	1	0.98	1	1	1	1
Untreated control 2	0.02	0	0.99	0.97	1	1	1	1
Stamina (0.075 ml/1 000 seed)	0.02	0	0.99	0.99	1	1	1	1
Stamina (0.15 ml/1 000 seed)	0.04	0	0.99	0.99	0.99	0.99	1	1
Stamina + Flite (0.075 ml each/1 000 seed)	0.03	0	0.99	0.97	0.99	0.99	0.99	0.99

[1]Each value is a mean of two replicates of 50 seeds
*MJGT = Mean just germinated; MGT = Mean germinated
**dap = days after planting

4.3.3 Standard germination and germination vigour tests

As can be seen in Table 12, one of the untreated Monsanto controls had a significantly lower germination in the standard germination test than any of the treated seed. There were no significant differences between any of the treatments in the cold soil test and after 2-day accelerated ageing there were again no significant differences except for the fact that the Celest® XL treatment had a lower germination than any of the other treatments. After 4-day accelerated ageing, all the treated seed had a lower germination than the untreated controls, except for the Stamina+Flite treatment.

Table 12: Percentage germination of treated Monsanto DKC78-15B maize seed after standard germination, the cold soil test and the accelerated ageing (AA) test.

Treatment	Germination (%)			
	Standard	Cold soil test	2-day AA	4-day AA
Untreated control 1	94.0[1]a*	90.0[2]a*	99.0[2]a*	99.5[2]a*
Untreated control 2	96.5ab	86.0a	100.0a	94.5a
Stamina (0.075 ml/1 000 seed)	97.5b	88.0a	99.0a	65.5b
Stamina (0.15 ml/1 000 seed)	97.3b	87.0a	98.0a	64.5b
Stamina + Flite (0.075 ml each/1 000 seed)	98.3b	87.0a	100.0a	100.0a
Celest® XL (0.33 ml/1 000 seed)	Nt	Nt	85.0b	63.0b
LSD	3.19	7.77	3.03	5.6
SED	1.465	3.56	1.50	2.8

[1]Each value is a mean percentage of four replicates of 100 seeds
[2]Each value is a mean percentage of four replicates of 50 seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)
nt = not tested

When the vigour tests were repeated (Table 13), it was found that there were no significant differences in the cold test or in either of the two accelerated ageing tests. Stamina at both a high and a low concentration (Figure 15) showed 100% germination after 4-day accelerated ageing, significantly higher than the untreated control (Figure 16).

Table 13: Percentage germination of treated Monsanto DKC78-15B maize seed after the cold soil test and the accelerated ageing (AA) test (Repeat: September-October 2011).

Treatment	Germination (%)		
	Cold soil test	2-day AA	4-day AA
Untreated control	99.0[1]a*	100[1]a*	98.5[1]a*
Stamina (0.075 ml/1 000 seed)	98.5a	98.5a	100b
Stamina (0.15 ml/1 000 seed)	94.5a	98a	100b
Stamina + Flite (0.075 ml each /1 000 seed)	99.5a	99a	99.5ab
Celest® XL (0.33 ml/1 000 seed)	98.0a	97a	99ab
LSD	8.11	3.667	1.462
SED	3.72	1.683	0.671

[1]Each value is a mean percentage of four replicates of 100 seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

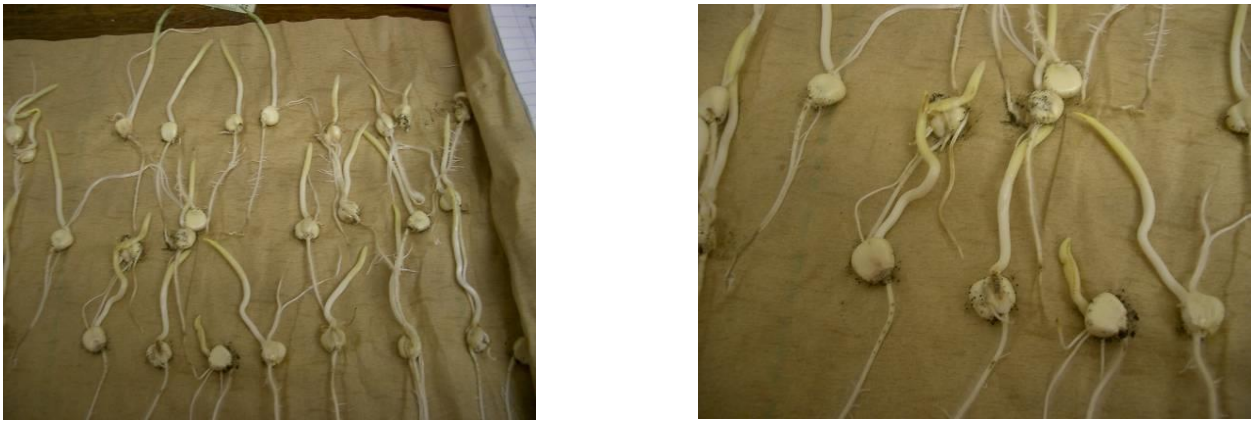

Figure 15: 4-day accelerated ageing test. Left = Untreated control 1, right = Fungal saprophytes on Monsanto DKC78-15B seeds of control 1.

Figure 16: 4-day accelerated ageing test. Left = Untreated control 2, Right = Monsanto DKC78-15B seed treated with Stamina (0.075 ml/1 000 seed).

After 2-day accelerated ageing none of the treatments differed from the two controls in shoot and root length except Stamina (0.15 ml/1 000 seed), which was greater than control 1, and Celest® XL (0.33 ml/1 000 seed), which was lower than control 2 (Table 14). After 4-day accelerated ageing the root length of seedlings treated with Stamina (0.075 ml/1 000 seed), Stamina (0.15 ml/1 000 seed) and Celest® XL were significantly less than both control 1 and 2 and Stamina + Flite. The same trend was evident for shoot lengths (Table 14). The seeds treated with a combination of Stamina and Flite had the longest root and shoot lengths.

Table 14: Root and shoot length of seedlings of treated Monsanto DKC78-15B maize seed after 2-day and 4-day accelerated ageing (AA).

Treatment	Length (mm)			
	2-day AA		4-day AA	
	Root	Shoot	Root	Shoot
Untreated control 1	137.5[1]a*	133.3[1]ab*	148.9[1]b*	109.9[1]cd*
Untreated control 2	164.5bc	162.6bc	141.2b	96.96c
Stamina (0.075 ml/1 000 seeds)	153.7abc	149.1ab	100.9a	78.8b
Stamina (0.15 ml/1 000seeds)	172.1c	183.1c	96.9a	60.9a
Stamina + Flite (0.075 ml each /1 000 seed)	145.7ab	142.7ab	152.8b	114.1d
Celest® XL (0.033 ml/1 000 seed)	139.5ab	133.3a	92.1a	64.9ab
LSD	26.26	26.04	26.4	16.3
SED	13.03	12.92	13.1	8.1

[1]Each value is a mean of four replicates of 50 seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

No germination of either the treated or untreated seed of the two maize cultivars occurred after either 3- or 6-month long-term storage (Tables 15 and 16).

Table 15: Percentage germination of treated Monsanto DKC78-15B maize seed after 3- and 6-month storage under extreme conditions of high temperature and moisture.

