Incidence and etiology of maize seedling blight and control of soil borne pathogens using seed treatments

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It all starts here ™

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"*Choosing a goal and sticking to it changes everything*" – Scott Reed.

ABSTRACT

Seedling blight of maize has significantly influenced field crop stands and seedling vigour over various localities and seasons. The extent of the problem is influenced by a number of factors which includes soil temperature (generally below 13 °C), waterlogged soils, inadequate fertilization, herbicide damage and fungal pathogens. The fungi generally causing seedling damping off are often involved in a complex and succession over time varying in importance depending on the field circumstances at a given time. These generally include the *Pythium* spp., *Rhizoctonia* spp. and various *Fusarium* spp. These have been recorded in a number of studies conducted by local researchers in the late 1980's and early 1990's on sorghum but to a lesser degree on maize. Uncertainty regarding the status of the etiology of maize seedling blights as maize production practices have changed dramatically in the last 10 years with increased plant populations, reduced tillage, increased crop rotation options and new short season maize hybrids. It is therefore essential to determine the present status of seedling blights in South Africa to confirm the necessity of fungicide seed treatments to ensure adequate plant densities and seedling vigour.

Cob and tassel smut caused by *Sphacelotheca reiliana* is a disease of maize that was a problem in the 1970's. Due to improved fertilisation, fungicide seed treatments and hybrid resistance this disease was reduced to such levels that the disease was only found to occur on research farms where seedlings were inoculated. Since 2007, the disease was reported to reach epidemic proportions on the heavy clay soils in the Standerton area. This disease has since spread over the last seven seasons to a range including northern KwaZulu/Natal, namely as far as Underberg/Swartberg, the Witbank, Ermelo, Middelburg and Delmas area in Mpumalanga and to Harrismith in the eastern Free State maize production area. This may be due to susceptible hybrids coming onto the local market or the inability of traditional fungicide seed treatments to contain infection. New and unregistered seed treatments

available will be tested for their ability to control cob and tassel smut in two fields over two seasons.

The aims of this dissertation were to determine the extent of the seedling blight problem in commercial fields throughout the maize industry. To determine the efficacy of fungicide seed treatments for the control of maize seedling blights using both field and greenhouse studies, and to determine the efficacy of fungicide seed treatments for the control of cob and tassel smut of maize in field trials. A total of 101 localities were sampled throughout the maize producing region of South Africa with root discolouration varying from 0 to 90 % root discolouration. Seventy different fungal species were isolated from the maize seedlings roots which include species such as *Aspergillus*, *Clonostachus*, *Fusarium*, *Trichoderma* and *Penicillium*. The most commonly isolated fungi which included *Aspergillus niger*, *Fusarium solani*, *Fusarium verticillioides* and *Fusarium oxysporum* were evaluated in glasshouse studies to determine their pathogenicity. Pathogenicity differed between isolates of the same fungal species, which were collected from different geographical regions, in the glasshouse studies. Field trials for seedling blight disease showed significant differences between the localities ($P < 0.001$) the trials were planted at, and between seed treatments. Significant season ($P < 0.001$) and locality ($P < 0.05$) differences were also found for cob and tassel smut trials planted at Potchefstroom, North-West province and Greytown, KwaZulu/Natal Province respectively. Fungicide seed treatments also showed significant differences for cob and tassel smut regarding plants infected ($P < 0.001$) and yield loss ($P < 0.05$). Overall seed treatments can be seen as an effective controlling agent for the control of seed- and soil-borne fungi on maize.

Key words: Seedling blight, etiology, efficacy, fungicide, seed treatments, root discolouration, *Sphacelotheca reiliana, Aspergillus* spp., *Clonostychus* spp., *Fusarium* spp., *Trichoderma* spp., *Penicillium* spp.

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Chapter 1

General introduction

1.1 The production of maize in South Africa

Maize (*Zea mays* L.) is the most important summer grain crop in South Africa. Maize has a long history of breeding and cultivation, and was introduced to South Africa in 1655, shortly after the Dutch colonists arrived (Saunders, 1930). Currently, maize is the most important crop in the world following wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) (Tilman, 1999). Maize is grown in tropical, subtropical and temperate regions, where it is produced between 30 ºC and 55 ºC (Shaw, 1988). It is mostly produced where rainfall is between 450 and 600 mm per annum under irrigation, or with rainfall between 600 to 900 mm per annum (Sprague and Dudley, 1988; Tekwa and Bwade, 2011). In South Africa maize is primarily used for human consumption (white maize), and animal feed (yellow maize) (SAGIS, 2014). Maize is relatively easy to cultivate and can produce a large yield of kernels, which is high in starch that can be metabolised into energy (Sprague and Dudley, 1988). In South Africa maize is the most commonly planted crop followed by soybean, sunflower, sorghum, groundnut and dry beans (SAGIS, 2014).

Maize production in South Africa is largely rain dependent as 80 % of maize is cultivated on dry land while only 20 % is irrigated (SAGIS, 2014). The percentage dry land maize production in relation to irrigated production (Figure 1.1) indicates that South African maize farmers are more dependent on rainfall than on irrigation (SAGIS, 2014). South Africa is largely dependent on seasonal rain for crops to be produced, but is prone to extreme climatic conditions which often results in a poor national yield (Martin *et al*., 2000). Walker and Schulze (2007) also highlighted the importance on the El Nino phenomenon as it plays a major role in food and economic security for South African farmers.

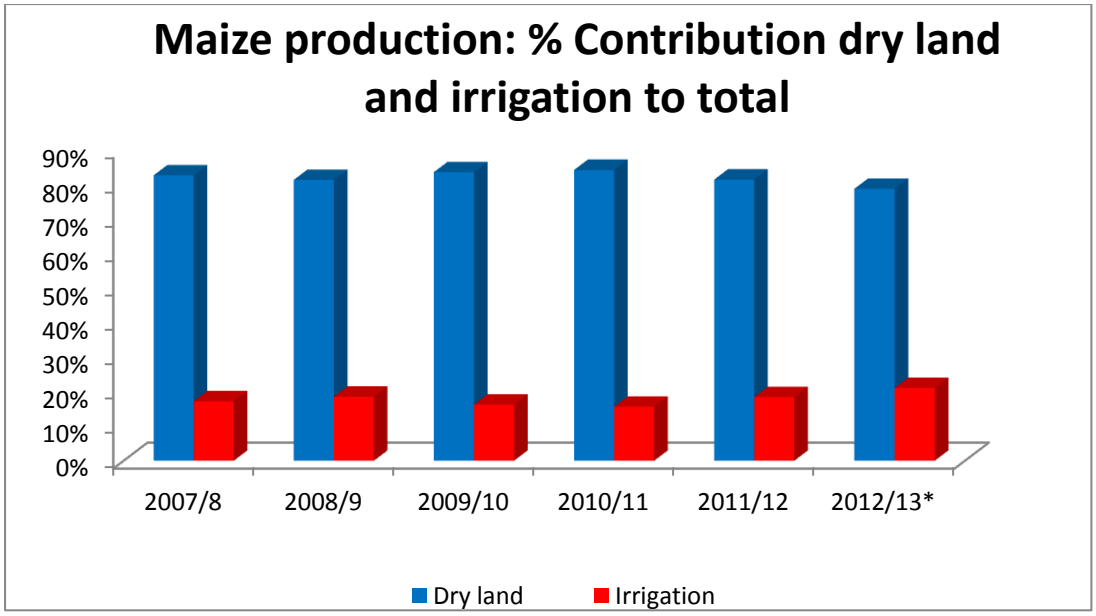

Figure 1.1: Maize production on dry and irrigation lands in South Africa (SAGIS, 2014).

Maize is planted in seven of the nine provinces of South Africa and includes the Free State, Mpumalanga, North-West, Gauteng, KwaZulu-Natal, Limpopo and the Northern Cape provinces on a total area of 2.78 million ha. A total of 11.72 million tons of maize were produced in the 2012/13 season and increased to 13.029 million tons in the 2013/14 season (SAGIS, 2014). South Africa produced a total of 5.606 million tons of white maize and 6.203 million tons of yellow maize for the 2012/13 season. Dry land farmers produced an average of 3.58 tons/ha while irrigation farmers produced an average of 10.03 tons/ha in the 2013/14 season (SAGIS, 2014).

Maize plants are susceptible to biotic diseases from the seedling stage up to maturity which reduces the yield potential of the maize plant resulting in yield loss at harvest (Sprague and Dudley, 1988).

1.2 Effect of soil- and seed borne fungi on maize

Soil can be seen as a complex and dynamic environment, in which microorganisms play a major role in the biological activities (Newton *et al*., 2003). Soil microorganisms contribute greatly to the soil habitat and helps with nitrogen fixation, decomposition of organic matter,

breakdown of metabolic by-products and agrochemicals (Bridge and Spooner, 2001). Newton *et al.* (2003) stated that soil microorganisms increase the availability of nitrates, sulphates, phosphates and essential minerals to maize plants. Fungi also play an important role in the cycling of nutrients, plant health and development (Newton *et al*., 2003). Certain fungi are known to be antagonistic to plant pathogens by means of chemical mobilisation, outcompeting pathogens by growing much faster, to decompose plant debris in the soil on which certain pathogens survive and supply the plant with nutrients to stimulate growth and reduce stress (Newton *et al*., 2003).

Many biotic factors and agricultural practices such as insect and nematode damage, plant depth, row spacing as well as herbicide and fertilizer damage can influence the soil and its fungal composition at any given time (Sabatini and Innocenti, 1999). Beneficial soil fungi such as the arbuscular mycorrhizae complex colonise the roots of a wide range of plants as obligatory symbiotic fungi (David *et al*., 1999). These mycorrizal fungi are beneficial to the plants and can result in increased growth, improved water and nutrient uptake, pest and disease resistance, as well as modification of root morphology that helps with increased growth and improved water uptake throughout globose, lipid rich structures in intracellular areas of the root cortex (David *et al*., 1999). Agricultural practices also influence the fungal soil community and the pathogenicity of certain soil fungi (Soonthornpoct *et al.*, 2000). For instance, no-tillage systems often increase specific pathogenic organisms that survive on crop debris retained on the soil surface, it also influences the soil temperature and moisture which can have diverse effects on the type of crop planted and the fungi found to colonise a specific field (Soonthornpoct *et al.,* 2000). Govaerts *et al.* (2005) also stated that changes in tillage, residue and rotation practices can induce a number of major shifts in the composition of the soil pathogens and beneficial organisms.

Many fungi can cause a range of plant diseases that can completely destroy maize (Newton *et al*., 2003). Soil-borne phytopathogenic fungi are responsible for causing diseases of the root, stem and vascular tissues (Pane *et al*., 2013). These diseases are damaging to a wide

range of susceptible hosts under diverse environmental conditions and are very difficult to control without fungicides, resulting in serious economic losses (Pane *et al*., 2013). Many species of pathogenic fungi can be present in agricultural soils, which are reservoirs of inoculum that infect living plants or survive on dead plant residues in and on the soil from where they infect and invade host plant tissue (Sabatini and Innocenti, 1999). Fungicides are effective for controlling pathogens but also affect non-target mycorrhizal fungi, which are favourable to plant growth and development (Hongyan *et al*., 2013).

These phytopathogenic fungi infecting crops are not only soil-borne but some are seed borne. A study conducted by Debnath *et al*. (2012) indicated that seed rot, seedling blight, *Bipolaris* leaf spot and *Curvularia* leaf spot are all seed borne diseases that infect maize seedlings. The use of chemical control for these seed borne diseases was not effective, resulting in poor control (Debnath *et al*., 2012).

Fungi often associated with seedling blights of maize are seed- and soil-borne pathogens and include *Aspergillus* spp*., Fusarium* spp*., Gibberella* spp*., Nigrospora* spp*., Penicillium* spp*., Pythium* spp*., Rhizoctonia* spp*., Stenocarpella* spp*.* and *Trichoderma* spp. (Solorzano and Malvick, 2011). If seeds are infected with soil- and/or seed borne pathogens it may lead to the infection of the seedling roots as pathogenic hyphae penetrate the root epidermis and grow systemically throughout the plant and often continue to infect the stalks and ears of maize (Solorzano and Malvick, 2011). These systemic fungi may influence seed quality through the increase of fatty acids, reduction in emergence, plant growth and stand as well as the spoilage of grain (Niaz and Dawar, 2009). In most cases maize seeds that are severely infected with fungal pathogens are removed in the seed cleaning process, if seeds have moderate to low infection levels they may not be seen and therefore not removed prior to planting (Solorzano and Malvick, 2011).

1.3 Seedling blight and damping off of maize in South Africa

Seedling blight of maize has significantly influenced field crop stands and seedling vigour over various localities and seasons in South Africa. Seedling blight is a disease complex caused by a combination of different species of fungi that are found in soil or on/in seed. This complex is affected by the environment conditions, the host (maize) resistance and presence of fungal inoculum (Agrios, 2005). A study conducted by Bacon *et al*. (1994) screened maize hybrids for resistance to seedling blight disease and found that as the pericarp of the seed breaks and the roots start to develop, the seedling blight fungi present in the soil will grow and infect the maize seedling. The fungi associated with seedling blight can vary in terms of the aggressiveness of diverse biotypes, which in turn will influence hybrids differently and will cause varying disease severities (Bacon *et al*., 1994). Disease severity may also vary between localities due to different soil types, soil moisture levels, soil temperatures, soil biology and soil nutrient imbalances (Dodd and White, 2000). The most common fungi reported to be involved in seedling blight include *Pythium* spp., *Fusarium* spp. and *Rhizoctonia* spp. (Dodd and White, 2000; Solorzano and Malvick, 2011).

Seedling blight or damping off can be divided into two groups, namely the pre-emergence and post-emergence seedling blight (Govender, 2008). Pre-emergence seedling blight affects the maize seedlings before they have emerged from the soil (Govender, 2008). Various factors interact with one another to negatively affect the emergence of seed under adverse conditions (Berjak and Villiers, 1972). These conditions can include the environment in which the seed was produced, the genetics of the seed, the stage of maturity at seed harvest, seed size, seed mass, structural integrity, deterioration after harvest, herbicides used, compaction of the soil and the presence of pathogens found in the soil (Berjak and Villiers, 1972). One of the primary contributing factors to seedling blight is the stress the seed finds itself subjected to, due to cool wet soils. Berjak and Villiers (1972) stated that maize seeds can take up soil water above freezing, but the seeds metabolism will be extremely low if the temperature of the soil in which the seed's is planted is below 13 °C.

The cell tissues rupture during water uptake, causing disruption of the seeds protein production and energy transformation in the activated cells (Berjak and Villiers, 1972). If the conditions are warmer the membranes of the seed can repair themselves which will limit the damage to emergence, but at continued low temperatures this reparation of the damaged membranes can cause death of the seed (Berjak and Villiers, 1972). Another factor contributing to pre-emergence seedling blight is when maize seeds discharge or naturally releases carbohydrates and proteins after water uptake has been initiated and the seeds with damaged membranes leak these released carbohydrates and proteins into the soil. These carbohydrates and proteins that leaked from the damaged seeds stimulate spore emergence of numerous fungi (Berjak and Villiers, 1972). Seeds die or roots start to develop with various root rotting fungi present in the seedling (Figure 1.2).

Figure 1.2: Seeds from water logged soils, with fungi present on the seed coat.