Treatment	Germination after 3-month storage (%)	Germination after 6-month storage (%)
Untreated control	0[1]a*	0[1]a*
Stamina (0.075 ml/1 000 seeds)	0a	0a
Stamina (0.15 ml/1 000seeds)	0a	0a
Stamina + Flite (0.075 ml each /1 000 seed)	0a	0a
Celest® XL (0.033 ml/1 000 seed)	0a	0a
LSD	NS	NS
SED	NS	NS

[1]Each value is a mean of four replicates of 50 seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

Table 16: Percentage germination of treated PANNAR 6Q308B maize seed after 3- and 6-month storage under extreme conditions of high temperature and moisture.

Treatment	Germination after 3-month storage (%)	Germination after 6-month storage (%)
Untreated control	0[1]a*	0[1]a*
Stamina (0.075 ml/1 000 seeds)	0a	0a
Stamina (0.15 ml/1 000seeds)	0a	0a
Stamina + Flite (0.075 ml each/1 000 seed)	0a	0a
Celest® XL (0.033 ml/1 000 seed)	0a	0a
LSD	NS	NS
SED	NS	NS

[1]Each value is a mean of four replicates of 50 seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

When PANNAR 6Q308B seed was subjected to standard germination, the cold soil test and 4-day accelerated ageing (Table 17), it was found that there were no significant differences after standard germination or the cold soil test. After 4-day accelerated ageing, only the Stamina+Flite treatment had a significantly higher germination than the untreated control.

Table 17: Percentage germination of treated PANNAR 6Q308B maize seed after standard germination, the cold soil test and the accelerated ageing (AA) test.

Treatment	Standard germination (%)	Germination cold soil test (%)	Germination 4-day AA (%)
Untreated control	69[1]a*	35[2]a*	77[2]b*
Stamina (0.075 ml/1 000 seeds)	64a	25a	83ab
Stamina (0.15 ml/1 000 seeds)	63a	16a	95ab
Stamina + Flite (0.075 ml each/1 000 seed)	70a	5a	99a
Celest® XL (0.033 ml/1 000 seed)	84a	28a	86ab
LSD	28.25	33.91	18.96
SED	11.87	12.83	6.96

[1]Each value is a mean percentage of four replicates of 100 seeds
[2]Each value is a mean percentage of four replicates of 50 seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

When the above-mentioned germination tests were repeated (Table 18), it was found that there were again no significant differences in standard germination. In the cold soil test, only the Celest® XL treatment had a higher germination percentage than the untreated control. After 2-day accelerated ageing, the low concentration of Stamina and Celest® XL unexpectedly had a lower percentage germination than any of the other treatments. There were no significant differences found after 4-day accelerated ageing.

Table 18: Percentage germination of treated PANNAR 3Q308B maize seed after the cold soil test and the accelerated ageing (AA) test (Repeat: September-October 2011).

Treatment	Germination (%)			
	Standard germination	Cold soil test	2-day AA	4-day AA
Untreated control	90.50[1]a*	44[1]a*	79.5[1]b*	91[1]a*
Stamina (0.075 ml/1 000 seed)	93.50a	52.5ab	63.0a	89a
Stamina (0.15 ml/1 000 seed)	94.50a	53.5ab	76.5b	85a
Stamina + Flite (0.075 ml each /1 000 seed)	94.00a	59.0ab	78.5b	91.5a
Celest® XL (0.33 ml/1 000 seed)	94.50a	64.05b	66.0a	88.5a
LSD	4.839	19.26	8.91	7
SED	2.221	8.84	4.09	3.21

[1]Each value is a mean percentage of four replicates of 100 seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

4.4 Discussion and conclusion

The results of this study showed that treating maize seeds with Stamina, Flite, a combination of Stamina and Flite, and Celest® XL did not have any negative effects on the germination and vigour of PANNAR 6Q308B and Monsanto DKC78-15B maize seed. Govender (2005) also found that Celest® XL did not reduce the germination or vigour of maize seed and did not cause any phytotoxic effects. Furthermore, Kahn (1992) reported that two systemic fungicides applied to wheat increased germination percentage by 4.1%. Bradley *et al.* (2001) found that fungicide seed treatments do not affect the vigour and viability of maize seeds. Munkvold & O'Mara (2002) found that seeds treated with fludioxonil (the active ingredient of Celest® XL) showed an increased radicle length, thus indicating a higher vigour.

In the current study the cold soil test showed a decreased germination percentage when compared to the standard germination test, which could thus be an effective indication of field emergence under adverse conditions. Both the cold soil test and the accelerated ageing test had high LSD and SED values, and thus the experiments were repeated in order to confirm the results. The LSD and SED of the cold soil test in the repeat were still high, but this is most likely because the germination in the cold soil test dropped drastically. Two different seed lots of PANNAR 6Q308-15B (due to availability) were also used for the standard germination and vigour tests.The first lot had a lower percentage germination than the second; however, the trends between the two sets of experiments and seed lots were similar. Namely, the cold soil test caused a significant drop in germination when compared to standard germination, and the combination of Stamina and Flite treatment resulted in the highest percentage germination after 2-day and 4-day accelerated ageing. Nijenstein and Kruse (2000) found that even with all the problems encountered when attempting to standardize the cold soil test, it still remains a test that is used on maize to simulate field conditions and predict field behaviour. These results were confirmed by Noli *et al*. (2008), who found that the cold soil test was the most accurate vigour test to predict field performance as long as the laboratory test was conducted at a low temperature and the soil microflora was similar to that found in the field. For this reason, the soil used for the cold soil test in this study was taken from a maize field.In the vigour tests, Monsanto DKC 78-15B displayed a higher seed vigour than the PANNAR 6Q308B seed.

Root and shoot length in the 2-day and 4-day accelerated ageingtests was only done on the Monsanto DKC78-15B seed and the experiment was only carried out once, since the results, even among the controls, were very variable and the LSD and SED were very high, indicating variability. After 3- and 6-month long-term storage, none of the maize seedsof either cultivars survived. This was unexpected since the Monsanto DKC78-15B seed exhibited high vigour in all the other tests that were carried out. It was also an unexpected result, since Govender (2005) found that untreated maize (unknown cultivar), had a 71.5% germination after 3-month long-term storage, and the same seed treated with Celest® XL had a 15% germination.

Results from the current study further confirmed the results of BASF (2008), namely that Stamina mitigated the damage caused by short exposures to cold and light freezing conditions. BASF (2008) conducted growth chamber studies with plants exposed to -5°C for 3 h at the seedling spike stage. Only 68% of seedlings in the untreated control survived, while 83% of the Stamina-treated seeds survived. Stamina-treated maize seeds also showed an increased emergence and the plants were taller than the untreated control.

In the chapter that follows, the efficacy of Stamina, Flite and Celest® XL for controlling *Pythium*, *Fusarium* and *Rhizoctonia* spp. is evaluated *in vivo* in the greenhouse. Greenhouse trials are important to corroborate or refute the results obtained in this chapter as they give a better idea of how seedlings will behave in the field than laboratory germination and vigour tests.

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Chapter 5
Efficacy of Stamina, Flite and Celest®XL for Controlling Damping-off Diseases of *Zea mays* in the Greenhouse

Abstract

In Chapter 3 of this dissertation the efficacy of Stamina, Flite and Celest® XL for controlling damping-off diseases *in-vitro* was tested.The fungicides controlled the pathogens effectively; however, there are many external factors that play a role in biological systems and thus it is essential that the fungicides be tested *in vivo* as well. In this chapter the efficacy of Stamina, Flite and Celest® XL for controlling *Pythium*, *Fusarium* and *Rhizoctonia* spp. on maize was tested in the greenhouse. Isolates of *Pythium*, *Fusarium* and *Rhizoctonia* spp. that were found to be pathogenic in previous experiments were used in greenhouse trials with two maize cultivars (Monsanto DKC78-15B and PANNAR 6Q308 B). The treatments consisted of a) an untreated, uninoculated control; b) an untreated inoculated control; and the following seed treatments planted in soil inoculated with the various pathogens: c) a low concentration of Stamina; d) a high concentration of Stamina; e) a combination of Stamina and Flite; and f) Celest® XL. When Monsanto seed was planted in *Fusarium* sp. inoculated soil,there was a large disease percentage difference between the inoculated and the uninoculated control, and all the treatments controlled the disease effectively. When PANNAR seed was planted in soil inoculated with *Fusarium* sp., it was found that the higher concentration of Stamina as well as the combination of Stamina and Flite treatments had significantly higher emergence percentages than the inoculated control. When Monsanto seed was planted in *Pythium* sp. inoculated soil, no significant differences were found in emergence percentages between any of the treatments. However, the low concentration of Stamina and the Stamina+Flite treatments resulted in plant heights that were significantly greater than that of the inoculated control, thus indicating some disease control. In the repeat of this experiment a low emergence percentage was found in the inoculated control when compared to all other treatments, thus indicating that this isolate of *Pythium* sp. caused pre-emergence damping-off. When the PANNAR seed was planted in soil inoculated with *Pythium* sp., it was found that the combination of Stamina and Flite as well as the Celest® XL treatments unexpectedly had a lower emergence than any of the other treatments, possibly indicating phytotoxicity. However, this was not found in any of the other experiments. Monsanto seed was planted in *Rhizoctonia* sp. inoculated soil and all the seedlings emerged. The only significant difference found was that Celest® XL had a significantly lower dry shoot mass than the uninoculated control.However, there was no significant difference between the inoculated and

uninoculated controls. Lastly, PANNAR seed was planted in *Rhizoctonia* sp. inoculated soil and it was found that none of the treatments differed significantly from the inoculated control in terms of emergence. All treatments effectively controlled the disease and also had a higher dry root mass than the inoculated control. Overall, it was very difficult to find continuity between the repeats of the various experiments conducted. However, PANNAR seed planted in *Rhizoctonia* sp. inoculated soil appeared to produce the most consistent results, showing that all the treatments effectively controlled *Rhizoctonia* sp.

5.1 Introduction

Maize is an essential part of the average South African's staple diet (Saunders, 1930). The maize plant is prone to many diseases, some of the most devastating being seed rots caused by *Pythium*, *Fusarium* and *Rhizoctonia* spp. (Garrett, 1970; Kloeper, 1991; Harvey *et al*., 2008). Pathogens of seeds and seedlings decrease germination and emergence (Campbell, 1985). The resistance to disease in young seedlings is very low and thus they need to either escape the presence of damping-off diseases (caused by *Pythium*, *Fusarium* and *Rhizoctonia* spp.) or be pre-treated with fungicides in order to survive (Garret, 1970; Ugoji & Lang, 2008).

There are several fungicides that can be used for control of maize damping-off diseases (Rodriguez-Brljevich *et al.*, 2010). The ones discussed in this dissertation are: Stamina, which has the active ingredient pyraclostrobin and acts by binding to the Q_o centre of the cytochrome bc_1complex on the mitochondria of the fungus, thus inhibiting ATP synthesis (Jin *et al.*, 2009); Flite, which has the active ingredient triticonazole which is part of the triazole group of fungicides and whose mode ofaction is to act as an ergosterol biosynthesis inhibitor (Schwin, 1984); and Celest® XL, which has two active ingredients:fludioxonil, a phenol-pyrrole fungicide (Julia & Senn, 2005) which inhibits transport-associated phosphorylation of glucose, thus reducing mycelial growth (European Commission: Health and Consumer Protection Directorate – General, 2010), and mefenoxam, which is a phenylamide fungicide (O'Neil, 2001) and inhibits protein synthesis in fungi by interfering with the synthesis of ribosomal RNA (Tomlin, 2009).

In the previous chapters of this dissertation, the efficacy of Stamina, Flite and Celest® XL was tested *in vitro* for controlling *Pythium*, *Fusarium* and *Rhizoctonia* spp. by means of the agar amendment method. Subsequently, the effects of these fungicides were tested on the germination and vigour of two cultivars of maize seed. It was found that Celest® XL was the most effective at controlling these pathogens *in vitro* and that none of the fungicides tested had any negative effects on the germination and vigour of the maize seeds. These results should, however, not be

viewed in isolation. It is also important to test the effects of fungicides *in vivo* since different environmental conditions as well as interactions between the soil, the seed and the pathogens may also have an effect on the results (Allen *et al.*, 2004). Emergence of fungicide-treated seeds in pathogen-infested soil under greenhouse conditions gives a good indication of which fungicides are effective in protecting seeds in the presence of pathogens (Govender, 2005).Thus as described in the current chapter, treated and untreated seeds of both maize cultivars (Monsanto DKC78-15B and PANNAR 6Q308B) were planted in greenhouse trials in soil inoculated with *Pythium*, *Fusarium* and *Rhizoctonia* spp.

5.2 Materials and methods

5.2.1 Pathogen preparation

Isolates of each of the three pathogens (*Rhizoctonia solani* RPPR-11034, *Fusarium oxysporum* UPGH 107, *Fusarium solani* UPGH121 and *Pythium ultimum* var *ultimum* Py495 and Py496), which showed high pathogenicity (Section 3.2.4) on PANNAR 6Q308B seed were prepared for use in greenhouse trials by plating them onto potato dextrose agar (PDA, Biolab) and incubating at 25°C (12h day/12h night light cycle) for 7 days. Enough fungal culture was prepared so that two agar plugs of 5mm in diameter could be used per well for inoculation in the seedling trays.There were 6 wells per seedling tray and 6 seedling trays per treatment, as well as 12 treatments (excluding the uninoculated control), therefore there were 432 wells per trial that needed to be inoculated with fungus.

5.2.2 Seed treatment

Monsanto DKC78-15B and PANNAR 6Q308B seeds were treated with the following: Stamina at a rate of 0.075ml/1 000 seed, Stamina at a rate of 0.15ml/1 000 seed, a combination of Stamina and Flite at a rate of 0.075ml/1 000 seed each, and Celest® XL at a rate of 0.33ml/1 000 seed. The treatments were applied by means of a slurry (Section 3.2.5).