Post-emergence seedling blight (damping off) affects the roots, mesocotyl, radical, coleoptile, wet and dry mass (Figure 1.3) or lower stems of the young seedlings from emergence to the second or third leaf stage (Govender, 2008). The incidence of damping off that causes root rot is enhanced by various environmental factors such as strong winds,

drought stress, shallow soil and tillage practices that can result in soil compaction (Dodd, 1980). Typical damping off symptoms observed above ground are typified by a poor maize plant stand with uneven seedling growth, while some seedlings may be stunted and chlorotic (Figure 1.4) (Dodd, 1980). In such cases, the seedlings are still in a primary root development stage or only a limited number of secondary roots have developed. Hornby and Ullstrup (1967) reported that root rot of young maize plants follows a specific sequence where at the tip of the roots small lens-shaped lesions form which will start to turn brown, followed by necrosis of the root tips and decortication with complete root discolouration (Figure 1.5). Sumner and Bell (1983) also reported that lesions on the maize roots begin as light brown lesions with a water-like appearance, these lesions will enlarge as the secondary roots develop and will start to appear as elongated dark sunken areas (Sumner and Bell, 1983). Du Toit (1968) found that although discolouration and lesions can be observed on the roots, the younger maize seedling roots will produce young healthy roots out of the discoloured roots and concluded that plants are never killed by only one fungus species. However, the lack of well-developed lateral branching when roots are infected can be significant as this will result in reduced water and nutrient uptake (Sumner and Bell, 1983). Rotted roots that disintegrate two to five centimetres beneath the soil, display symptoms such as lodging, stunting, and chlorosis (Sumner and Bell, 1986).

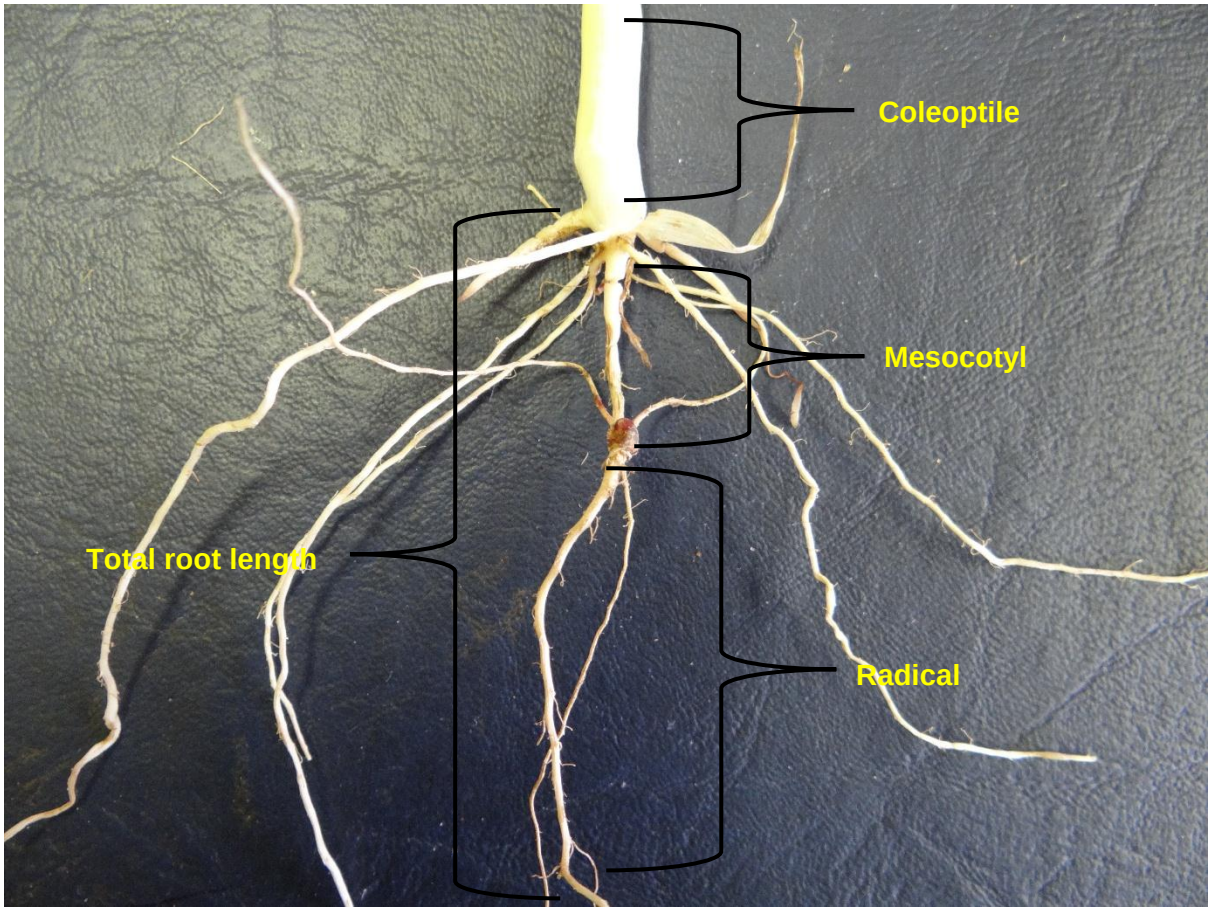

Figure 1.3: Different plant parameters that are potentially affected by the seedling blight complex.

Figure 1.4: Chlorotic seedlings with a yellow appearance three weeks after emergence.

Figure 1.5: Roots from a maize seedling grown in wet soil showing discolouration.

1.4 Pathogens involved in the seedling blight complex of maize in South Africa

Many studies have been conducted on root rots, and were based on isolation of various fungi from maize roots during a growing season (Hoppe and Middleton, 1950; Obendorf, 1972; White, 2000). Researchers from these studies all agreed that root rot is a disease complex, involving many different fungi, with the fungal complex found on the roots varying depending on the growth stage of the maize plant, the environmental conditions, the hybrids resistance and the previously planted crops, as well as the fact that fungi vary in their ability to cause root rot (White, 2000).

1.4.1 *Pythium* as a seedling blight pathogen of maize

Biesbrock *et al*. (1967) initially identified different *Pythium* spp. isolated from various maize samples by examining their oospore, oogonium and antheridium morphology. They found that positive identification of many *Pythium* spp. is a difficult task but for the purpose of this study, *Pythium* in general will be discussed.

Pythium spp. are renowned for their role as pathogens causing seed rot, damping off, seedling blight and root rot (White, 2000*). Pythium* spp. as root rot pathogens are known to result in low maize yields in poorly drained clay soils, with continuous maize and conservation tillage systems (Rao *et al*., 1978).

1.4.1.2 Symptoms

Phytopathogenic fungi like *Pythium* spp. affect many plant species, and are regarded as important pathogens infecting seeds or seedlings prior to emergence from the soil, resulting in seedling blight (Frank and Loper, 1999). According to Farr *et al*. (1989) maize plants that are severely infected have visible lesions and discolouration on their root systems and it usually results in yellowing and stunting of seedlings. The fungus also infects the root cortex which becomes discoloured and rotten while the stele may remain intact (Farr *et al*., 1989; Frank and Loper, 1999). *Pythium* spp. infect roots which significantly reduces the maize plants growth and yield without any obvious necrotic symptoms which are usually associated with infection (Frank and Loper, 1999). Confirmation of diagnosis that *Pythium* spp. are the causal organism is done by observing oospores in rotting tissue, although saprophytic species of *Pythium* may also be present in rotted roots (Farr *et al*., 1989).

1.4.1.3 Disease cycle and epidemiology

Frank and Loper (1999) noted that the oospores of *Pythium* spp. are the primary survival structures during overwintering in plant and soil debris. These thick-walled sexual oospores are resistant to long periods of drought, and will survive in soils for long periods of time without organic matter or favourable conditions (Frank and Loper, 1999). Once favourable conditions occur the oospores will germinate and produce mycelium or sporangia, which again produce and releases oospores (Hendrix and Campbell, 1983). In these pathogenic fungi the mycelium and the oospores can infect seedling roots and are abundant after rain when they are more active at high soil moisture levels (Hendrix and Campbell, 1983). The behaviour of *Pythium* spp. in soils are regulated by environmental factors such as moisture,

temperature, soil pH and the presence of specific soil minerals (Frank and Loper, 1999). However, *Pythium* spp. are generally seen as invaders of different host type root systems and poor competitors with other soil-borne fungi, the disease is favoured by high moisture levels combined with low temperatures, which results in low oxygen levels in soil (Hendrix and Campbell, 1983). If suitable soil conditions are met for infection by *Pythium* spp., they are able to colonise maize roots while they are capable of outcompeting other microorganisms in the soil (Hendrix and Campbell, 1983).

1.4.1.4 Control

To control *Pythium* spp. infections and damage, one must improve soil drainage or plant later when soil temperatures are higher (Rao *et al*., 1978). Seeds treated with metalaxyl in combination with other systemic fungicides protect seedlings against *Pythium* spp. infections (Rao *et al*., 1978; Frank and Loper, 1999). As the plant grows, the less effective the seed treatments become, and crop rotation has been shown to provide little to almost no control. This can be because as soon as *Pythium* infects, it produces large numbers of spores which enable the pathogen to re-infect growing roots of lupin, canola, peas, wheat, barley and maize throughout the season and across different phases of crop rotation (Rao *et al*., 1978).

1.4.2 *Rhizoctonia* spp. as seedling blight pathogens of maize

Rhizoctonia spp. are major soil-borne fungal pathogens in various agricultural systems occurring worldwide on a number of susceptible hosts (Anees *et al*., 2010). These fungi infect maize roots at temperatures ranging from 8 to 34 °C but only cause root rot to seedlings between 8 and 28 °C (Sumner and Dowler, 1983). This pathogen can cause severe damage to crops in areas and can reduce yield of maize up to 30 % (Sumner and Bell, 1986). *Rhizoctonia* spp. are soil saprotrophs, which colonise maize seedling roots and feed on organic matter as the seedling roots die off. *Rhizoctonia* spp. identification was divided intra-specifically based on their hyphal anastomosis reactions (Donn *et al*., 2014).

1.4.2.1 Symptoms

The first visible appearance of *Rhizoctonia* spp. in the root systems of maize, are brown lesions on the mesocotyl and primary and lateral seminal roots of seedlings and young plants (Sumner and Bell, 1994). The most characteristic symptoms are the large reddish-brown cankers that can be noticed on the adventitious crown and brace roots of mature plants. These symptoms usually result in a terminal decay and disintegration of the roots two to five centimetres below the soil (Sumner and Bell, 1994). *Rhizoctonia* spp. infections in fields form patches with dead, chlorotic and stunted plants within an infected area (Anees *et al*., 2010). These patches have distinct edges, and outside the patches maize plants show no symptoms of the disease, but may have root rot caused by late infections (Anees *et al*., 2010). Patches appear when the maize plants are at the seedling stage and when the seedlings are most susceptible to the disease. These patches also appear between seasons and affect new areas, grow, shrink or disappear from one season to another (Donn *et al*., 2014).

1.4.2.2 Disease cycle and epidemiology

Rhizoctonia spp. primarily survive in the soil as sclerotia, because sexual spores (basidiospores) are rarely produced (Sumner and Bell, 1986; Anees *et al*., 2010). The sclerotia also survive in colonised maize plant debris or on the roots of weeds and susceptible hosts (Sumner and Bell, 1986). As *Rhizoctonia* spp. are soil-borne pathogens they have limited means of spreading throughout the soil, it is usually the maize plant that accidently grows towards the pathogen because the disease can only be found in patches in the soil of a maize field (Anees *et al*., 2010). Spatial distribution of inoculum in maize fields is crucially important for the pathogen because of unpredictable occurrence of root rot in patches throughout a maize field. The diseases produced by *Rhizoctonia* spp. in the field are dependent on the balance between primary and secondary inoculum that are produced because the disease severity in the fields are strongly dependent on environmental

conditions like soil moisture and pH (Anees *et al*., 2010). The quantity of primary inoculum is theoretically the sum of the disease produced and the saprotrophic growth until the end of the previous season (Anees *et al*., 2010). The method of infection is not known but some *Rhizoctonia* spp. form dome-shaped infection cushions or lobate appressoria on the roots. These infections produce one or several infection pegs that help with the penetration of the cuticle, epidermis and can enter through natural openings or wounds on the plant (Sumner and Bell, 1986). After the pathogen has invaded the susceptible host plant tissue the hyphae will grow throughout the tissue, while the pathogen produces pectolytic and celluloytic enzymes to destroy the maize plant's root system completely (Sumner and Bell, 1986).

1.4.2.3 Control

Fungicides like azoxystrobin, carboxin with thiram, fludioxonil and ipconazole are all likely to be effective against *Rhizoctonia* spp. when screened in glasshouse trials but to a lesser extent in the field (Anees *et al*., 2010; Ruden, 2013). Fields naturally infested with the pathogen and rotated with an alternate crop between maize crops may not reduce the severity of the disease, but over several years of no host crops with adequate weed control inoculum levels may be controlled which will reduce root infections (Sumner and Dowler, 1983). Tillage practices have no effect on the disease severity, but under irrigated maize severity may increase and decrease under dry land production systems. The herbicide pendimethalin may also increase disease severity early on in the planting where soil temperatures are low. Resistant hybrids are yet to be developed against *Rhizoctonia* spp. infection (Sumner and Dowler, 1983).

1.4.3 *Fusarium* spp. as seedling blight pathogens of maize

The *Fusarium* genus contains a large number of soil-borne pathogen species that have a worldwide distribution and have been shown to be important plant pathogens (Roncero *et al*., 2003). These fungi are generally isolated from the roots of maize plants, but are also known pathogens of maize ears and stalks while some are only secondary pathogens to a susceptible host (Leslie, 1995). The majority of the *Fusarium* spp. that are pathogenic to maize plants will infect other plant species as well.

1.4.3.1 Symptoms

When root rot develops, the infected roots have a very slight brownish colour, but a dark black discolouration when roots are completely rotted. Depending on the species, the colour of the roots may also differ from red to pink (Leslie, 1995). The cortex tissues are initially infected, but lesions will spread to the vascular tissue beneath the endodermis. The reduction in root biomass due to infection reduces water and nutrient uptake. Should healthy roots develop from the older ones, that are not completely rotted, the plants may then compensate for the infection and survive the disease (Futrell and Kilgore, 1969). Should the roots be infected it is not always obvious in terms of clear distinct aerial symptoms. When infection by *Fusarium* spp. does occur and appears to be severe, poor plant stands and uneven growth would be observed (Kedera *et al*., 1994). Plants may also become stunted and chlorotic as they grow, while roots are rotted to such an extent that only a few secondary roots remain functional (Leslie, 1995). Lodging may also occur due to the rotted roots which break off below the soil surface, or between the fourth and fifth internode due to stalk rot (Leslie, 1995). The frequency of lodging may vary from season to season.

1.4.3.2 Disease cycle and epidemiology

Most seedling rots caused by *Fusarium* spp. result from the successful survival of chlamydospores, mycelia and conidia in the soil or crop residue (Young and Kucharek,

1977). Infection takes place as the plant's root system grows towards the pathogen in the soil. Damaged roots will promote infections but is not necessarily a requirement for infection (Leslie, 1995). Environmental conditions that promote plant stress increase the plant's susceptibility to root rot due to the allocation of photosynthate to the roots (Leslie, 1995). As the plants mature their roots also become more susceptible to infection (Leslie, 1995).

Herbicides and fertilisers that are incorrectly applied may also affect the stimulation of root rot infections on the maize plant. Some *Fusarium* infections and root rot symptoms can be found in almost all fields every year, but severity is not always significant enough to cause economic losses (Leslie, 1995). Losses mainly occur when extreme conditions are met, like high or low soil temperatures, or when other stresses predispose maize plants to infection.