5.2.3 Greenhouse preparation, inoculation and planting

The soil (sandy loam topsoil containing 77.5% sand and 22.5% clay and silt, obtained from Schoeman Vervoer, Pretoria) was steam sterilized twice and placed into plastic, 6-well seedling trayswhich had been sterilized in 1.3% formaldehyde overnight and left to dry. Each of the wells in each of the 6-well trays was inoculated with *Rhizoctonia solani* RPPR-11034, *Fusarium oxysporum* UPGH 107, *Fusarium solani* UPGH121 or *Pythium ultimum* var *ultimum* Py495 and

Py496 (depending on the trial) by placing two agar plugs (5mm) of 7-day-old culture in the centre of each well. There were 6 treatments of both Monsanto DKC78-15B and PANNAR 6Q308B seeds: a positive control (untreated maize seeds left uninoculated), a negative control (inoculated, but untreated maize seeds), Stamina at a rate of 0.075ml/1 000 seed, Stamina at a rate of 0.15ml/1 000 seed, a combination of Stamina and Flite at a rate of 0.075ml/1 000 seed each, and Celest® XL at a rate of 0.33ml/1 000 seedas an industry control (except for the first *Fusarium* sp. trial). Six replicates of 6-well seedling trays were done for each treatmentand the experiment was arranged in a randomized complete block design (with randomization being done using a random number table). The trials were conducted in a greenhouse with temperatures ranging from 18°C (night) to 32°C maximum in the day, andthe plants were watered daily. Each greenhouse experiment was evaluated as a separate trial and thus a completely randomized design was used.

5.2.4 Measurements and observations

The plants were observed for phytotoxicity and disease incidence. Shoot length was measured 28 days after planting and the dry mass of shoots and roots was obtained. No phytotoxicity was observed, but this would have been expected to present as yellowing/browning of the leaves. Disease incidence was assessed as a percentage of plants showing any disease symptoms. These trials were repeated twice over two years.

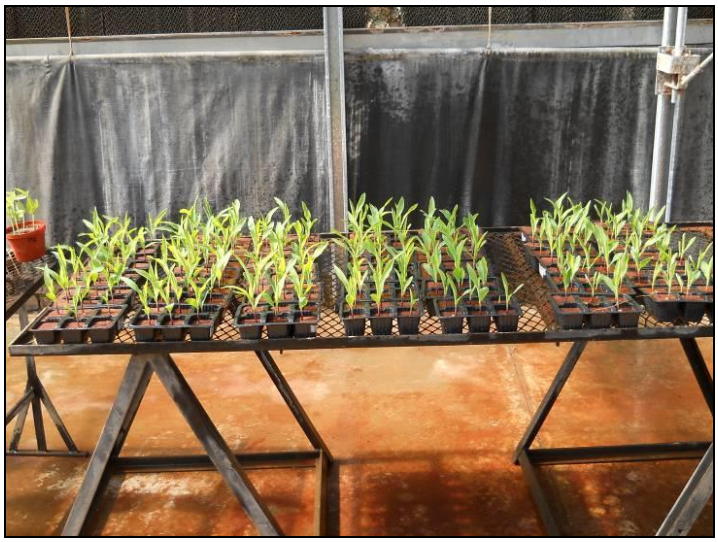

Figure 17: Seedling trays planted with maize in greenhouse trial.

5.3 Statistical analysis

A general analysis of variance (ANOVA) was performed on all data and means were separated with Fischer's least significant difference test (LSD) at the 5% level of significance ($P \leq 0.05$).Standard errors of differences (SED) were determined using GenStat®software (Payne *et al*., 2009)

5.4 Results

In the first greenhouse trial in which Monsanto DKC78-15B seed was planted in soil inoculated with *Fusarium oxysporum* UPGH 107 it was found that there were no significant differences and neither were there any disease symptoms that could be observed (Table 19).

Table 19: Emergence, plant height and dry mass of shoots and roots of treated Monsanto DKC78-15B maize seed planted in *Fusarium oxysporum* UPGH 107 inoculated soil (04/05/2010 to 01/07/2010).

Treatment	Emergence (%)	Plant height (mm)	Dry mass shoots (g)	Dry mass roots (g)
Uninoculated control	100 [1]a*	151.3 [2]a*	1.629 [2]a*	1.06 [2]a*
Inoculated control	94.4 a	151.7 a	1.676 a	1.01 a
Stamina (0.075 ml/1 000 seed)	97.2 a	151.2 a	1.657 a	0.93 a
Stamina (0.15 ml/1 000 seed)	97.2 a	155.1 a	1.636 a	1.11 a
Stamina + Flite (0.075ml each/1 000 seed)	100 a	148.2 a	1.533 a	1.04 a
LSD	7.42	25.5	0.452	0.302
SED	3.68	12.7	0.224	0.150

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

When the above experiment was repeated a year later with *Fusarium solani* UPGH 121and a Celest® XL treatment added, it was found that there were still no significant differences in emergence or plant height (Table 20). There was, however, a large difference in the percentage

of disease incidence between the inoculated and uninoculated control – all the treatments appeared to control the disease effectively. Symptoms of disease included stunting of plants and roots, chlorosis of leaves and browning of roots. Celest® XL showed a slightly lower shoot dry mass than the uninoculated control and the high concentration of the Stamina treatment. There was no significant difference between the root dry mass of any of the Stamina or Flite treatments, and neither between the inoculated and uninoculated control. The Celest® XL treatment showed a lower root dry mass than the uninoculated control.

Table 20: Emergence, disease percentage, plant height and dry mass of shoots and roots of treated Monsanto DKC78-15B maize seed planted in *Fusarium solani* UPGH 121 inoculated soil (17/06/2011 to 15/07/2011).

Treatment	Emergence (%)	Disease (%)	Plant height (mm)	Dry mass shoots (g)	Dry mass roots (g)
Uninoculated control	91.7[1]a*	2.8[1]a*	94.8[2]a*	0.48[2]b*	0.615[2]b*
Inoculated control	86.2a	25b	80.1a	0.383ab	0.378a
Stamina (0.075 ml/1 00 0 seed)	94.5a	5.5a	90a	0.418ab	0.517ab
Stamina (0.15 ml/1 000 seed)	100a	5.5a	91.5a	0.45b	0.537ab
Stamina + Flite(0.075 ml each/1 000 seed)	91.7a	8.3a	84.2a	0.37ab	0.523ab
Celest® XL (0.033 ml/ 1 000 seed)	91.7a	11.2a	71.2a	0.263a	0.352a
LSD	14.65	12.35	25.88	0.1657	0.227
SED	7.27	6.13	12.84	0.0822	0.113

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

In the *Fusarium solani* UPGH 121 inoculated soil planted with PANNAR 6Q308B seed (Table 21), none of the treatments differed from the uninoculated control, but Stamina (0.15ml/1 000 seed) and Stamina + Flite had a significantly higher percentage emergence than the inoculated

control. None of the treatments reduced the percentage of diseased plants when compared with the inoculated control; however, there was also no significant difference between the inoculated and uninoculated controls. Stamina + Flite had a significantly higher dry shoot and root mass than the inoculated control, while Stamina (0.075ml/1 000 seed) also had a higher root mass than the inoculated control. None of the treatments differed from the uninoculated control with respect to dry shoot and root mass.

Table 21: Emergence, plant disease and dry mass of shoots and roots of treated PANNAR 6Q308B maize seed planted in *Fusarium solani* UPGH 121 inoculated soil (07/10/2010 to 02/11/2010).

Treatment	Emergence (%)	Diseased (%)	Dry mass shoots (g)	Dry mass roots (g)
Uninoculated control	72.23[1]ab*	33.3[1]ab*	0.3[2]ab*	0.917[2]ab*
Inoculated control	58.3a	36.1ab	0.26a	0.654a
Stamina (0.075 ml/1 000 seed)	76.68ab	30.6ab	0.348ab	1.12b
Stamina (0.15 ml/1 000 seed)	86.12b	52.8a	0.372ab	1.095ab
Stamina + Flite (0.075 ml each/1 000 seed)	83.34b	30.6ab	0.427b	1.408bc
Celest® XL (0.033 ml/1 000 seed)	72.23ab	16.7b	0.425ab	1.235b
LSD	18.70	26.19	0.166	0.4537
SED	9.28	12.99	0.082	0.2252

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

The above experiment was repeated and it was found that there was no significant difference in emergence percentage (Table 22). There was a significant difference in plant height between the inoculated and uninoculated controls. However, none of the other treatments differed significantly from either of these, except for Celest® XL, which showed aneven greater plant height than the uninoculated control. All treatments, except the high concentration of Stamina, significantly reduced the disease percentage when compared to the uninoculated control. The

inoculated control and low and high concentrations of Stamina had lower dry root masses than the uninoculated control, Stamina+ Flite and Celest® XL. There was no significant difference between the dry shoot mass of the inoculated and uninoculated controls. The possibility of a physiological interaction between the fungicide and the fungus was noted since there was a higher percentage disease at the higher concentration of Stamina however the root and shoot dry masses stayed similar.

Table 22: Emergence, plant height, plant disease and dry mass of shoots and roots of treated PANNAR 6Q308B maize seed planted in *Fusarium solani* UPGH 121 inoculated soil (01/04/2011 to 29/04/2011).

Treatment	Emergence (%)	Plant height (mm)	Diseased(%)	Dry mass roots (g)	Dry mass shoots (g)
Uninoculated control	100.00[1]a*	160.1[2]b*	18.3[1]ab*	1,857[2]bc*	1.007[2]ab*
Inoculated control	97.17a	145.7a	50.8d	1.185a	0.845a
Stamina (0.075 ml/ 1 000 seed)	100.00a	156.4ab	35.0c	1.242a	1.037b
Stamina (0.15 ml/1 000 seed)	97.17a	153.3ab	42.5cd	1.177a	0.980ab
Stamina + Flite (0.075 ml each/1 000 seed)	100.00a	156.0ab	29.2bc	1.715bc	0.907ab
Celest® XL (0.033 ml/ 1 000 seed)	100.00a	164.2b	14.2a	2.000c	0.888ab
LSD	4.725	13.04	14.16	0.2872	0.1663
SED	2.359	6.33	6.88	0.426	0.0840

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

There were no significant differences in the percentage emergence of Monsanto DKC78-15B seed among the treatments and controls in the *Pythium ultimum* var *ultimum* Py 495 inoculated soil. The emergence percentages ranged from 94 to 100% (Table 23). The plant height of Stamina (0.075ml/1 000 seed) and Stamina + Flite treatments was significantly greaterthan that of the inoculated control. The plant height of Celest® XL (0.033ml/1 000 seed) was significantly less than that of the uninoculated control. None of the treatments differed significantly from the uninoculated control in shoot mass except Stamina (0.15ml/1 000 seed). All treatments had root

dry mass values higher than that of the inoculated control, although not significantly. No disease was observed.

Table 23: Emergence, plant height and dry mass of shoots and roots of treated Monsanto DKC78-15B maize seed planted in *Pythium ultimum* var *ultimum* Py 495 inoculated soil (01/04/2011 to 29/04/2011).

Treatment	Emergence (%)	Plant height (mm)	Dry mass shoots (g)	Dry mass roots (g)
Uninoculated control	100[1]a*	200[2]bc*	1.377[2]bc*	1.658[2]a*
Inoculated control	100 a	152.5ab	0.732 ab	1.197 a
Stamina (0.075 ml/1 000 seed)	100 a	219.2 c	1.308 bc	1.602 a
Stamina (0.15 ml/1 000 seed)	94.5 a	153.3 ab	0.558 a	1.243 a
Stamina + Flite (0.075 ml each/1 000 seed)	97.2 a	219.2 c	0.732 ab	1.65 a
Celest® XL (0.033 ml/1 000 seed)	97.2 a	129.2 a	0.852 abc	1.917 a
LSD	6.61	50.08	0.601	0.884
SED	3.28	24.85	0.298	0.439

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

The second time that Monsanto DKC78-15B seed was planted in soil inoculated with *Pythium ultimum* var *ultimum* Py 495 (Table 24), it was found that the emergence was consistently high, except in the inoculated control, thus *Pythium ultimum* var *ultimum* Py 495 caused pre-emergence damping-off. Disease symptoms on plants which did emerge included stunting, chlorosis of leaves and browning of roots. There was no significant difference in plant height or disease percentage among the seedlings that did emerge. The uninoculated control had a lower dry shoot mass than any of the other treatments as can be expected due to its much lower emergence.

Table 24: Emergence, plant disease and dry mass of shoots and roots of treated Monsanto DKC78-15B maize seed planted in *Pythium ultimum* var *ultimum* Py 495 inoculated soil (28/11/2011 to 14/01/2012).

Treatment	Emergence (%)	Plant height (mm)	Disease (%)	Dry massshoots (g)	Dry massroots (g)
Uninoculated control	91.7[1]a*	14.83[2]a*	22.2[1]a*	0.88[2]b*	0.833[2]b*
Inoculated control	38.9b	13.87a	13.9a	0.255a	0.265a
Stamina (0.075 ml/ 1 000 seed)	94.4a	14.06a	27.8a	0.933b	0.897b
Stamina (0.15 ml/1 000 seed)	83.3a	13.88a	16.7a	0.845b	0.853b
Stamina + Flite (0.075 ml each/1 000 seed)	91.7a	15.2a	8.3a	1.188b	1.143b
Celest® XL (0.033 ml/ 1 000 seed)	94.4a	14.39a	13.9a	0.953b	1.032b
LSD	18.64	4.396	21.35	0.419	0.4664
SED	9.05	2.134	10.37	2.035	0.2266

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

When PANNAR 6Q308B seeds were planted in soil inoculated with *Pythium ultimum* var *ultimum* Py 495(Table 25), it was found that the Stamina+Flite and Celest®XL treatments unexpectedly had a lower emergence than any of the other treatments. No disease symptoms could be found and thus no disease assessment could be done for this experiment. There was also no significant difference between the dry root and shoot mass of any of the treatments.

Table 25: Emergence, plant disease and dry mass of shoots and roots of treated PANNAR 6Q308B maize seed planted in *Pythium ultimum* var *ultimum* Py 495 inoculated soil (01/04/2011 to 29/04/2011).

Treatment	Emergence (%)	Dry mass shoots (g)	Dry mass roots (g)
Uninoculated control	100.00[1]a*	0.672[2]a*	1.055[2]a*
Inoculated control	94.33ab	0.630a	1.068a
Stamina (0.075 ml/1 000 seed)	91.58ab	0.690a	1.028a
Stamina (0.15 ml/1 000 seed)	97.17a	0.745a	1.218a
Stamina + Flite (0.075 ml each/1 000 seed)	77.50b	0.800a	1.167a
Celest® XL (0.033 ml/1 000 seed)	77.67b	0.717a	1.057a
LSD	17.56	0.2295	0.3881
SED	8.52	0.114	0.1884

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

The above experiment was repeated in November 2011 to January 2012 and it was found that there were again no significant differences between any of the variables in any of the treatments (Table 26).