1.4.3.3 Control

The control of maize root rot caused by *Fusarium* spp. is complex due to different species influencing the plant at different plant growth stages and at different environmental conditions. Seed treatments with fungicides such as Kodiak HB, carboxin with thiram, fludioxonil with mefenoxam, ipconazole, metaconazole and trifloxystrobin are all effective against *Fusarium* root rot diseases (Ruden, 2013). Crop rotation and tillage systems like mulch-till have both been reported to be effective (Leslie, 1995).

1.5 Economic impact on maize production of seedling blight

These different genera (*Pythium*, *Rhizoctonia* and *Fusarium*) all play a significant role in the maize seedling blight and root rot complex in South Africa. Some of these pathogens may infect a maize plant at any given time under various environmental conditions (Agrios, 2005). These pathogens can cause diseases on their own, but more likely in combination with one another at different development stages during the maize plants growth stages. Smit (2000) researched maize root rot diseases over a three year period and found that yield losses, caused by these pathogens, can be as high as 22 % when maize is produced under

monoculture. In many cases where disease severity is high, farmers will most likely replant fields due to poor emergence, which has great economic implications for farmers. In South Africa maize is constantly under threat from pathogens and insects at any given time where not all losses are reported but in certain cases high yield losses can be experienced (Smit, 2000).

1.6 *Trichoderma* spp. and seedling blight of maize

Trichoderma spp. are common saprophytic filamentous fungi that can be found in almost any soil type worldwide where they colonise cellulosic materials (Hajieghrari, 2010; Schuster and Schmoll, 2010). The first description of *Trichoderma* dates back to 1794 but due to poor species description and characterisation, it was redescribed by Rifai in 1969 (Rifai, 1969). Jaklitsch *et al*. (2006) and Samuels *et al*. (2006) have intensively reassessed descriptions of related *Trichoderma* spp. and managed to report the three *Trichoderma gamsii* (Samuels and Druzhin) species found in South Africa in the Western Cape province, in soil from *Eucaluptus* plantations and from Protea plantation's soil. However, the diversity and impact of *Trichoderma* spp. in South African maize fields causing seedling blight are unknown.

1.6.1 *Trichoderma* spp. as biocontrol agents against seedling blight pathogens

Trichoderma spp., such as *T. harzianum* (Rifai), have been known to increase maize plant growth which includes shoot length, root biomass, crop yield and plant nutrient uptake which are of significance to plants in agricultural systems worldwide (Shoresh and Harman, 2008). *Trichoderma* spp. can form symbiotic relationships with infected plant roots through chemical communication (Yedidia *et al*., 1999, 2000, 2003). This induces the maize plant to prevent the invading *Trichoderma* hypha so that the organism is restricted to the outer layers of the roots (Shoresh and Harman, 2008; Hajieghrari, 2010), where they induce a localised resistance to maize plant pathogen infection. The *Trichoderma* spp. induces systemic acquired resistance responses within the plant (Shoresh and Harma, 2008). *Trichoderma*

spp. defence mechanisms comprise both enzymatic and chemical defence, which makes it an efficient mycoparasite, antagonist and biocontrol agent that can be used as biological fungicides due to their secretion of these metabolites (Schuster and Schmoll, 2010). *Trichoderma harzianum* is the most studied biocontrol species and have been intensively exploited for its abilities to control other pathogenic fungi like *Fusarium verticillioides* (Sacc.) Nirenberg which is a seedling blight pathogen on maize (Srobarova and Eged, 2005; Sobowale *et al*., 2007; Ferrigo *et al*., 2014b.) and *Fusarium solani* (Mart.) Sacc. on soybean (Rojo *et al*., 2007). Studies conducted by Ferrigo *et al*. (2014a) showed that maize seeds treated with *T. harzianum* and inoculated with *F. verticillioides* had lower levels of infection when compared to untreated seeds. Another study conducted by Ferrigo *et al*. (2014b) found that *T. harzianum* colonised 90 % of maize roots and detected that *T. harzianum* decreased the severity of symptoms caused by *F. verticillioides.* These results indicated certain *Trichoderma* spp. and their role in maize production practices are of importance to farmers due to their successful colonisation and biocontrol abilities by preventing seedling blight pathogens to colonise maize seedling roots.

1.7 Cob and tassel smut of maize in South Africa

Cob and tassel smut caused by the soil-borne fungus *Sphacelotheca reiliana* (J. G. Kühn) G. P. Clinton, is a systemic disease of maize which was first described in the 1900s (Li *et al*., 2008). In the early 1970s the pathogen became a problem in both seed and commercial maize production areas at Bloemfontein in the Free State province in South Africa (Reed *et al*., 1927). This disease can occur worldwide where maize is cultivated, but reached epidemic proportions over the last five to six seasons in South Africa in the KwaZulu-Natal province as far as Underberg, the Standerton and Witbank area in Mpumalanga and in the eastern Free State maize production area (Dr. R. Kloppers, Pannar Seed South Africa. Personal communication, 2014). Li *et al*. (2008) reported that yield losses attributed to cob and tassel smut can be estimated to be more than 80 % during an epidemic.

1.7.1 Symptoms

At the maize seedling stage no visible symptoms are observed, the first symptoms appear after the ears and tassels have been formed (Halisky, 1963). The ears and tassels of the maize plant are a mass of black spores called smut sori (Figures 1.6 and 1.7). Each sorus is covered by a periderm layer which ruptures and then the black teliospores become visible (Halisky, 1963). The tassel infection may only be visible on individual spikelets, causing a shoot like growth, and in some cases the entire tassel may proliferate to cause bizarre leafy structures. In all of the cases when the tassel is infected, each of the ears will be infected as well, and rudiments of leafy tissue will replace the normal ears (Halisky, 1963). The ears that are infected will be rounded and sponge-like, the ears will also lack silks. In some cases the tassels will not be infected, but the ears will be, with a few smut free kernels still intact inside the husk leaves (Halisky, 1963). Plants that are infected with *S. reiliana* may be dwarfed and have a stunted growth, and the sori on the leaves will develop as long thin stripes.

Figure 1.6: A maize ear infected with cob and tassel smut spores (smut sorus).

Figure 1.7: Tassels of maize infected with spores of *Sphacelotheca reiliana.*

1.7.2 Disease cycle and epidemiology

The spores of *S. reiliana* (Figure 1.8) overwinter in soil, or on plant material found in the soil. The pathogen infects young maize seedlings where the mycelium penetrates young roots or the coleoptile of infected seedlings and grows systemically within the plant, once it has established itself in the meristem it can invade undifferentiated tissue (Halisky, 1963; Ali and Baggett, 1990; Li *et al*., 2008). The disease is more prevalent in clay soils that are cold (21 - 28 °C) and wet. The pathogen is even more infectious when soil nitrogen levels are low because maize plants are more susceptible to the pathogen and take longer to grow and develop, which in turns give *S. reiliana* a longer timeframe to infect roots (Ali and Baggett, 1990). When the correct fertilisers are applied and temperatures are higher the pathogen is less infectious to maize seedlings.

Figure 1.8: *Sphacelotheca reiliana* spores that overwinter in soil.

1.7.3 Control

Maize hybrid resistance is the most effective measure to control cob and tassel smut. Significant differences in the resistance of maize germplasm to *S. reiliana* infections under natural and artificial inoculation have been reported (Li *et al*., 2008). Hybrids with rapid seedling emergence may escape disease infection completely (Ali and Baggett, 1990). In recent studies in China, which focused on maize resistance that was quantitatively inherited, it was found that 70 maize landraces were highly resistant to cob and tassel smut (Li *et al*., 2008). Systemic fungicide seed treatments are also effective in controlling the pathogen. These are azoxystrobin, carboxin and thiram, fludioxonil with mefenoxam, thiabendazole and tebuconazole which all to keep the seed and seedling protected against infection by *S. reiliana* (Ruden, 2013). Foliar applications of fungicides to control cob and tassel smut are not effective, while crop rotation can be questionable due to spores being able to survive in a dormant state in the soils for several years (Ali and Baggett, 1990).

1.8 Seed treatments of maize in South Africa

According to the International Seed Testing Association (ISTA, 2011) a seed treatment is the application of a fungicide to seed of any product considered beneficial or necessary in maintaining or enhancing the genetic yield potential of a crop. Seed treatments have a long history and were initially used 4000 years ago. The Egyptians were the first to use seed treatments in 2000 BC where they soaked various seeds for planting in onion or cypress sap until 100 AD which also became the norm later on for Greeks and Romans (ISTA, 2011). In the 1700's the use of copper salts was initially recorded and in the 1740's arsenic was introduced, but was banned in the 1800's due to high toxicity (ISTA, 2011). However, it wasn't until 1890 that Bayer produced a *Bacillus* seed treatment for various seeds due to the plant growth promoting abilities of the *Bacillus* spp. In the mid 1900's more traditional chemical pesticide seed treatments were introduced like captan and thiram, as well as mercury based products and seed contact and systemic fungicides such as carboxin and chloroneb in the 1970 (ISTA, 2011). Modern day seed treatments are applied on the seed prior to planting. Modern seed treatments usually consist of fungicide and insecticide treatments (ISTA, 2011).

Initial infections of damping off, root rots and cob and tassel smut infections occur during the seedling stage of the maize plant. Seeds are treated with fungicides to control soil- and seed borne fungal disease organisms, as well as surface-borne pathogens on seeds, such as the smuts, to control seedling and young plant diseases (ISTA, 2011). More recently, seeds are treated with multiple products that are applied simultaneously to provide protection against all pathogens and insects that may reduce the population stand of plantings over a wide range of geographic areas (ISTA, 2011).

The role of seedling blight in poor plant stands over a number of seasons are not fully understood in South Africa and clarification on the role that seedling blight may play in stand reduction and poor seedling vigour is needed (Prof. Bradley Flett, ARC – Grain Crops

Institute, South Africa. Personal communication, 2014). Since 2007, cob and tassel smut has become more of an economical challenge to farmers throughout certain maize production areas of South Africa. To overcome these challenges the agrochemical companies are continuously testing new fungicide treatments or combinations on maize seed in an attempt to improve control of seedling blight and/or cob and tassel smut.

Most fungicide seed treatments are focused on soil- and seed borne fungal diseases, and are classified regarding the movement of the seed treatment product in relation to the seed itself within the plant (Ruden, 2013). Fungicides that are used as a protectant like captan, maneb, thiram, or fludioxonil on the seed surface are only effective on the maize seed surface where it provides protection against seed surface-borne pathogens and to soil-borne pathogens within a close radius of where the seed is placed in the soil (Ruden, 2013). Products associated with seed surface protection have a short residual effect, while protectant fungicides such as Captan, fludioxonil, Maneb and Thiram control most types of soil-borne pathogens, with the exception of the root rotting organisms (Ruden, 2013). On the other hand systemic fungicides are absorbed into the seedling as it emerges and inhibit or kill the fungus inside the plant tissue. The following are examples of systemic fungicide seed treatments: azoxystrobin, carboxin, mefenoxam, metalaxyl, thiabendazole, trifloxystrobin (Ruden, 2013). An indication of seed treatments which are available for cob and tassel smut, seedling blight, seedling root rots and *Fusarium* root diseases in South Africa are provided in table 1.1:

Maize seeds are treated with fungicides to reduce the risks of seedling blight and seedling borne diseases (Solorzano and Malvick, 2011). Captan was widely used to control soil and seed borne fungal diseases but it has been replaced with more efficient treatments (Solorzano and Malvick, 2011). In the USA most maize seeds are treated with mefenoxam or metalaxyl and accompanied by a fungicide like fludioxonil and/or strobilurins (Solorzano and Malvick, 2011). Other products like Maxim® XL include a combination of mefenoxam and fludioxonil which inhibit energy production and disrupt respiration in many fungi. Many soil-

borne fungicide disease interaction studies have been conducted and there is still room for improvement (Solorzano and Malvick, 2011).

Table 1.1: A list of seed treatments available for fungal diseases on maize.

Diseases			Seed treatment products	Application rate	References
Cob and tassel smut	Seedling blight	*Fusarium* root diseases			
x	**x**		Azoxystrobin (Dynasty)	0.153 ml/kg (0.0025 mg/kernel)	Ruden, 2013
	x	**x**	*Bacillus subtilis* GB03 (Kodiak HB)	4 ml/kg	Ruden, 2013
	x		Captan (Captan 400 and Captan 400-C)	1.25-2.375 ml/kg, 1.25-2.375 ml/kg	Ruden, 2013
x	**x**		Carboxin (Vitavax-34)	2-4 ml/kg	Ruden, 2013
x	**x**	**x**	Carboxin and thiram (Vitaflo 280)	4.5 ml/kg control of seedling rots, *Fusarium* and *Rhizoctonai* root rots 8.5-11 ml/kg control of seed borne head smut	Ruden, 2013
x	**x**	**x**	Fludioxonil (Maxim 4FS)	0.08-0.16 ml/kg	Soloranzano and Malvick, 2011
x	**x**	**x**	Fludioxonil and mefenoxam (Celest XL)	0.009-0.018 mg/kernel	Soloranzano and Malvick, 2011
x	**x**	**x**	Fludioxonil, mefenoxam, azoxystrobin and thiamethoxam (Maxim Quattro)	0.46 ml/ 80, 000, kernel count	Soloranzano and Malvick, 2011 Ruden, 2013.
x	**x**	**x**	Metconazole (Metlock)	0.045-0.09 ml/kg for seedling blight 0.18-0.52 ml/kg for head smut	Ruden, 2013
x	**x**	**x**	Tebuconazole (Raxil 2.6F), (Sativa 309 FS)	0.075-0.1 ml/kg soil and seed borne *Fusarium.* 0.37-0.74 ml/kg soil and seed borne head smuts	Ruden, 2013 Soloranzano and Malvick, 2011

1.9 Objective of this study

1.9.1 General objective

The aim of the study was to evaluate the incidence and etiology of maize seedling blight and control of soil-borne pathogens using seed treatments.

1.9.2 Specific objectives

The specific objectives were to:

- Evaluate the extent of the seedling blight problem in fields in the maize production region of South Africa.
- Evaluate the *Trichoderma* species associated with maize seedlings roots in South Africa.
- Evaluate the efficacy of fungicide seed treatments for the control of maize seedling blights.
- Evaluate the efficacy of fungicide seed treatments for the control of cob and tassel smut of maize in field trials.

The results of this study are presented in the form of chapters with the following titles:

- Chapter 2: Seedling blight of maize in the South African maize production system.
- Chapter 3: *Trichoderma* species associated with maize seedling roots in South Africa.
- Chapter 4: The efficacy of fungicide seed treatments for the control of maize seedling blights.
- Chapter 5: The efficacy of fungicide seed treatments for the control of cob and tassel smut on maize.