Table 26: Emergence, plant height, disease and dry mass of shoots and roots of treated PANNAR 6Q308B maize seed planted in *Pythium ultimum* var *ultimum* Py 495 inoculated soil (28/11/2011 to 14/01/2012).

Treatment	Emergence (%)	Plant height (mm)	Disease (%)	Dry massshoots (g)	Dry massroots (g)
Uninoculated control	80.6[1]a*	14,97[2]a*	28.6[1]a*	0.768[2]a*	0.68[2]a*
Inoculated control	80.6a	13.99a	30.6a	0.743a	0.578a
Stamina (0.075 ml/1 000 seed)	91.7a	13,29a	13.9a	0.918a	0.895a
Stamina (0.15 ml/1 000 seed)	86.1a	15a	30.6a	0.842a	0.862a
Stamina + Flite(0.075 ml each/1 000 seed)	83.3a	15,24a	22.2a	0.89a	0.858a
Celest® XL (0.033 ml/1 000 seed)	72.2a	14,01a	11.1a	0.768a	0.673a
LSD	28.89	2.863	24.66	0.5454	0.5165
SED	14.03	1.390	11.97	0.2648	0.2508

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

All Monsanto DKC78-15B seedlings of all the treatments emerged in soil inoculated with *Rhizoctonia solani* RPPR-11036 (Table 27). There were no significant differences in plant height among all treatments and both controls. There were also no significant differences in dry mass of shoots between all treatments and both controls, with the exception of Celest® XL, which had a significantly lower value than the inoculated control. There were no significant differences in dry mass of roots among all treatments.

Table 27: Emergence, plant height and dry mass of shoots and roots of treated Monsanto DKC78-15B maize seed planted in *Rhizoctonia solani* RPPR-11036 inoculated soil (15/10/2010 to 10/11/2010).

Treatment	Emergence (%)	Plant height (mm)	Dry mass shoots (g)	Dry mass roots (g)
Uninoculated control	100[1]a*	196.0[2]a*	1.052[2]b*	4.892[2]a*
Inoculated control	100 a	178.6 a	0.792 ab	1.99 a
Stamina (0.075ml/1000 seed)	100 a	191 a	0.935 b	2.373 a
Stamina (0.15 ml/1 000 seed)	100 a	185.1 a	0.792 ab	2.078 a
Stamina + Flite (0.075 ml each/1 000 seed)	100 a	192.6 a	0.903 ab	2.697 a
Celest® XL (0.033 ml/1 000 seed)	100 a	186.4 a	0.605 a	1.795 a
LSD	Ns	20.82 a	0.3088	3.437
SED	Ns	10.33	0.1533	1.706

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

Monsanto DKC78-15B seed was again planted in soil inoculated with *Rhizoctonia solani* RPPR-11036 (Table 28), and it was found that there were no significant differences in emergence. Unexpectedly, the Celest® XL treatment showed a higher disease percentage than any of the other treatments,although it was still lower than that off the inoculated control. Symptoms included stunting of the plants and roots, brown lesions on the roots and base of the stem and chlorosis on the leaves. All the treatments successfully increased the plant height and dry mass of the shoots when compared to the inoculated control.

Table 28: Emergence, plant height and dry mass of shoots and roots of treated Monsanto DKC78-15B maize seed planted in *Rhizoctonia solani* RPPR-11036 inoculated soil (17/06/2011 to 15/07/2011).

Treatment	Emergence (%)	Disease (%)	Plant height (mm)	Dry mass shoots (g)	Dry mass roots (g)
Uninoculated control	97.2[1]a*	2.8[2]a*	99.9[2]b*	0.548[2]b*	0.84[2]cd*
Inoculated control	97.2a	66.6c	71.6a	0.298a	0.302a
Stamina (0.075 ml/1 000 seed)	97.2a	5.5a	102.9b	0.565b	0.942d
Stamina (0.15 ml/1 000 seed)	97.2a	8.3a	98.1b	0.51b	0.753cd
Stamina + Flite(0.075 ml each/1 000 seed)	100a	13.8a	94.7b	0.513b	0.653bc
Celest® XL (0.033 ml/1 000 seed)	100a	30.5b	92.5b	0.482b	0.475ab
LSD	6.6	14.05	17.58	0.1551	0.264
SED	3.3	6.97	8.72	0.0770	0.131

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

In the PANNAR 6Q308B cultivar in soil inoculated with *Rhizoctonia solani* RPPR-11036 only Stamina + Flite had a higher emergence than the inoculated control and none of the treatments differed significantly from the uninoculated control although all the treatments had higher values (Table 29). The uninoculated control and Stamina (0.075ml/1 000 seed) had the lowest level of disease but did not differ from Celest® XL. The inoculated control had significantly the highest percentage of diseased plants. There were no statistical differences in dry shoot mass among treatments and the two controls. All the treatments had a higher dry root mass than the inoculated control, and Stamina (0.15ml/1 000 seed) and Stamina + Flite had a higher dry root mass than even the uninoculated control.

Table 29: Emergence, plant disease and dry mass of shoots and roots of treated PANNAR 6Q308B maize seed planted in *Rhizoctonia solani* RPPR-11036 inoculated soil (07/10/2010 to 02/11/2010).

Treatment	Emergence (%)	Diseased plants (%)	Dry mass shoots (g)	Dry mass roots (g)
Uninoculated control	77.84[1]ab*	8.3[1]a*	0.613[2]a*	0.937[2]ab*
Inoculated control	75a	50c	0.342a	0.578a
Stamina (0.075 ml/1 000 seed)	91.67ab	2.8a	0.81a	1.263bc
Stamina (0.15 ml/1 000 seed)	86.17ab	30.0b	0.417a	1.35cd
Stamina + Flite (0.075 ml each/1 000 seed)	94.45b	30.6b	0.397a	1.447cd
Celest® XL (0.033 ml/1 000 seed)	88.89ab	20.0ab	0.457a	1.212bc
LSD	16.74	25.48	0.55	0.4124
SED	8.31	12.65	0.27	0.2047

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

When the experiment was repeated (Table 30), it was found that there were no significant differences in emergence, plant height or dry shoot mass. All the treatments significantly reduced the disease percentage when compared to the inoculated control. Only the low concentration of Stamina and the combination treatment of Stamina and Flite produced a significant increase in dry root mass when compared to the inoculated control.

Table 30: Emergence, plant disease and dry mass of shoots and roots of treated PANNAR 6Q308B maize seed planted in *Rhizoctonia solani* RPPR-11036 inoculated soil (17/06/2011 to 15/07/2011).

Treatment	Emergence (%)	Disease (%)	Plant height (mm)	Dry mass shoots (g)	Dry mass roots (g)
Uninoculated control	94.5[1]a*	13.8[2]ab*	92.7[2]a*	0.433[2]a*	0.507[2]b*
Inoculated control	94.5a	72.2c	82.8a	0.418a	0.318a
Stamina (0.075ml/1000 seed)	94.5a	27.8b	89.1a	0.475a	0.528b
Stamina (0.15 ml/1 000 seed)	94.5a	30.5ab	83.8a	0.407a	0.392ab
Stamina + Flite (0.075 ml each/1 000 seed)	91.7a	2.8a	88.6a	0.418a	0.495b
Celest® XL (0.033 ml/ 1 000 seed)	94.5a	25.0b	85.9a	0.427a	0.322a
LSD	12.5	15.5	16.38	0.1279	0.1601
SED	6.2	7.7	8.13	0.0635	0.0795

[1]Each value is a mean percentage of six replicates of six seeds
[2]Each value is a mean of six replicates of six seeds
*Means within a COLUMN not followed by the same letter are significantly different ($P \leq 0.05$)

5.5 Discussion and conclusion

In Chapter 3 of this dissertation, it was found that Celest® XL controlled *Pythium*, *Fusarium* and *Rhizoctonia* spp. most effectively *in vitro*.Loper & Buyer (1991) as well as Paulitz and Loper (1991) did, however, warn against associating *in vitro* inhibition with *in vivo* activity.In the greenhouse, Celest® XL did not perform as well as Stamina and the combination of Stamina and Flite. Both concentrations of Stamina as well as a combination of Stamina and Flite treatments appeared to provide an adequate level of disease control against *Rhizoctonia solani* RPPR-11034, *Fusarium oxysporum* UPGH 107, *Fusarium solani* UPGH121 and *Pythium ultimum* var *ultimum* Py495 and Py496 and even seemed to have a growth-promoting effect on the plants. This confirms the research done by BASF (2008), wheremaize seeds treated with Stamina were

planted in cold conditions and it was found that the treated seeds had a greater emergence and increased plant height when compared to the untreated control.

Allen *et al.* (2004) evaluated the effect of three systemic fungicides, a protectant fungicide and a disinfectant on the germination of longleaf pine seed as well as the efficacy of these fungicides for controlling *Fusarium* spp. They recorded differences in product performance between the laboratory and greenhouse trials and attributed these differences to different seed sources, although the differences could also simply have been due to external factors in the greenhouse. Mavrodi *et al.* (2012) also found that laboratory test results could not always be correlated to greenhouse trials. Thus it is essential that greenhouse trials are conducted.

In Chapter 4 of this dissertation it was found that none of the fungicides tested had any negative effect on the germination or vigour of either of the maize cultivars under laboratory conditions. In the greenhousetrials (Chapter 5) however, in one experiment where Celest® XL and the combination of Stamina and Flite were applied to PANNAR 6Q308B seeds planted in soil inoculated with *Pythium ultimum* var *ultimum* Py 495, poor emergence and some stunting of seedlings occurred, possibly due to phytotoxicity. The stunting did not occur in the other experiments, thus indicating that stunting was not due to the fungicide as found by Govender (2005), who showed that Celest® XL did not have any phytotoxic effects on maize seed.

In the current chapter of this dissertation, the same two maize cultivars (Monsanto DKC78-15B and PANNAR 6Q308B) as in previous chapters were used in greenhouse trials with *Rhizoctonia solani* RPPR-11034, *Fusarium oxysporum* UPGH 107, *Fusarium solani* UPGH121 and *Pythium ultimum* var *ultimum* Py495 and Py496 and the same treatments as in previous chapters. When Monsanto DKC78-15B seed was planted in *Fusarium oxysporum* UPGH107 inoculated soil, no significant differences were found. When the experiment was repeated there were again no significant differences in emergence or plant height, but there was a significant difference in disease incidence. This could indicate that for this*Fusarium oxysporum* UPGH107, pathogenicity does not manifest in reduced emergence or plant height under these trial conditions.All the treatments lowered the disease incidence significantly when compared to the inoculated control. When PANNAR 6Q308B seed was planted in soil inoculated with *Fusarium solani* UPGH121, the low concentration of Stamina and the Stamina + Flite treatments showed a higher emergence percentage than the inoculated control. When the experiment was repeated, no significant differences in emergence percentage was found, but the Celest® XL treatment did show a greater plant height than even the uninoculated control. This indicates that Celest® XL could have a

plant growth promoting effect apart from pathogen inhibition. A similar observation was made in the case of Stamina in chapter 3.

When Monsanto DKC78-15B seed was planted in soil inoculated with *Pythium ultimum* var. *ultimum* Py495, the only significant differences found were that the low concentration of Stamina and the Stamina + Flite treatments showed a significantly greater plant height when compared to the inoculated control. When the experiment was repeated the level of emergence was consistently high except in the inoculated control, thus indicating pre-emergence damping-off. When PANNAR 6Q308B maize was planted in soil inoculated with *Pythium ultimum* var. *ultimum* Py495. It was found that the Stamina + Flite as well as the Celest® XL treatments unexpectedly had a lower emergence than that of any of the other treatments. No disease symptoms could be observed, and when the experiment was repeated no significant differences were found in any of the variables. These results indicate that under the trial conditions in this study Stamina alone and the combination of Stamina and Flight treatments were effective in suppressing *Pythium ultimum* var. *ultimum* Py495 therby causing an increase in plant height and seedling emergence on Monsanto DKC78-15B seed. Whereas on PANNAR 6Q308B effective disease control was not obtained. This could possibly be due to the fact that PANNAR 6Q308B seedlings are more susceptibleto *Pythium ultimum* var. *ultimum* Py495, confirming previous observations in chapter 3 of this study.

When Monsanto DKC78-15 seed was planted in soil inoculated with *Rhizoctonia solani* RPPR-11036, all the seedlings emerged. The Celest® XL treatment had a significantly lower dry mass than the uninoculated control. When the experiment was repeated it was found that all the treatments significantly decreased the disease incidence and also resulted in greater plant heights when compared to the inoculated control. When PANNAR 6Q308B seed was planted in soil inoculated with *Rhizctonia* spp., it was found that the Stamina + Flite treatment significantly increased the emergence percentage and that the low concentration of Stamina as well as the Stamina + Flite treatment displayed a higher dry root mass than even the uninoculated control. When the experiment was repeated, all the treatments significantly reduced the disease incidence when compared to the inoculated control; the low concentration of Stamina as well as the Stamina + Flite treatment also showed a significant increase in dry root mass. This indicates that the Stamina+ Flite treatment effectively controlled the pathogen and also possibly had a growth-promoting effect on the plants. Aveling *et al.* (2012) found that the use of pesticides was sometimes associated with increased shoot and root biomass of maize plant in the presence of pathogens. Pereira *et al.* (2008) also found that maize seed treated with fludioxonil and

metalaxyl, among other fungicides, significantly improved the field emergence of maize cultivars with low vigour. Jorgensen *et al.* (2012) found that triticonazole significantly reduced seedling blight cause by *Fusarium* on wheat.

As was shown in previous chapters and confirmed by the current chapter, the Monsanto DKC78-15B maize had higher seedling vigour than the PANNAR 6Q308B maize, showing consistently greater plant heights and higher dry root and shoot masses throughout all experiments (Matthews & Khajeh-Hosseini, 2006). It was difficult to obtain consistency in results when repeating experiments, since these are biological systems and are influenced by a variety of different factors such as temperature, humidity and interactions between the seedlings, pathogens and the soil (Allen *et al.*, 2004, Mavrodi *et al.*, 2012).

In future these experiments could be improved by implimenting a factorial-type design in order to quantify any pathogen x genotype internactions and thus obtain more reliable results. In light of the high variability observed between repeats of the seedling tray trials it would be advisable to use bigger containers with larger volumes of soil which can be expected to decrease variability.