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Chapter 2

Seedling blight of maize in the South African maize production system

2.1 Abstract

Seedling blight of maize is a problem in localised areas worldwide and also in South Africa. Disease severity depends on prevailing environmental conditions and is more prevalent in cool wet soils with temperatures below 13 °C. Poor maize plant stand with uneven growth results and some seedlings are stunted and chlorotic. Below the soil, seeds rot and die prior to emergence and in some cases where the seeds do emerge, roots will be discoloured with dark brown to black sunken lesions. A total of 101 maize fields were sampled in the South African commercial maize producing areas over two seasons. In the 2012/13 a total of 75 maize fields were sampled and 26 in the 2013/14 planting season. Maize seedlings were sampled randomly 10 m from the edges of commercial fields and inwards. Zero to 10 % root discolouration was found in 73 % of the seedling samples. Only eight of the maize seedling samples had more than 70 % discolouration. Seventy fungal genera were isolated from the roots, with the most prevalent groups being *Aspergillus* spp., *Clonostachys* spp., *Fusarium* spp. and *Penicillium* spp. Isolates were sequenced to confirm visual identification of the four most prevalent *Fusarium* species identified in maize seedling roots. These species were *F. fujikuroi*, *F. oxysporum*, *F. solani* and *F. verticillioides*. Glasshouse studies were conducted to determine the pathogenicity of different isolates from different localities using *A. niger*, *F. solani*, *F. verticillioides* and *F. oxysporum*. Significant ($P < 0.05$) isolate differences were found for parameters which included root, coleoptile, mesocotyl and radical length as well as wet and dry root mass and seedling emergence, root discolouration and reisolation percentages. Significant isolate x line interaction ($P = 0.01$) differences were found for *A. niger* regarding mean percentage root discolouration. Significant interactions regarding isolate x line were found for mean percentage emergence ($P = 0.04$), seedling wet root mass ($P = 0.02$) and seedling dry root mass ($P = 0.001$) for *F. solani* in a glasshouse trial. There

were also significant isolate x soil interactions observed for root length (P = 0.00) and mean percentage discolouration (P = 0.02) for *F. verticillioides* in a glasshouse trial. *Fusarium oxysporum* showed significant differences (P < 0.05) for all the parameters evaluated except coleoptile length. Although commercial seeds are pretreated with fungicides, seedling blight is still a problem in certain maize producing areas/seasons in South Africa which result in financial losses due to the replanting of badly infected maize fields.

Key words: Maize seedling blight, root discolouration, *Aspergillus* spp., *Clonostachys* spp., *Fusarium* spp., *Penicillium* spp., *Aspergillus niger*, *Fusarium solani*, *Fusarium verticillioides*, *Fusarium oxysporum*

2.2 Introduction

Maize (*Zea mays* L.), is one of the most important staple food sources produced worldwide and ranked first in South Africa with a total yield of 13.03 million tons planted on 2.68 million hectares of land (SAGIS, 2014). According to continued reports farmers are experiencing problems with poor seed emergence and seedling vigour (Prof. Bradley Flett, ARC – Grain Crop Institute. Personal communication, 2014). These problems can be attributed to various factors that influence maize seed and seedlings such as insect damage (Stotz *et al*., 1999; Kessler and Baldwin, 2002; Toepfer *et al*., 2010), nematodes (Thomas and Norton, 1986; De Waele and Jordaan, 1988; Mc Donald and Nicol, 2005; Ismail and Kheir, 2014), agronomic practices such as planting depth, row spacing, seed placement and environment (Graven and Carter, 1999; Baron *et al*., 2006; Msuya and Stefano, 2010; Hussen *et al*., 2013; Yoti and Malik, 2013), herbicide application to seedlings that have emerged (Adesina *et al*., 2012), fertiliser application (Karthika and Vanangamudi, 2013; Miraj *et al*., 2013) and the seedling blight complex (Lunsford *et al*., 1974).

Seedling blight of maize is a widespread disease that can be a problem in localised areas where it contributes to poor seed emergence, plant stand and seedling vigour (Dodd and White, 2002). Isolated cases of maize seedling blight may be widespread. Sharma *et al*. (1993) reported yield losses of up to 12 % in the USA, 13.2 % in India and 9.4 % in Asia. Maize seedling blight or damping off can be seen as a pathogen complex that can vary over time and space due to different environmental conditions and soil types (Dodd and White, 2002). The determination of whether the fungi involved in the disease complex, are soil- or seed borne are critical in identifying the source of the disease complex (Solorzano and Malvick, 2011) and will thus influence the control thereof. The most commonly found species involved in the maize seedling blight complex, irrespective of whether they are soil- or seed borne, are *Acremonium* spp., *Aspergillus* spp., *Fusarium* spp., *Nigrospora* spp., *Penicillium* spp., *Pythium* spp., *Rhizoctonia* spp., and *Trichoderma* spp. (Laxminarayan *et al*., 1987; Solorzano and Malvick, 2011). Disease severity is influenced by various factors, but seedling

blight is most prevalent when maize seed are planted in cool soils with temperatures below 13 °C. This could be ascribed to seeds still taking up soil moisture at temperatures above freezing, although seed metabolism is still slow below 13 °C (Obendorf, 1972; Dodd and White, 2000). During water uptake, the cells in the embryo may rupture and cause a disruption of protein production and energy transformation in the activated cells (Obendorf, 1972). When the soil temperatures are higher, the membranes will repair and damage will be limited, but low temperatures result in slow membrane repair. Seeds with damaged membranes leak carbohydrates and proteins into the surrounding soil. These leachates stimulate spore emergence of various fungi in the soil or on the seed (Dodd and White, 2000). Below the soil, seeds may rot and die prior to seedling emergence (Sumner and Bell, 1983), whilst above ground the effect of seedling blights can be seen as poor maize plant stand with uneven seedling growth, with some seedlings being stunted and chlorotic (Dodd, 1980). Roots of seeds that germinate and emerge may be discoloured with dark brown to black sunken lesions on the surface (Sumner and Bell, 1983).

In South Africa, the etiology of the seedling blight status of this disease complex and the factors that influence the disease are not fully understood.

The objective of this study was to determine the status and etiology of seedling blight in the maize production areas of South Africa. Isolates from different survey points were screened for pathogenicity on maize seedlings to establish the variation in pathogenicity within each species.

2.3 Materials and Methods

2.3.1 Field survey

A total of 101 commercial maize fields in the maize producing area in South Africa was randomly selected from various maize producing provinces in South Africa in an attempt to make sampling as representative as possible of the entire maize industry. During the

2012/13 season, 75 farms were visited and 26 farms in the 2013/14 maize planting season. All the seed planted was treated with a standard fungicide as preselected by the seed companies, namely captab, thiram, or celest, prior to planting. Ten seedlings were sampled from each of these fields within three weeks after seedling emergence. Random sampling of seedlings was done 10 m from the field edges and inwards. The seedlings were transported in 25 x 50 cm brown paper bags in a cooler box and GPS coordinates of each locality were noted. Seedlings roots and crowns were washed with distilled water to remove all soil particles. Primary and secondary root and coleoptile discolouration were visually assessed and recorded using the root disease index (RDI). The disease ratings were based on an adjusted scale of 0 – 4 where 0 indicated no symptoms, 1: 1– 25 % rot, 2: 26 – 49 % rot, 3: 50 – 74 % rot, and 4: 75 – 100 % rot (Soonthornpoct *et al*., 2000). Roots were cut into 1 mm pieces and sterilised in a 1 % sodium hypochlorite solution for one minute (Lamprecht, 2007).The root pieces were washed again with sterile water and placed under a layer of filter paper to dry. Fifty root pieces per sample were selected at random and plated out on a *Pythium* selective medium (Eckert and Tsao, 1962) to determine if any *Pythium* spp. infections were present. A further 80 root pieces were selected at random, and four pieces per Petri dish were plated out on a half strength potato dextrose agar (Bilolab, Merck Pty. Ltd) (Leslie and Summerell's, 2006). These Petri dishes were then incubated in an incubation chamber at 25 for 5 days with a non-ultraviolet base lamp and a 12 hour light–dark cycle (Nel *et al*., 2006). The resultant fungi were reisolated and single spored using the method described by Leslie and Summerell's (2006), and kept in the incubation chamber and left to grow for two weeks. The sporulation structures that were generally visible after two weeks incubation were used for visual identification. Visual identification of the *Fusarium* species were confirmed by means of a DNA sequencing using the ABI PRISM 3100 (Applied Biosystems, Foster city, CA, USA).

2.3.2 Morphology and culture comparisons

All fungal isolates obtained from the survey were visually identified using the following reference sources; *Aspergillus* spp. (Klich and Pitt, 1988), *Clonostachys* spp. (Schroers, 2001), *Fusarium* spp. (Nelson *et al.,* 1983; Leslie and Summerell's, 2006), *Penicillium* spp (Pitt, 1979; Pitt, 1988), *Paecilomyces* spp. (Samson, 1974), *Rhizoctonia* spp. (Sneh *et al*., 1991; Sneh *et al*., 1996), and other species (Ellis, 1971; Barnett and Hunter, 1972; Ellis, 1976; Carmichael *et al*., 1980; Sutton, 1980; Vonarx, 1980; Nag-Raj, 1993). Further morphological identifications were carried out on the *Fusarium* species to confirm by means of culture comparisons*. Fusarium* isolates were grown on potato dextrose agar (PDA), carnation leaf agar (CLA) (Nelson *et al*., 1983), and synthetic low nutrient agar (SNA) (Nirenberg, 1976). Colony morphology was determined on PDA and colony colour was assigned using the colour charts of Rayner (1970). Species demarcation based on morphological characters was based on Leslie and Summerell's (2006) method on species demarcation.

2.3.3 DNA extraction, amplification and sequencing

The most frequently isolated *Fusarium* spp. obtained from the survey were grown on PDA at 25 ºC for 10 days. DNA was isolated using the DNAeasy® Plant Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's specifications. Extracted DNA was used as template in PCR reactions to amplify a part of the translation elongation factor 1α (TEF) region using the primer set EF1 (5'– CGAATCTTTGAACGCACATTG– 3) and EF2 (5'– CCGTGTTTCAAGACGGG – 3') (O'Donnell *et al*., 2012). The PCR reaction consisted of 1x Dream Taq reaction buffer with $MgCl_2$, dNTPs (250 µM each), primers (0.2 µM each), and template DNA (25 ng) and Dream Taq polymerase (0.5 U). The PCR reaction conditions for the TEF gene region was amplified by initial denaturation at 94 °C for 2 min. This was followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, elongation at 72 °C for 1 min and elongation step at 72 °C for 5 min. The resulting amplicons

were purified with a QIAquick PCR Purification kit (QIAGEN, Germany) and subjected to automated DNA sequencing using the same primers that were used for the respective PCRs and the ABI PRISMTM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, (Applied Biosystems, UK) and Applied Biosystems' 3730 DNA Analyzer. Raw sequence trace files were analysed with BioEdit. All of the sequences generated in this study have been deposited in GenBank. The partial sequence obtained from the Fusarium data for translation elongation factor1– α gene was compared against the NCBI (GenBank) database, the *Fusarium* Database (Geiser *et al*., 2004) and *Fusarium* MLST databases (O'Donnell *et al*., 2012).

2.3.4 Glasshouse study

Preparation of inoculum

Ten single spore isolates of four fungal species that occurred most frequently across all localities surveyed were selected for pathogenicity studies. Inoculum of these fungal species were prepared as follows; selected isolates were plated out in Petri dishes containing half strength potato dextrose agar and incubated at 25 °C for 5 days. From an active growing colony, each isolate was plated onto 52 Petri dishes of half strength PDA and kept in an incubation chamber for two weeks at 25 °C. These isolates were used to inoculate the consol jars to produce the inoculum being used in the glasshouse. A total of 520 one litre consol jars were half-filled with a mixture of small bird seed (Nel *et al*., 2006), 50 ml of water were added and left to stand for 24 hours. The jars were autoclaved for 20 minutes at 121 °C, left to cool down and autoclaved again for 20 minutes at 121 °C. After cooling, the jars were inoculated with actively growing colonies of the selected isolates, sealed and the inoculum was left to grow in a culture room at 26 °C for 50 days. The inoculum was removed from the jars and transferred to brown paper bags and air dried (Dumroese *et al*., 1998) after which each set of inoculum were milled using a Cyclotec 1093 Foss mill to grind the inoculum to a fine powder. The milled inoculum was kept in brown paper bags in a plastic

container kept in a cold room at 1° C for 2 days to be used as inoculum in the glasshouse. Inoculum of the following species was used for the purpose of this study: *Aspergillus niger* Tiegh, *Fusarium solani* (Mart.) Sacc, *Fusarium verticillioides* (Sacc.) Nirenberg (synonym: *F. moniliforme* Sheldon) and *Fusarium oxysporum* Schltdl.

Glasshouse trial

The soil for the glasshouse experiments was collected from a field at the ARC– Grain Crops Institute in Potchefstroom South Africa (S 26°43'43.16; E 27°04'47.71). Half the soil was sterilised (1000 kg) and the other half (1000 kg) were kept unsterilised. Each pathogen species experiment was planted in a randomized complete block design using ten isolates in both sterilised and unsterilised soil. Each species pathogenicity experiment of *A. niger*, *F. solani*, *F. verticillioides* and *F. oxysporum*, was planted independently to determine the variation in pathogenicity within each species in four separate glasshouse experiments. Five litre plastic pots (320 in total) of sterilised and unsterilised soil were inoculated at a rate of 150 g inoculum which was mixed independently into the soil of each pot. There were 8 replications of sterilised and unsterilised soil as well as an uninoculated control treatment. Two maize lines, namely I 137 TN W and V 0617 YZ were planted per treatment. Four seeds of each line were planted per pot and placed in a glasshouse at 26 °C day and 18 °C night temperatures with natural day and night lengths.

Seedlings were removed from the soil in a cautious manner, three weeks after emergence, washed with distilled water and primary and secondary root discolouration were visually assessed and recorded using the root disease index (RDI). Disease ratings were based on an adjusted scale of 0 – 4 where 0 is no symptoms, 1 = 1– 25 % rot, 2 = 26 – 49 % rot, 3 = 50 – 74 % rot, and 4 = 75 – 100 % rot (Soonthornpoct *et al*., 2000). The coleoptile, mesocotyl, radical and root length were measured and seedling wet root mass was also determined. The maize seedling roots were dried at 105 °C for 24 h. (Srobarova and Eged, 2005) and dry mass determined. Root pieces (1 mm), were cut from both the inoculated and

uninoculated seedlings, sterilised with a 3.5 % sodium hypochlorite solution for 1 minute and washed with sterlised distilled water. Filter paper was placed on the root pieces to dry. Four root pieces from each plant used in the glasshouse trial were plated out on a half strength potato dextrose agar. The fungi that grew on the Petri dishes were allowed to grow for five days at 25 °C in an incubation chamber and the percentage reisolation was determined.

The data for the different glasshouse trials were analysed by means of factorial analyses of variance (ANOVA), followed by Fisher's protected least significant difference (LSD) test using Statistica® 12th edition (StatSoft Inc., Tulsa, UK).

2.4 Results

2.4.1 Field survey

The maize fields sampled during the survey, are indicated in Figure 2.1, and all the fungal species isolated from maize roots at the respective sampling points are provided in Table 2.1. The first sampling was carried out in the 2012/13 maize planting seasons which are indicated by the black spots in Figure 2.1. The second seasons sampling in the 2013/14 maize planting season are indicated in Figure 2.1 by the brown spots. More localities were sampled in the 2012/13 planting season compared to the 2013/14 planting season (Figure 2.1). There were 70 root inhabiting fungal species isolated from the maize seedling roots over all localities and both seasons (Table 2.1). From the 101 samples obtained, 73 samples had 0 – 10 % discolouration, while 28 of samples had 10 – 90 % discolouration, with only 8 of the 28 samples having more than 70 % discolouration (Table 2.1). Seedling blight fungal species diversity for surveyed localities is reported in Table 1. Root discolouration varied between 0 and 90 % at the localities sampled (Table 1). Fungi that were most frequently isolated from the maize seedling samples were *A. niger* which were identified on 40 % of the maize seedling samples, *F. solani* from 60 % of the maize seedling samples, *F. verticillioides* from 50 % of the samples, *F. oxysporum* from 55 % of the maize seedling samples and *F.*

graminearum (Schwabe) was only found on 11 % of the maize seedling samples. Maize seedlings sampled from 50 % of the localities showed root discolouration while 15 % of those samples had 50 % and more root discolouration. Localities with more than 50 % root discolouration showed low maize seedling root fungal biodiversity which includes localities such as 9, 28, and 88 in the Mpumalanga province, 58 in central Limpopo province, 66 and 80 in the Free State province, 90 in the Gauteng province and 99 in the North-West province (Table 2.1). From all these localities, *F. solani* was the most frequently isolated fungus followed by *F. verticillioides* in the maize seedling roots. However, the majority of seedling samples showing 50 % or less root discolouration had higher root inhabiting fungal biodiversity but discolouration was primarily found on the primary roots of the seedlings (Table 2.1). In all of the samples from the various localities more than two different fungal species were isolated from maize seedling roots (Table 2.1). Maize seedling samples with no root discolouration still had root inhabiting fungi present which included *Fusarium* spp. (Table 2.1).