5.6 Literature cited

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Chapter 6
Summary

Maize (*Zea mays* L.) is a cereal crop grown throughout the world. It plays an important role in the diet of millions of African people due to its high yields per hectare, its ease of cultivation and adaptability to different areas, its versatile food uses and storage characteristics (Asiedu, 1989). Maize is a staple crop in Southern Africa where it accounts for 70% of total human intake of calories (Martin *et al*., 2000). Thus it is essential that maize can be sustainably produced in South Africa and that maize seeds are of the highest possible quality.

Fungi rank as the second biggest cause of deterioration and loss of maize (Ominski *et al.*, 1994). At the very early stages of seedling development, maize seedlings are attacked by fungi such as *Pythium, Fusarium* and *Rhizoctonia* spp., which cause severe diseases, including pre-emergence damping-off, which lead to yield losses (Dodd & White, 1999). These diseases can be effectively controlled by applying fungicidal seed treatments (Peltier *et al.*, 2010). However, these seed treatments should be tested to ensure that they provide an acceptable level of control against the pathogens and that they do not have any negative effects on the germination and vigour of the maize seed.

In Chapter 3 of this dissertation, three important fungal genera, namely *Pythium, Fusarium* and *Rhizoctonia* spp., were isolated from diseased maize plant samples and soil. The beet seed baiting method was used for *Rhizoctonia* sp. and the citrus leaf disk baiting method for *Pythium* sp. *Fusarium* sp. was isolated by means of serial dilution on a selective medium. The selective media used were agar containing chlorotetracycline hydrochloride and streptomycin sulfate for *Rhizoctonia*, pimaricin and vancomycin, PARP (pimaricin + ampicillin + rifampicin + pentachloronitrobenzene (PCNB) agar) for *Pythium* sp. and Rose Bengal Glyceraldehyde Urea (RBGU) for *Fusarium* sp. These fungal isolates, as well as some isolates revivedfrom the University of Pretoria's culture collection and obtained from the Agricultural Research Council (ARC-PPRI), were used for pathogenicity trials conducted on maize in the between-paper method (BP), and in six-celled plastic seedling trays in the greenhouse (described in Chapter 5).

In order to test the efficacy of Stamina, Flite and Celest® XL for controlling *Pythium* spp., *Fusarium* spp. and *Rhizoctonia* spp. *in vitro*, each of the three fungicides was added to PDA at concentrations of 1, 2 and 3ppm. In order to mirror the treatments used in other experiments, a

combination of Stamina and Flite was also incorporatedinto PDA at concentrations of 1, 2 and 3ppm each. A 5mm^2 block of each of the fungi was plated onto the centre of the media and incubated at 25°C. The diameter of the fugal growth was measured at regular intervals depending on the rate of growth of the fungus. It was found that Celest® XL was very effective in controlling all three of these pathogens *in vitro*, confirming research done by Govender (2005), who found that Celest® XL effectively controlled these pathogens on maize. The combination of Stamina and Flite also controlled these pathogens although to a lesser extent. Research done by BASF in 2008 showed that Stamina is able to control *Pythium*, *Fusarium* and *Rhizoctonia* spp. Pyraclostrobin (the active ingredient of Stamina) has also been found to effectively control all three of these pathogens in numerous *in vitro* and *in vivo* experiments (Broders *et al*., 2007; Peltier *et al.*, 2010; Solorzano & Malvick, 2011).

In Chapter 4 of this dissertation, the effect of three different fungicides (Stamina, Flite and Celest® XL) on the germination and vigour of two *Zea mays* cultivars (Monsanto DKC78-15B and PANNAR 6Q308B) was assessed. This was achieved by carrying out a standard germination test, a cold soil test, short accelerated ageing and long-term storage tests according to the guidelines of the International Seed Testing Association (ISTA, 2012). It was found that none of the fungicides had a detrimental effect on either seed germination or vigour and no phytotoxic effects were observed. The combination of Stamina and Flite treatment also led to an increased percentage germination after the cold soil test when compared to the untreated control. This confirms the research of Govender (2005), who showed that Celest® XL had no negative effects on the germination or vigour of maize, and BASF (2008), which showed that Stamina could even lead to increased germination and an increased yield of maize under cold conditions when compared to an untreated control. Bradley *et al*. (2001) found that fungicide seed treatments do not affect the vigour and viability of maize seeds. Seeds treated with fludioxonil also showed an increased radicle length in some cases (Munkvold & O'Mara, 2002). Increased radicle length could indicate increased vigour of the seeds (Matthews & Khajeh-Hosseini,2006).

In Chapter 5 of this dissertation the efficacy of Stamina, Flite and Celest® XL for controlling *Pythium*, *Fusarium* and *Rhizoctonia* spp. *in vivo* was tested by means of greenhouse trials. The trials were conducted in seedling trays using a randomized complete block design and agar plugs of the three pathogens as the inoculum. In most instances results varied between repeat experiments, possibly indicating sensitivity of the seedling tray system to variations in conditions such as soil moisture (drying of soil) and ambient temperatures. This fact notwithstanding, in general it was found that seed treatment with Celest® XL was not as effective

in controlling the three fungi as seed treatments with Stamina and the combination of Stamina and Flite respectively. In three instances all the fungicide seed treatments significantly lowered disease incidence. These were the repeat experiment with Monsanto DKC 78-15B inoculated with *Fusarium* and the repeat experiments with Monsanto DKC 78-15B and PANNAR 6Q308B inoculated with *Rhizoctonia* respectively. In two experiments seed treatments with low concentrations of Stamina and Stamina + Flite resulted in increased emergence in comparison with the untreated pathogen-inoculated control, namely in the first experiment with PANNAR 6Q308B inoculated with *Fusarium* and the first experiment with PANNAR 6Q308B inoculated with *Rhizoctonia.* Seed treatments with low concentrations of Stamina and Stamina + Flite respectively also resulted in increased root dry mass in the two experiments with PANNAR 6Q308B inoculated with *Rhizoctonia*. In the greenhouse experiments *Rhizoctonia* proved to be the most pathogenic of the three fungi based on disease incidence. Typical symptoms of disease observed for *Rhizoctonia* spp. were stunted root and shoot growth as well as chlorotic lesions on the leaves and browning of the roots. In the repeat experiment with Monsanto DKC78-15B inoculated with *Pythium* spp., the *Pythium* spp. caused pre-emergence damping-off.

In conclusion,of the three fungicides tested, Celest® XL provided the highest level of inhibition of fungal growth *in vitro*, whereas Stamina as well as the combination of Stamina and Flite treatments provided better levels of disease control *in vivo* in the greenhouse experiments.The greenhouse trials in seedling trays gave varying results between repeat experiments, possibly indicating that the system was too sensitive to factors such as drying out of the soil and ambient temperature variations. Overall, the combination of Stamina and Flite appears to have greater efficacy at controlling each of the tree pathogens than either Stamina or Flite on their own. None of the fungicide treatments had any negative effects on the seed germination and vigour of eitherMonsanto DKC78-15B or PANNAR 6Q308B maize cultivars. Monsanto DKC78-15B had a higher germination and vigour across all experiments when compared to the PANNAR 6Q308B cultivar.

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