2.4.2 Glasshouse study

2.4.2.1 Aspergillus niger

There was a significant isolate x line interaction in terms of mean percentage maize seedling root discolouration (Table 2.2). The uninoculated control for line I 137 TN W had a significantly lower mean percentage maize seedling root discolouration than maize seedlings inoculated with isolates 4, 5, 6, 7 and 10 (Table 2.2.1). The uninoculated control for line V 0617 YZ had a significantly lower mean percentage seedling root discolouration than maize seedlings inoculated with isolates 1, 3, 5, 6, 7, 8, 9, 10 and 11 (Table 2.2.1). Isolates 3 and 8 resulted in a significantly increased mean percentage root discolouration of maize seedlings on line V 0617 YZ when compared to line I 137 TN W. However, isolate 6 resulted in a greater mean percentage root discolouration on line I 137 TN W compared to line V 0617 YZ (Table 2.2.1).

The longest mesocotyl was recorded for isolate 2, but it did not differ from the mesocotyl lengths of isolates 1, 3, 5 and 7 infected seedlings. The shortest mesocotyl length was recorded for isolate 9, but it did not differ significantly in length from seedlings infected with isolate 3, 4, 6, 7, 8, 10, 11 and the uninoculated control (Figure 2.2). A significantly higher percentage reisolation of *A. niger* isolates 4, 5 and 7 from maize seedling roots were recorded compared to isolate 2 (Figure 2.3). A significantly higher percentage reisolation of all *A. niger* isolates occurred compared to the uninoculated control. The maize lines planted differed in terms of root and radical length as well as seedling wet and dry root mass of seedlings (Table 2.2). Mean maize seedling root length for line I 137 TN W was significantly shorter than mean maize seedling root length for line V 0617 YZ ($F_{1,144}$ = 6.9; P < 0.05). The radical lengths of seedlings from line V 0617 YZ was significantly longer compared to line I 137 TN W ($F_{1,144}$ = 40.8; P < 0.001). Seedling wet root mass of line V 0617 YZ was higher when compared to line I 137 TN W ($F_{1,144}$ = 40.8; P < 0.5). Seedling root dry mass of line V 0617 YZ was also higher when compared to line I 137 TN W ($F_{1,144}$ = 6.5; P < 0.5). Maize seedling root lengths also differed between sterilised and unsterilised soil inoculated with *A. niger*, with the longest root lengths from seedlings grown in sterilised soil ($F_{1,144}$ = 6.5; P < 0.5.). The isolate x soil; line x soil, and isolate x line x soil interactions were not significantly different for any of the parameters evaluated (P > 0.05) (Table 2.2). There was no significant difference in mean percentage emergence of maize seeds planted in soil that was inoculated with any of the *A. niger* isolates.

2.4.2.2 Fusarium solani

There were significant isolate x line interactions in terms of wet and dry root mass of seedlings as well as mean percentage emergence of seed (Table 2.3). There were no differences in isolate effects on line V 0617 YZ, but there were significant *F. solani* isolate effects on line I 137 TN W (Table 2.3.1). Isolate 4 showed the highest seedling mean wet root mass but did not differ significantly from isolates 5, 6, 7, 8 and the uninoculated control. However, isolate 10 had the lowest mean seedling wet root mass on I 137 TN W which

differed significantly from the previously mentioned isolates and the uninoculated control but not from isolate 1, 2, 3, 9 and 11. Mean seedling wet root mass from seedlings infected with isolates 4, 5, 6 and 7 were significantly greater on line I 137 TN W than line V 0617 YZ (Table 2.3.1). Mean maize seedling wet root mass of line V 0617 YZ seedlings were significantly lower compared to seedlings of line I 137 TN W (Table 2.3.1). There were significant differences in isolate effects on both line V 0617 YZ and line I 137 TN W in terms of mean seedling root dry mass (Table 2.3.2). Mean dry mass of seedling roots infected with isolate 4 did not differ significantly from that of any other isolate or the uninoculated control on line V 0617 YZ, however, it was significantly lower than the mean seedling root dry mass inoculated with isolate 9. Seedlings of line I 137 TN W infected with isolate 4 had a significantly greater mean seedling root dry mass compared to seedlings infected with all other isolates and the uninoculated control, except for isolate 7. Seedlings infected with isolate 10 had the lowest mean seedling root dry mass but did not differ significantly from those infected with isolate 2, 3, 9 11 and the uninoculated control. The dry mass of seedling roots infected with isolates 4, 5 and 7 were significantly greater on line I 137 TN W than line V 0617 YZ (Table 2.3.2). Mean seedling root dry mass of line V 0617 YZ were significantly lower compared to seedlings of line I 137 TN W (Table 2.3.2).

A significant isolate x line interaction was also recorded for mean percentage seed emergence. There were significant differences in isolate effects on both line V 0617 YZ and line I 137 TN W in terms of mean percentage seed emergence (Table 2.3.3). The lowest mean percentage seed emergence for line V 0617 YZ was caused by isolate 7, however, mean percentage seed emergence caused by this isolate did not differ significantly from that caused by isolates 2, 3, 4, 5, 6, 8, 10 and 11. Mean percentage seed emergence from seed infected with isolate 7 differed significantly from that caused by isolate 1, 9 and the uninoculated control. Within line I 137 TN W the lowest mean percentage seed emergence was caused by isolate 10, which did not differ significantly from isolates 3, 6, 9 an the uninoculated control. The highest mean percentage seed emergence resulted from

inoculation with isolate 8 which did not differ significantly from isolates 1, 2, 3, 4, 5, 7, 9, 11 and the uninoculated control. Percentage emergence of maize seed in soil inoculated with isolate 10 differed significantly ($P < 0.05$) from all other isolates as well as the uninoculated control. For isolate 10 line I 137 TN W had the lowest percentage emergence when compared to V 0617 YZ. *Fusarium solani* inoculum from isolate 10 affected the emergence of line I 137 TN W significantly and reduced the overall emergence if compared to the uninoculated control. Percentage emergence of maize seed in soil inoculated with isolates for 1, 2, 3, 4, 5, 6, 7, 8, 9 and 11 did not differ significantly from one another (Table 2.3.3). Mean percentage emergence of seed from soil inoculated with all isolates did not differ significantly over both lines except for isolate 10 which significantly reduced percentage seed emergence of line I 137 TN W compared to that of line V 0617 YZ (Table 2.3.3).

Significant differences in radical length, percentage root discolouration, and percentage reisolation between the *F. solani* isolates evaluated were observed (Table 2.3). The longest radicals were recorded from seedlings infected with isolate 1, but it did not differ significantly from that of seedlings infected with isolates 3, 4, 5, 6, 8, 9 and the uninoculated control (Figure 2.4). The shortest mean radical length was recorded for seedlings infected with isolate 10 which differed significantly from the uninoculated control and seedlings infected with all isolates, except for isolates 7 and 11. The highest mean percentage root discolouration was caused by isolate 11, but it did not differ from that caused by isolates 4, 7, 8, 9 and 10. Percentage root discolouration caused by isolate 5 was significantly less than that caused by isolates 7, 8, 10 and 11 (Figure 2.5). All isolates caused a significantly higher percentage root discolouration than the uninoculated control (Figure 2.5).

Isolate 7 showed the lowest mean percentage reisolation of *F. solani* and did not differ significantly from isolates 2, 3 and 10. Isolates 4, 8 and 11 had the highest mean percentage reisolation of *F. solani*, but did not differ significantly from isolates 1, 3, 5, 6 and 9. The mean percentage reisolation of all *F. solani* isolates was significantly higher compared to the uninoculated control (Figure 2.6).

The lines planted also differed significantly regarding coleoptile, mesocotyl and radical lengths (Table 2.3). The coleoptile - ($F_{1,144}$ = 9.2; P < 0.05) and mesocotyl lengths ($F_{1,144}$ = 9.4; P < 0.05) of line V 0617 YZ were significantly longer than line I 137 TN W, but line I 137 TN W seedlings had a significantly longer radical length ($F_{1,144}$ = 13.7; P < 0.001). Coleoptile length was significantly longer in the sterilised compared to the unsterilised soil to which the *F. solani* isolates were added. ($F_{1,144}$ = 5.7; P < 0.05) as was radial length ($F_{1,144}$ = 19.0; P < 0.001).

The isolate x soil, line x soil and isolate x line x soil interactions were not significant for any of the parameters evaluated (Table 2.3).

2.4.2.3 Fusarium verticillioides

There was a significant isolate x soil interaction for mean root length (Table 2.4) of the different *F. verticillioides* isolates in sterilised and unsterilised soil (Table 2.4.1). The shortest mean seedling root length was observed for seedlings infected with isolate 10 in both sterilised and unsterilised soil. In the sterilised soil, the mean root length of seedlings from isolate 10 infected soil differed significantly from that of seedlings from isolate 1 infected soil, but not from seedlings from any other isolate infected soil and the uninoculated control. In the unsterilised soil, mean root lengths of seedlings infected with isolate 10 differed significantly from mean root lengths of seedlings infected with isolates 1, 2, 3, 4, 5, 7, and the uninoculated control. Seedlings infected with isolate 3 had the longest mean root length in sterilised and unsterilised soil and differed significantly from seedlings infected with all other isolates and the uninoculated control (Table 2.4.1).

There was a significant line x soil interaction on mean percentage root discolouration resulting from *F. verticillioides* infections. Line I 137 TN W did not differ significantly between sterilised and unsterilised soil and line V 0617 YZ in the unsterilised soil. However, line V 0617 YZ had a significantly lower mean percentage root discolouration in the sterilised soil (Table 2.4.2).

Fusarium verticillioides also had a significant effect on wet and dry root mass of seedlings, percentage root discolouration and percentage reisolation (Table 2.4). Seedlings infected with *F. verticillioides* isolate 3 had the highest mean seedling wet root mass and differed significantly from mean seedling wet root mass of seedlings infected with isolates 4, 5, 6, 7 8, 9 and 10, but not from the uninoculated control ($P < 0.05$) (Figure 2.7). No isolates differed significantly from the uninoculated control ($P > 0.05$) (Figure 2.7). Isolates 6, 9 and 10 reduced mean seedling wet root mass significantly compared to isolates 1, 2 and 3 (Figure 2.7).

The mean root dry mass of seedlings infected with isolate 3 was significantly higher than mean root dry mass of seedlings infected with all other isolates except for isolate 1, 2, 8 and the uninoculated control. Isolate 9 and 10 significantly reduced mean seedling root dry mass compared to isolate 1, 2, 3 and the uninoculated control. (Figure 2.8).

There was no significant difference in percentage root discolouration caused by any of the isolates, but all isolates differed significantly from the uninoculated control which showed lower than 5 % root discolouration (Figure 2.9).

The percentage reisolation of isolate 4 was significantly lower than percentage reisolation of isolates 1, 8 and 10. Reisolation percentage of isolate 8 was significantly greater than isolates 3, 4 and 7. Percentage reisolation of the control differed significantly from all *F. verticillioides* isolates (Figure 2.10).

The mean mesocotyl and radical length as well as mean percentage seed emergence were significantly affected by soil sterilisation or not (Table 2.4). The seedling mesocotyl – ($F_{1,132} = 5.9$; $P < 0.05$) and radical lengths ($F_{1,132} = 7.7$; $P < 0.05$) were significantly longer in the sterilised soil than in the unsterilised soil (Table 2.4).

There was a significant effect on wet root mass, dry root mass and mean percentage seed emergence between maize lines (Table 2.4). Mean wet root mass of the seedlings of the line I 137 TN W, were significantly higher than that of V 0617 YZ ($F_{1,132} = 13.9$; $P < 0.001$) (Table

2.4). The root dry mass of I 137 TN W seedlings was significantly higher compared to that of V 0617 YZ ($F_{1,132}$ = 16.5; P < 0.001) (Table 2.4).

The line I 137 TN W had a significantly lower mean percentage emergence than line V 0617 YZ ($F_{1,132}$ = 10.8; P < 0.05) (Table 2.4).

The mean wet root mass of seedlings infected with isolates 4, 7 and 8 did not differ from each other but did differ from the mean wet root mass of seedlings infected with isolate 3 (Figure 2.7).

No significant differences were recorded for isolates x line and isolate x line x soil interactions. Isolate x soil significantly affected the root length while line x soil interaction significantly affected the mean percentage root discolouration (Table 2.4). There was a significant line x soil interaction for maize seedling root discolouration. The mean root discolouration in the unsterilised soil was higher when compared to the sterilised soil. Line V 0617 YZ had a higher mean percentage root discolouration in the unsterilised soil than in the sterilised soil (Table 2.4.2) (Figure 2.10).

2.4.2.4 Fusarium oxysporum

The line x soil interaction influenced root, mesocotyl and radical length significantly (Table2.5). Mean maize seedling root length for line I 137 TN W did not differ significantly between sterilised and unsterlised soils. However, mean maize seedling root length for line V 0617 YZ was significantly shorter in the sterilised than the unsterilised soil (Table 2.5.1). Mean maize seedling radical length for line I 137 TN W was significantly longer in the sterilised than unsterilised soil. However, for line V 0617 YZ radical length did not differ significantly.

The *Fusarium oxysporum* isolate x soil interaction significantly influenced mean seedling root dry mass (Tabel 2.5.3). The mean dry mass of maize seedling roots grown in sterilised soil to which isolate 8 was added was significantly greater compared to all other *F. oxysporum*

isolate inoculated seedlings. The mean dry mass of maize seedling roots from unsterilised soil inoculated with isolate 8 was significantly lower compared to isolate 8 in the sterilised soil. However, isolate 8 did not differ significantly from any other *F. oxysporum* isolates in the unsterilised soil, but was significantly higher than the uninoculated control. Sterilised soil inoculated with isolate 6 had the lowest mean seedling root dry mass but did not differ from the uninoculated control and all isolates except isolates 1, 7, 8 and 9 in sterilised and isolate 3, 7 and 8 in unsterilised soil. The mean maize seedling root discolouration between *F. oxysporum* isolates inoculated into sterilised soil did not differ significantly. Maize seedlings in sterilised soil inoculated with isolates 5 and 8 had significantly higher seedling root discolouration than the sterilised soil uninoculated control. In the unsterilised soil, isolate 1 had the highest percentage seedling root discolouration compared to all other isolates but did not differ significantly from isolate 4. All isolates inoculated into unsterilised soil caused significantly higher percentage seedling root discolouration than the uninoculated control, except for isolate 7 which did not differ significantly from the uninoculated control.

A significant isolate x soil interaction for the percentage reisolation of *F. oxysporum* from maize seedling roots was observed. All isolates in both sterilised and unsterilised soil differed significantly from the uninoculated control, except for isolates 2 and 4 inoculated into sterilised soil which did not differ from the uninoculated control (Table 2.5.5).

There was a significant seedling coleoptile length difference between I 137 TN W and V 0617 YZ ($F_{1,120}$ = 6.2; $P < 0.05$). Maize seedlings inoculated with *F. oxysporum* isolates significantly influenced root, mesocotyl and radical lengths as well as wet root mass, and percentage seed emergence (Table 2.5).

Significant differences were found between the seedling root lengths inoculated with the different *F. oxysporum* isolates (Figure 2.11). Roots of maize seedlings from soil inoculated with isolates 1, 2, 3, 7, 8 and 9 were the longest when compared to the uninoculated control, with no significant difference in seedling root lengths of maize infected with isolates 2, 3, 4,

5, 8 and 9 (Figure 2.11). The mean root length of maize seedlings inoculated with isolate 6 did not differ significantly from the uninoculated control which had the shortest mean root length (Figure 2.11). The mesocotyl lengths of isolate 2, 3, 8 and 9 were significantly longer than that of isolate 5 and the uninoculated control, but did not differ significantly in length from isolate 1, 4, 6 and 7 (Figure 2.12). The longest maize seedling radical length was recorded seedlings infected with *F. oxysporum* isolate 3 which significantly differed from radical lengths of maize seedlings infected with all the isolates except isolate 1, 2 and 7 (Figure 2.13). All isolates differed significantly in terms of mean seedling radical length from the uninoculated control except for isolates 5 and 6 (Figure 2.13). There were also significant differences between maize seedling wet root mass, with the heaviest seedlings recorded for seedlings grown in soil infected with isolate 1, 3 and 8 while the seedlings with the lowest wet root mass was recorded from soil infected with isolate 6 which did not differ significantly from the mean seedling wet root mass of maize in soils infected with isolates 2, 4, 5, 9 and the uninoculated control (Figure 2.14).

The mean percentage maize seed emergence was the highest for maize seedling inoculated with isolates 1, 3 and 8 but did not differ significantly from isolates 4, 5 and 6. The uninoculated control had the lowest mean seed emergence percentage and differed significantly from all isolates, except for isolate 2.

2.5 Discussion

The maize seedling blight survey delivered high quantities of fungal isolates which were isolated from seedling roots. The majority of these species have previously been associated with maize seedling blight at some point (Solorzano and Malvick, 2011). Many of the fungi found during the survey are either soil- or seed borne (Solorzano or Malvick, 2011). The influence of fungal species, such as *Acremonium* spp, *Aspergillus* spp, *Fusarium* spp, *Nigrospora* spp, *Penicillium* spp, *Pythium* spp, *Rhizoctonia* spp, and *Trichoderma* spp., on maize seedlings and related seedling blights were studied by various researchers (Sharma

et al., 1993: Solorzano and Malvick, 2011: Laxminarayan *et al*., 1987). Some fields such as 9, 55, 56, 58, 66, 74, 87 and 88 showed high levels of root discolouration compared to fields only 20 km away which showed no discolouration. Seedlings from localities with 70 % and higher root discolouration ratings were all infected with *Aspergillus* spp., *Clonostachys* spp., *Fusarium* spp., *Penicillium* spp., *Trichoderma* spp. and *Rhizoctonia* spp. These fungi were most commonly associated with seed decay, damping off as well as seedling blight. However, these localities, with the highest percentage root discolouration, had species like *Fusarium oxysporum*, *Fusarium solani* and *Fusarium verticillioides* that were the most frequently isolated from the maize seedling roots. These *Fusarium* spp. have been reported to be associated with maize seedling roots and to cause seedling blight and damping off (Soonthornpoct *et al*., 2001; Fandohan *et al*., 2003; Covarelli *et al*., 2012; Avedi *et al*., 2014). This can explain the higher percentages of root discolouration found in the maize seedling samples from these 8 localities.

There were 70 different fungal species isolated from maize seedling roots with the most common being *Aspergillus* spp. *Clonostachys* spp. *Fusarium* spp. and *Trichoderma* spp. Niaz and Dawar (2009) sampled 100 maize seed samples from localities in Pakistan and isolated 56 different fungal species, confirming the diversity of fungal pathogens found on maize seeds. McLauglin and Martyn (1982) reported where high percentages of pathogens were isolated from seedlings, they may negatively influence the plants growth which delays seedling emergence giving other fungi an opportunity to infect, which describes why many different fungi were found on maize seedling roots and that the most aggressive growers being those most commonly isolated. In this study no *Pythium* spp. were isolated and only a few *Rhizoctonia* spp. were isolated. This can be due to commercial maize being treated with an effective fungicide seed treatment that reduces the infection by these species.

This survey was conducted on maize seedlings from commercial farms that were planted with commercially available seed which were all treated with fungicide seed treatments. Seed treatments are effective against various *Fusarium* strains under greenhouse conditions

(Munkvold and O' Mara, 2002). Results from a study on seed treatments for control of seed borne fungi reported variations from 84 to 100 % of emergence for treated seeds with as low as 4 % disease symptoms (Debnath *et al*., 2012), which is in concordance with the results of the present study with regard to variation in disease incidence between different fields. More than one fungus species was isolated, from a single maize seedling, from localities with high incidences of root discolouration; for example, seedlings from locality 74 had a high biodiversity of fungi isolated from their root systems. Seedling blight is therefore more likely to infect maize seedlings as a complex. The poor seedling vigour as well as chlorotic symptoms of maize seedlings (Dodd and White, 2000) indicate that infection from various fungal species at one stage are possible.

A wide range of *Fusarium* spp. were found in maize seedling roots. Identification of the most commonly isolated *Fusarium* spp. isolates were confirmed based on DNA sequence data as part of a multiphasic approach which has become the norm. The workhouse gene region identified for this purpose is the internal transcribed spacer region (ITS) and it is now also recognised as the DNA barcoding gene for fungi (Schoch *et al*., 2012). Although the gene is used in combination with others to provide multigene phylogenies upon which species demarcation is based in Fusarium, it cannot be used alone due to the two highly divergent non orthologous ITS 2 types (Waalwijk *et al*., 1996). O'Donnell and Cignelik (1997) hypothesised that the major type of ITS exhibits a homoplastic pattern of evolution, thus obscuring true ancestral relationships. The most common identified *Fusarium* spp. were *F. fujikuroi*, *F. oxysporum*, *F. solani* and *F. verticillioides.* These fungi have been reported to cause 50 – 80 % damage to maize as root rot pathogens whilst negatively influencing yield. *Fusarium* spp. are common field fungi that infects more than 50 % of maize grains prior to harvest, which can influence maize seedlings, if the maize is used as seed the following season for planting (Fandohan *et al*., 2003). *Fusarium verticillioides* as found in 50 % of the maize seedling samples in this study, which confirms findings of Shephard *et al*. (1996) that *F. verticillioides* is a problematic pathogen in maize fields throughout the world whether

found on seedlings or physiologically mature plants. *F. verticillioides* is reported to be a common endophyte of maize that establishes a long term interaction with the maize plant, confirming that *F. verticillioides* is a common species isolated from maize plants and tissues worldwide and Africa (Fandohan *et al*., 2003).

Four of the most isolated fungal species *Aspergillus niger*, *Fusarium solani*, *Fusarium verticillioides* and *Fusarium oxysporum* were evaluated in glasshouse studies to determine the pathogenicity of randomly selected isolates on maize seedlings. Li *et al*., (2009) used *F. virguliforme* isolates from various geographic regions to quantify their pathogenicity on roots of soybeans. Significant differences were found between the various parameters evaluated which included wet and dry root mass, root, mesocotyl and coleoptile lengths as well as root discolouration, emergence and reisolation percentages.

Significant maize line root length differences and radical length differences were observed for the *Aspergillus niger* glasshouse experiment, this can be due to different maize lines having different ranges of genetic variation. This was also observed by Akram *et al*. (2007) where they screened different maize hybrids under salinity stress and observed that different hybrids react differently to the same conditions. The different maize lines used in this study were placed under the same condition in the glasshouse but line V 0617 YZ had significantly longer root lengths. In this study variations in mesocotyl length were observed for maize lines inoculated with *Aspergillus niger* inoculum. Isolate 9 showed to have the shortest mesocotyl length when compared to other isolates. Studies conducted by Hussain *et al*. (2013) confirm these findings as they observed that different *A. niger* isolates differ in their ability to be pathogenic and influence root lengths due to symptomless infections. Wet and dry root mass of the seedling roots were observed for both maize lines and line V 0617 YZ had the highest mass for both wet and dry root mass when compared to I 137 TN W. This can be due to different genetic traits maize plants have as stated by Akram *et al*. (2007) as there was no interaction effect observed from the different isolates. Line and isolate interaction was also observed for root discolouration. Line I 137 TN W had more root

discolouration interactions with isolates than V 0617. This was confirmed by findings of Li *et al*. (2009) who reported that different *Fusarium* isolates from various geographical areas can influence seedlings differently by means of their ability to grow more rapidly, colonise roots faster and their expression of pathogenicity when in maize plants roots. They also found that isolates from different areas caused roots to vary in discolouration, which is in concordance with findings in this study, that different isolates within the same species can influence the different maize line seedlings differently (Li *et al*., 2009).

Isolate 5 on line I 137 TN W had the highest percentage root discolouration, while isolate 2 had the highest mesocotyl length as well as low root discolouration and reisolation percentage. This may be because *A. niger* isolates can infect seedlings but show no symptoms, and this is confirmed by Li *et al*. (2009) who observed different isolates of *A. niger* and concluded that depending on the geographical origin and the substrate of which isolates was inoculated from, the species will differ in their ability to express symptoms on maize plants.

Maize line differences were observed for coleoptile length in the *Fusarium solani* glasshouse trial. Line I 137 TN W had a shorter coleoptile length than V 0617 YZ indicating the different genetic traits that different lines have which can influence the development of the roots (Akram *et al*., 2007). However, there were also sterilised and unsterilised soil differences observed in this study regarding the coleoptile length. Coleoptile length was much longer in the sterilised soil than in the unsterile soil and the same was observed for radical length. A study conducted by Giovannetti and Mosse (1980) on mycorrizal fungi and their ability to colonise maize roots in sterile and unsterile soil found that roots in the sterilised soil inoculated with mycorrizal fungi were much longer than those of the unsterile soil, and this can be attributed to the different microflora found in the soil where fungi and bacteria all compete for the same resources. *Fusarium solani* isolate effects on maize line radical length was also observed and findings in this study indicated that different isolates were shown to influence the maize seedling radical lengths. Studies conducted by Soonthornpoct *et al*.

(2001) indicated that *F. solani* is one of two other species that is the most frequently isolated from maize roots. Soonthornpoct *et al*. (2001) also confirmed that *F. solani* has the ability to cause excessive damage to maize seedling roots, as could be seen in this study, where the different isolates influenced root development regarding the coleoptile, mesocotyl and radical lengths. Significant wet and dry root mass differences were also observed for the different *F. solani* isolates with which the maize was inoculated. There is a direct correlation between wet and dry root mass as the same isolates influenced both wet and dry root mass. This indicates that *F. solani* can have either a negative or a positive effect on maize seedling roots. The same trend was observed for the emergence percentages with some maize lines that had higher emergence percentages with certain isolates than others. This was confirmed by a study conducted by Li *et al*. (2009) on different *Fusarium* spp. and their effect on maize seedling roots. They reported that isolates from different localities caused different levels of root rot in terms of shorter root length and root discolouration.

Fusarium solani isolates can be seen to negatively influence the maize seedlings throughout the different parameters evaluated. Isolates that showed high percentages of root discolouration also had high levels of reisolation which in turn also influenced the emergence of different maize lines as well as the wet and dry root mass of the seedling root system. This is also directly proportional to isolates that positively influenced the maize seedlings, if isolates showed higher root lengths lower root discolouration were observed as well as lower reisolation percentages of the different isolates.

There was a significant soil and isolate interaction observed for the *Fusarium verticillioides* glasshouse experiment. Giovanetti and Mosse (1980) reported that sterilised soil was shown to promote plant root length but this was not observed in this study. Unsterilised soil inoculated with *F. verticillioides* had the longest root length when compared to the sterilised soil. This can be due to the isolates ability to colonise maize seedling roots endophytically and that the soil, whether sterilised or not, had no influence on the isolates ability to promote plant root length (Oren *et al*., 2003). In contrast higher mesocotyl and radical lengths were

observed in the sterilised soil than in the unsterilised soil, this finding was in agreement with the study conducted by Giovanetti and Mosse (1980). Significant lower wet and dry root masses were observed for isolate 10 indicating that *F. verticillioides* influence maize seedling root growth. However, Pereira *et al*. (2011) found that *F. verticillioides* isolates had no effect on seedling root mass, but in this study isolates had significantly influenced the wet and dry root mass of seedling roots after three weeks. The results of this study indicated that certain isolates had no effect on the maize seedlings which is in agreement with findings by Venturini *et al*. (2013), when they screened a wide range of *F. verticillioides* isolates and found that, depending on the environment, isolates can either be pathogenic or endophytic. There were also higher percentages of root discolouration observed on the maize seedling roots after three weeks in the unsterilised soil when compared to the sterilised soil on line V 0617 YZ. Lei *et al*. (2011) also indicated that maize seedling roots infected with *F. verticillioides* caused large black lesions and necrosis which explains the findings of this study regarding the percentage root discolouration.

In the *F. verticillioides* glasshouse experiment maize seedlings inoculated with isolate 10 had low wet and dry seedling root masses and high percentage root discolouration as well as high levels of isolate reisolation. We can thus conclude that isolate 10 has the ability to influence seedlings no older than three weeks, it can cause disease like symptoms and has the ability to colonise maize roots, this findings is in concordance with research results reported by Oren *et al*. (2003) and Li *et al*. (2009).

A significant soil x isolate interaction was observed for *Fusarium oxysporum* isolates effects on maize seedling root length. In this study root lengths from the unsterilised soil were longer than root from the sterilised soil while radical length was shown to be the longest in sterilised soil compared to unsterilised soil, the same effect was observed in the *F. verticillioides* glasshouse experiment. Maize seedlings inoculated with isolate 6 had the shortest seedling root length while isolate 5 had the shortest mesocotyl length which can be due to *Fusarium oxysporum* being a rapid maize colonising species. that can influence root growth (Ocamb

and Kommedahl, 1993). In a study conducted by Fracchia *et al*. (2000) on *F. oxysporum* effects on maize plants in greenhouse conditions, they found that *F. oxysporum* increased the mass of sorghum roots but not of maize roots which is in accordance with findings in this present study where different isolates influenced the mass of the maize seedling roots after three weeks. Ocamb and Kommedahl (1993) also reported *F. solani*, *F. oxysporum* and *F. verticillioides* to be root colonisers, and that *F. oxysporum* was the most prevalent species found on maize roots. They also confirmed that when seeds are inoculated with different pathogens the maize seeds emergence will also be influenced, these results are confirmed by the findings of Ocamb and Kommedahl (1993), that emergence of seed planted in soil inoculated with different pathogens will be negatively effected.

2.6 Conclusion

Seedling blight of maize can be found throughout South Africa in localised maize fields where it can be problematic. Farmers are usually obliged to replant due to poor seed emergence, and in cases where they do not replant fields are left with patches where poor seedling vigour and growth can be seen. A range of on farm practices and environmental conditions can influence the disease complex ability to infect and damage seedlings more aggressively, and causes high levels of root discolouration that can lead to damping off. The results of this study confirm that more than one fungal species is present in the roots of maize seedlings and that seedling blight can be seen as a disease complex. There were 70 different fungal genera identified from the maize seedling root samples collected. The incidence of the seedling blight complex is localised in South Africa as only 8 of the 101 maize seedling samples had root discolouration of 70 % and higher. *F. oxysporum*, *F. solani* and *F. verticillioides* as well as *Aspergillus niger* remain the most frequently isolated fungi from maize seedling roots, in South Africa, and in combination with other soil-borne and seed borne fungi influence a wide range of maize seedling variables, related to emergence and seedling vigour, among which include; seed emergence, seedling root length, mesocotyl length, coleoptile length, radical length, wet and dry root mass as well is root discolouration.

Fusarium graminearum which is commonly associated with the seedling blight complex were only found in 11 % of the maize seedling samples. Glasshouse trials confirmed that the variation in pathogenicity of the most commonly isolated fungal species isolates varied significantly, within each species, indicating that within species pathogenicity differs from the geographical areas they were collected from.

2.7 References

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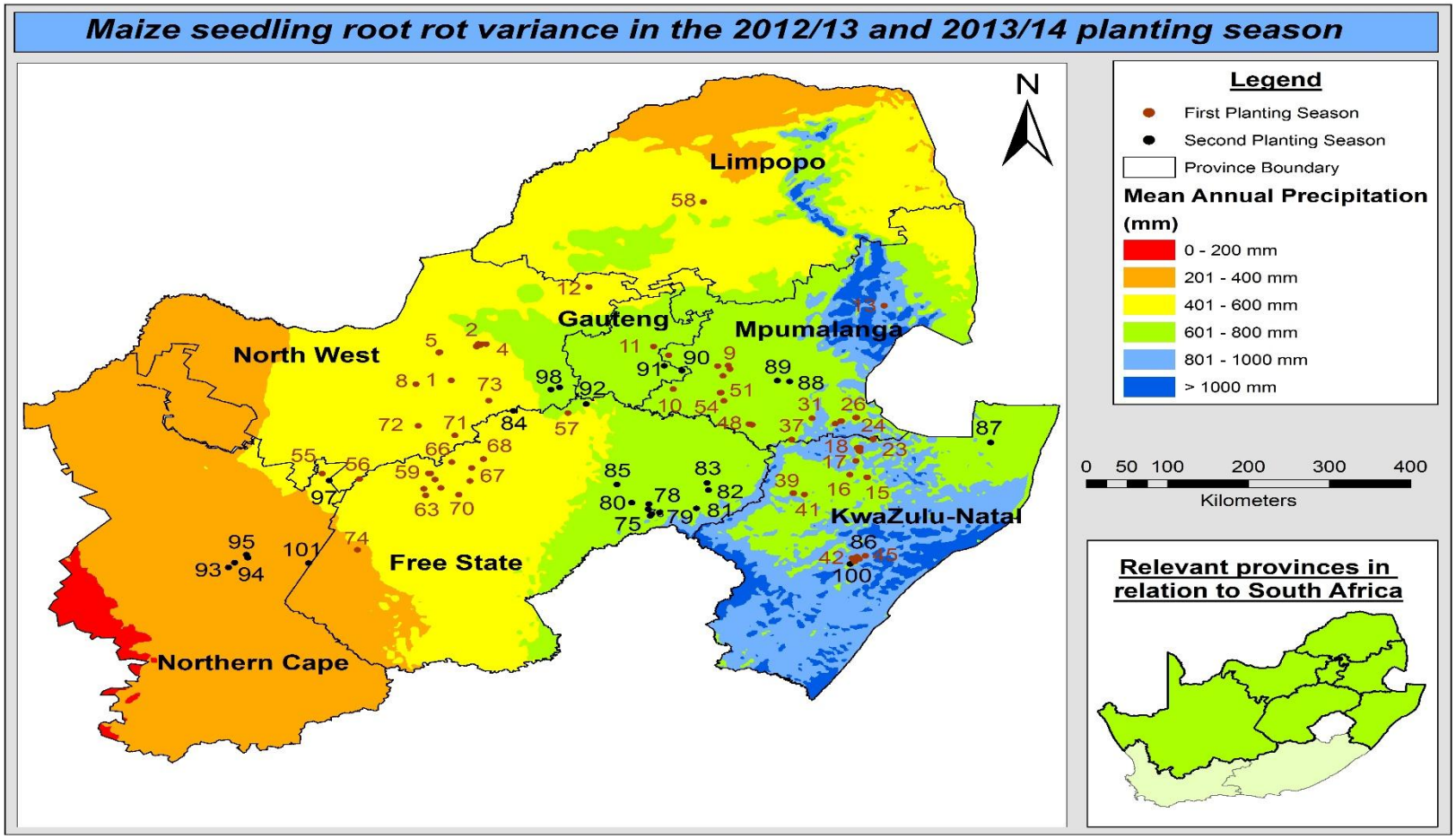

Figure 2.1: Maize fields where seedlings were sampled in the maize producing area of South Africa to determine the occurrence of seedling blight.

Table 2.1: List of localities corresponding with numbering on Figure 1 with percentage root discolouration and pathogens isolated per site.

Site number	**GPS coordinates**		**Percentage root discolouration**	**Pathogens isolated**
1	– 26.512S	26.196194E	40	3,6,7,8,9,10,11,12,13,15,16,19,22,29,36,3746,48,49,55,56,59,68,70
2	2.1) – 25.989389S	26.497278E	50	1,3,4,6,7,8,9,11,12,13,14,15,16,17,20,21,22,23,24,32,37,38,41,42,44,45,46,48,49,50,55,58,61,64,68,70
	2.2) – 25.989389S	26.497278E		
	2.3) – 26.018694S	26.477056E		
	2.4) – 26.017361S	26.482222E		
	2.5) – 26.025472S	26.479972E		
3	3.1) – 25.9905S	26.479972E	30	1,6,7,10,11,13,14,15,16,19,22,23,24,36,37,44,45,46,49,58,59,61,68
	3.2) – 26.003889S	26.532028E		
4	– 25.992889S	26.585083E	10	1,9,22,28,36,37,38,40,46,46,49,68
5	– 26.113861S	26.057972E	0	9,11,17,23,46
6	6.1) – 26.106417S	26.065778E	30	9,11,32,37,44,46,48
	6.2) – 26.107139S	26.069889E		
8	– 26.564556S	25.806111E	10	1,9,22,23,24,30,37,40,45,46,47,48,49
9	9.1) – 26.295778S	29.272417E	70	11,24,32,37,46,65,68,70
	9.2) – 26.444639S	29.212222E		
	9.3) – 26.304S	29.153472E		
	9.4) – 26.348444S	29.291472E		
10	10.1)–26.634167S	28.659528E	10	9,22,23,27,46

	10.2)–26.150139S	28.609083E		
11	– 26.024389S	28.442833E	0	11,22,28,46
12	– 25.174889S	27.723667E	30	9,11,22,32,37,68
13	– 25.442556S	31.002222E	60	1,2,9,21,22,23,24,27,35,37,45,46,47,68,69,70
15	– 27.895528S	30.815694E	0	8,22,23,24,61,68
16	– 27.858028S	30.619889E	0	2,11,22,23,33,36,37,43,46,49
17	– 27.660056S	30.68725E	0	1,11,22,23,37,46,63,66,68,69,70
18	– 27.516667S	30.722E	10	11,23,44,45,46,68
19	– 27.522S	30.731444E	10	17,32,46,68
20	– 27.473528S	30.704833E	0	6,9,11,17,21
21	– 27.476556S	30.731806E	10	20,22,23,37,44,45,46,68
22	– 27.475083S	30.734111E	10	23,46,62
23	– 27.355278S	30.877028E	20	11,23,37,43,46,49,68,70
24	– 27.34625S	30.879639E	0	1,11,17,22,37,46,50,68
25	– 27.345361S	30.877806E	10	17,22,23,24
26	– 27.043056S	30.685333E	50	11,22,24,46,49,68,69
27	– 27.042361S	30.689861E	60	1,9,11,15,16,23,24,37,43,45,46,59,60,61,68,70
28	– 27.042306S	30.690333E	50	24,33,65
29	– 27.042889S	30.694444E	10	23,46,61,68
30	– 27.043389S	30.694056E	50	22,23,24,33,37,43,46,49,61,64,68
31	– 27.0525S	30.202556E	0	23,37,43,46,49,61,68
32	– 27.092861S	30.526306E	0	11,23,24,36,37,45,46,49,6

				1,68
33	– 27.091556S	30.526111E	0	23,61,68
34	– 27.092333S	30.52325E	10	17,33,37,34,41,46,49,59,68
35	– 27.091778S	30.522167E	0	9,49,61
36	– 27.129889S	30.459944E	0	17,19,46,61
37	– 27.356278S	29.9735E	0	1,6,9,23,24,37,44,46,49,69,70
38	– 27.357889S	29.973722E	0	1,2,9,11,23,36,37,44,46,49,59,61,68,69,70
39	– 28.122833S	29.990722E	10	9,37,46,67
40	– 28.123778S	29.994944E	40	22,23,24,46
41	– 28.143028S	30.117917E	20	23,24,49,70
42	– 29.051361S	30.649056E	0	2,9,11,22,23,37,46,49,61,68
43	– 29.051528S	30.648556E	0	9,11,17,22,37
44	– 29.031944S	30.700667E	0	2,5,6,9,12,21,22,24,37,51,68,70
45	– 29.017472S	30.79475E	0	9,22,23,37,46,51
46	– 29.0645S	30.727083E	0	2,22,46,48,49,61
47	– 29.1025S	30.677667E	0	14,23,43,44,46,48,49,50,56,59,64,68,69
48	– 27.145556S	29.536444E	10	17,21,22,23,24,37,46,61,64,68
49	– 27.142333S	29.525611E	10	1,9,22,23,46,49,51
50	– 27.134056S	29.500472E	30	9,15,21,22,23,24,31,37,46,49,52,68
51	– 26.687333S	29.1855E	10	22,23,24,37,38,43,44,45,4

				6,48,49,61,68
52	– 26.687944S	29.182583E	10	9,20,22,23,24,37,44,46,49, 50,61,68
53	– 26.685528S	29.190639E	10	9,23,31,46,49
54	– 26.803972S	29.223778E	0	22,23,37,46,48,49,61,64
55	– 27.843556S	24.764694E	75	23,31,37,39,43,46,49
56	– 27.920503S	25.177389E	80	1,9,17,21,22,23,29,37,43, 44,46,49,61,65,68,69,70
57	– 26.97525S	27.491333E	30	9,17,22,23
58	– 23.955694S	28.994806E	80	23,31, 35, 37,43,46,49
59	– 27.841722S	25.965639E	10	9,37,46,49,68
60	– 27.841417S	25.943083E	10	1,5,9,11,17,43,49
61	– 27.92775S	26.015639E	0	6,9,17,22,23,37,46,58
62	– 27.927917S	26.016722E	0	9,17,23,37,49
63	– 28.154583S	25.91425E	10	24,27,45,46,49,55
64	– 28.062222S	25.890111E	0	6,8,9,11,37
65	– 28.048444S	26.080722E	0	22,23,49
66	– 27.678778S	26.20275E	80	22,31,37,49,61,68
67	– 27.762806S	26.422861E	10	22,37,44,49,60,61,62,68, 70
68	– 27.636528S	26.553222E	0	33,46,49,68,69,70
69	– 27.950139S	26.404667E	0	9,22,23,24
70	– 28.145222S	26.2785E	10	22,33,37,46,49,61,68,70
71	– 27.295861S	26.236E	0	1,9,22,61,68
72	– 27.158806S	25.829778E	0	19,22,23,24,61
73	– 26.799167S	26.613639E	0	23,37,49,51,61,68

74	– 28.935278S	25.156361E	90	22,33,37,46,49,50,68
75	– 28.422139S	28.420167E	0	2,8,37,43,45,55,61,68
76	– 28.445889S	28.405028E	0	9,30,33,37,46,49,68
77	– 28.354111S	28.382306E	0	33,37,44,49,51,60,61,68
78	– 28.277222S	28.390306E	10	1,9,11,21,22,23,24,68
79	– 28.400833S	28.508639E	30	7,9,22,24,37
80	– 28.25875S	28.200083E	60	20,23,33,37,43,46,49,68
81	– 28.339778S	28.919056E	0	29,49,59,68
82	– 28.080639S	29.052861E	10	21,61,62,68
83	– 27.97675S	29.037111E	0	22,61,62
84	– 26.945444S	26.885389E	40	20,22,33,37,43,44,62
85	– 27.999861S	28.035222E	10	2,18,37,46,49,57,61,62,65,68
86	– 29.017417S	30.794611E	0	9,43,68,70
87	– 27.399222S	32.183556E	70	9,20,25,26,30,37,43,46,49 54,57,61,62,65,68
88	– 26.525556S	29.952E	80	9,15,16,21,37,46,53,61
89	– 26.512694S	29.814778E	0	6,9,20,26,37,43,49,59,61,62,68
90	– 26.369972S	28.757139E	10	2,9,46,49,68
91	– 26.302611S	28.561833E	0	5,21,23,49,61,68
92	– 26.848028S	27.693639E	0	5,9,46,49
93	– 29.184139S	23.723E	50	46,49,61,62,68
94	– 29.117167S	23.7905E	0	61,62,68,70
95	– 29.003611S	23.921306E	0	61,68
96	– 29.0445S	23.936611E	0	20,46,68

97	– 27.944S	24.842194E	0	2,46,68
98	– 26.642472S	27.301611E	30	1,7,22,46,68
99	– 26.610833S	27.398444E	60	1,5,46,61,68
100	– 29.134778S	30.624917E	20	2,7,20,46,61,68
101	– 29.124472S	24.611806E	40	9,20,37,46,53,61,68

*1) *Acremonium* spp. 2) *Alternaria alternata*, 3) *Alternaria tenuissima*, 4) *Aureobasidium* spp. 5) *Aspergillus brevipes* 6) *Aspergillus flavus*, 7) *Aspergillus fumigatus*, 8) *Aspergillus ustus*, 9) *Aspergillus niger*, 10) *Aspergillus quadrilineata*, 11) *Aspergillus* spp, 12) *Aspergillus terreus*, 13) Basidiomycete spp. 14) *Basipetospora rubra*, 15) *Bipolaris australiensis*, 16) *Bipolaris holmii*, 17) *Botrytis* spp, 18) *Cephalophora tropica*, 19) *Chaetomium auratum*, 20) *Chaetomium* spp, 21) *Curvularia lunata*, 22) *Clonostachys catenulatum*, 23) *Clonostachys rosea*, 24) *Clonostachys. solani*, 25) *Didymosphaeria appendiculata*, 26) *Didymella effuse*, 27) *Fumago* spp, 28) *Fusarium anthophilum*, 29) *Fusarium chlamydosporum*, 30) *Fusarium crookwellense*, 31) *Fusarium dlamini*, 32) *Fusarium globosum* 33) *Fusarium graminearum*, 34) *Fusarium napiforme*, 35) *Fusarium nelsonii*, 36) *Fusarium nygamai*, 37) *Fusarium oxysporum*, 38) *Fusarium polyphialidicum*, 39) *Fusarium proliferatum*, 40) *Fusarium pseudoantophilum*, 41) *Fusarium redolens*, 42) *Fusarium sambucinum*, 43) *Fusarium scirpi*, 44) *Fusarium fujikuroi*, 45) *Fusarium subglutinans*, 46) *Fusarium solani*, 47) *Fusarium sporotrichiodes*, 48) *Fusarium thapsinum*, 49) *Fusarium verticillioides*, 50) *Gaeumannomyces graminis*, 51) *Gliocladium virens*, 52) *Humicola fuscoatra,* 53) *Macrophomina* spp, 54) *Microsphaeropsis* spp, 55) *Mucor* spp, 56) *Neocosmospora vasinfecta*, 57) *Nectaria asakawaensis*, 58) *Nigrospora* spp, 59) *Paecilomyces puntoni*, 60) *Pacilomyces. variabilis*, 61) *Penicillium spp*, 62) *Phoma* spp, 63) *Plectosporium tabacinum*, 64) *Ramichloridium anceps*, 65) *Rhizoctonia solani*, 66) *Taeniolelella phialosperma*, 67) *Torusla* spp, 68) *Trichoderma* spp. 69) *Verticillium nubilum*, 70) *Verticillium* spp.

Table 2.2: Results of the screening of maize seedlings against *Aspergillus niger* isolates in a glasshouse trial showing the factorial analyses of variance for parameters evaluated (n = 8).

Effects	Df	F– values								
		Length (cm)				Mass (g)		%		
		Root	Coleoptile	Mesocotyl	Radical	Wet	Dry	Discolouration	Emergence	Reisolation
Isolate	11, 144	0.9	1.1	1.8*	1.0	1.3	1.5	3.3**	1.2	3.6**
Line	1, 144	6.9*	0.6	0.3	40.8***	10.4*	6.5*	0.2	3.6	1.3
Sterilised/Unsterilised soil	1, 144	8.3*	0.0	0.0	3.3	0.0	1.1	0.8	0.0	0.0
Isolate x Line	11, 144	0.3	0.9	0.9	1.2	1.1	0.9	2.3*	1.2	0.6
Isolate x Sterilised/Unsterilised soil	11, 144	0.5	0.8	1.1	0.9	0.2	0.5	0.5	0.9	0.7
Line x Sterilised/Unsterilised soil	1, 144	0.1	0.0	0.0	0.3	0.0	0.2	0.2	0.1	0.0
Isolate x Line x Sterilised/Unsterilised soil	11, 144	0.7	0.7	1.3	0.5	0.2	0.2	0.4	0.6	0.5
CV%		12.9	22.3	20.7	15.5	47.4	31.9	21.9	7.5	20.2

Significance is indicated by *; *P < 0.05, **P < 0.001, *** P < 0.0001.

Table 2.2.1: The effect of isolates and lines on average percentage root discolouration in a glasshouse trial.

***Aspergillus niger* isolate treatments**	**Mean percentage root discolouration ± SE (n = 8)**		**Average**
	Lines		
	I 137 TN W	**V 0617 YZ**	
1	6.1 ± 1.3 abc	10.0 ± 2.1 cdef	8.1bc
2	8.8 ± 2.0 bcde	5.9 ± 2.2 abc	7.4b
3	8.6 ± 1.4 abcd	17.8 ± 7.1 fg	13.2cde
4	13.0 ± 2.3 cdefg	7.8 ± 1.6 abcd	10.4bcde
5	15.2 ± 2.4 defg	13.1 ± 1.1 cdefg	14.2 de
6	19.1 ± 5.4 g	10.0 ± 2.7 cdef	14.5e
7	14.8 ± 1.6 defg	11.7 ± 1.6 cdefg	13.2cde
8	5.6 ± 2.3 abc	16.8 ± 2.4 efg	11.2bcde
9	8.1 ± 2.1 abcd	9.6 ± 2.0 bcde	8.8bcd
10	12.5 ± 2.3 cdefg	10.0 ± 0.8 efg	11.2bcde
11	7.7 ± 1.8 abcd	14.7 ± 5.1 defg	11.2bcde
Control	1.7 ± 1.7 ab	0.6 ± 0.6 a	1.5a
Average	9.9 A	10.9 A	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

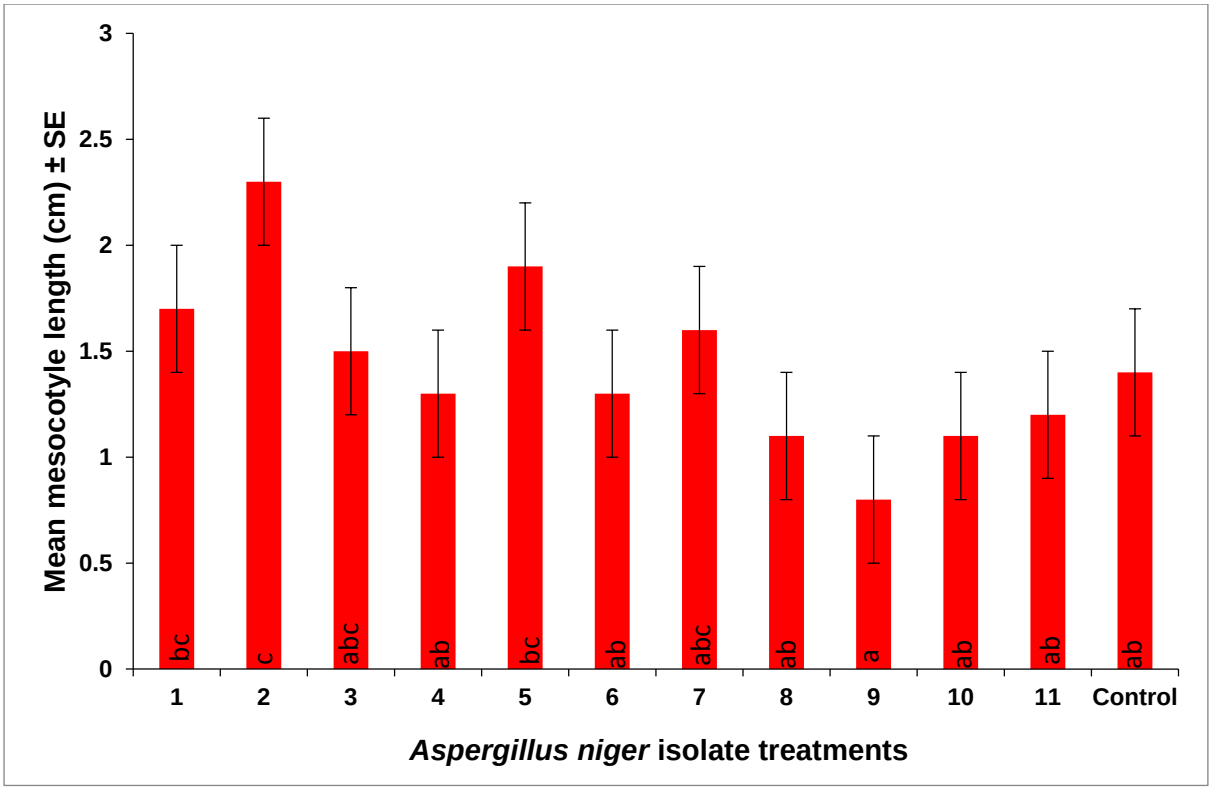

Figure 2.2: The effect of *Aspergillus niger* isolates on mean mesocotyl length of seedlings in a glasshouse trial.

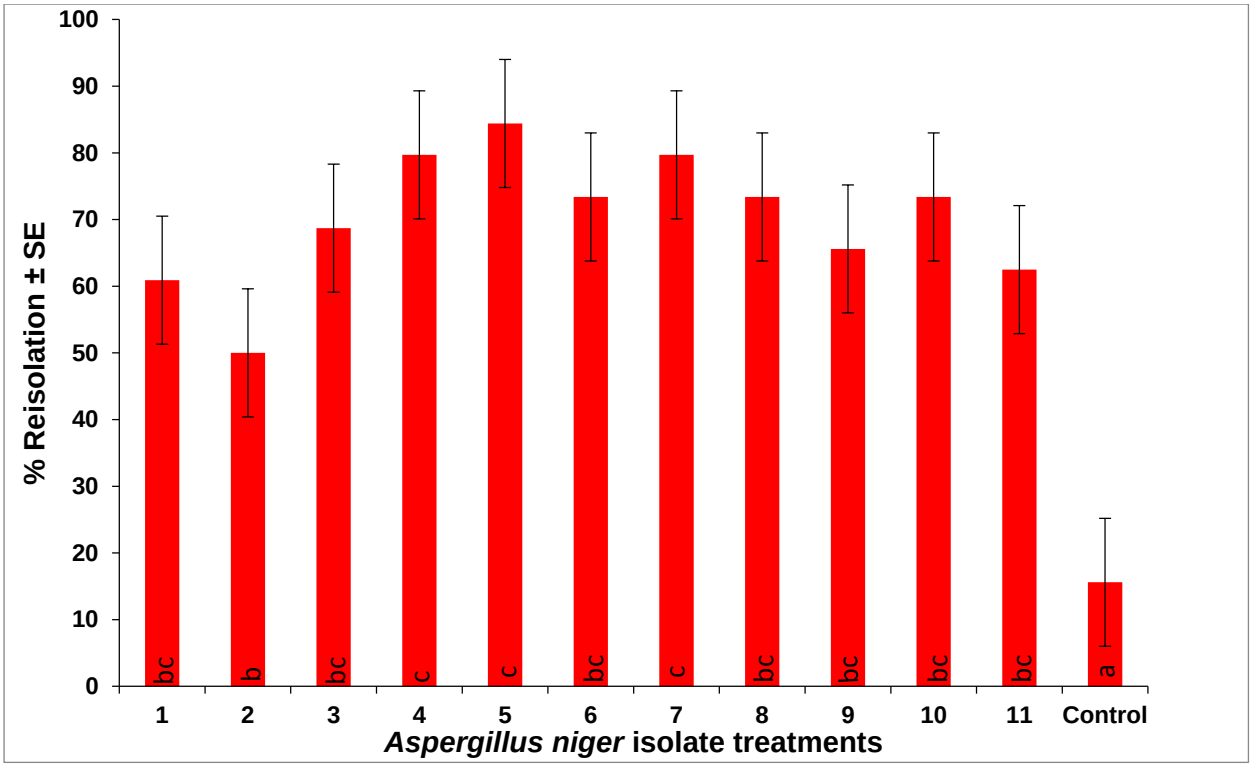

Figure 2.3: Mean percentage reisolation of *Aspergillus niger* isolated from the roots of maize seedlings in a glasshouse trial.

Table 2.3: Results of the screening of maize seedlings against *Fusarium solani* isolates in a glasshouse trial showing the factorial analyses of variance for parameters evaluated (n = 8).

Effects	Df	F– values								
		Length (cm)				Mass (g)		%		
		Root	Coleoptile	Mesocotyl	Radical	Wet	Dry	Discolouration	Emergence	Reisolation
Isolate	11, 144	0.6	1.6	0.9	2.3*	1.5	1.5	5.1***	0.9	10.2***
Line	1, 144	0.3	9.2*	9.4*	13.7**	33.0***	31.8***	2.5	0.0	0.0
Sterilised/Unsterilised soil	1, 144	0.1	5.7*	2.2	19.0***	0.5	0.0	1.7	0.8	0.3
Isolate x Line	11, 144	1.1	1.2	0.4	0.9	2.1*	2.7*	0.8	1.9*	0.8
Isolate x Sterilised/Unsterilised soil	11, 144	1.2	1.1	0.4	0.5	1.1	0.9	1.0	0.8	0.7
Line x Sterilised/Unsterilised soil	1, 144	1.1	0.4	0.5	3.2	0.1	0.0	1.3	3.7	0.0
Isolate x Line x Sterilised/Unsterilised soil	11, 144	1.2	1.1	0.7	1.2	0.9	0.9	0.5	0.7	1.1
CV %		24.7	10.3	18.5	22.3	33.3	23.3	15.5	4.0	8.1

Significance is indicated by *; *P < 0.05, **P < 0.001, *** P < 0.0001

Table 2.3.1: The effect of *Fusarium solani* isolates and maize lines on seedling root wet mass (g) in a glasshouse trial.

***Fusarium solani* isolate treatments**	**Mean root wet mass (g) ± SE (n = 8)**		**Average**
	Lines		
	I 137 TN W	**V 0617 YZ**	
1	1.2 ± 0.3 abcd	0.9 ± 0.2 abc	1.1abc
2	0.8 ± 0.2 ab	0.5 ± 0.1 a	0.7bcd
3	0.7 ± 0.1 ab	0.5 ± 0.1 a	0.6d
4	2.3 ± 0.7 e	0.3 ± 0.1 a	1.3a
5	2.0 ± 0.6 de	0.5 ± 0.1 a	1.3a
6	1.8 ± 0.7 cde	0.7 ± 0.2 ab	1.2ab
7	2.0 ± 0.4 de	0.4 ± 0.2 a	1.2ab
8	1.5 ± 0.2 bcde	0.8 ± 0.3 ab	1.2ab
9	1.1 ± 0.3 abc	1.1 ± 0.3 abcd	1.1abc
10	0.5 ± 0.2 a	0.6 ± 0.2 a	0.6d
11	1.3 ± 0.2 abcd	0.4 ± 0.1 a	0.8bc
Control	1.6 ± 0.6 bcde	0.9 ± 0.1 abc	1.3a
Average	1.4A	0.6B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 2.3.2: The effect of *Fusarium solani* isolates and lines on average root dry mass (g) in a glasshouse trial.

***Fusarium solani* isolate treatments**	**Mean root dry mass (g) ± SE (n = 8)**		**Average**
	Lines		
	I 137 TN W	**V 0617 YZ**	
1	1.0 ± 0.3def	0.6 ± 0.1abcde	0.8a
2	0.7 ± 0.2abcde	0.4 ± 0.1abcd	0.5ab
3	0.6 ± 0.1abcde	0.3 ± 0.1abc	0.5ab
4	1.8 ± 0.5g	0.2 ± 0.1a	1.0a
5	1.1 ± 0.2ef	0.4 ± 0.1abcd	0.7a
6	1.2 ± 0.4ef	0.5 ± 0.1abcde	0.9a
7	1.6 ± 0.4fg	0.3 ± 0.1ab	1.0c
8	1.1 ± 0.2ef	0.6 ± 0.2abcde	0.9a
9	0.7 ± 0.3abcde	0.8 ± 0.2bcde	0.8a
10	0.4 ± 0.2abc	0.4 ± 0.2abcd	0.4b
11	0.9 ± 0.2cde	0.3 ± 0.1ab	0.6a
Control	0.8 ± 0.2abcde	0.7 ± 0.1abcde	0.7a
Average	1.0A	0.5B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 2.3.3: The effect of *Fusarium solani* isolates and hybrids on average percentage emergences in a glasshouse trial.

***Fusarium solani* isolate treatments**	**Mean percentage emergence ± SE (n = 8)**		**Average**
	Lines		
	I 137 TN W	**V 0617 YZ**	
1	71.9 ± 11.0 bcdef	81.2 ± 9.1 def	76.6a
2	68.8 ± 11.3 bcdef	65.6 ± 14.1 bcdef	67.2a
3	62.5 ± 9.5 abcdef	68.7 ± 9.1 bcdef	65.6a
4	84.4 ± 9.4 ef	56.2 ± 9.2 abcde	70.3a
5	78.1 ± 8.8 cdef	65.6 ± 12.4 bcdef	71.9a
6	53.1 ± 8.8 abcd	71.9 ± 14.5 bcdef	62.5a
7	65.6 ± 13.3 bcdef	46.9 ± 12.0 ab	56.3a
8	87.5 ± 6.7 f	71.9 ± 8.8 bcdef	79.7a
9	59.4 ± 12.4 abcdef	81.2 ± 7.8 def	70.3a
10	34.4 ± 13.3 a	71.9 ± 12.9 bcdef	53.1b
11	78.1 ± 7.4 cdef	50.0 ± 11.6 abc	64.1a
Control	62.5 ± 14.9 abcdef	78.1 ± 5.7 cdef	70.3a
Average	67.2A	67.4A	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

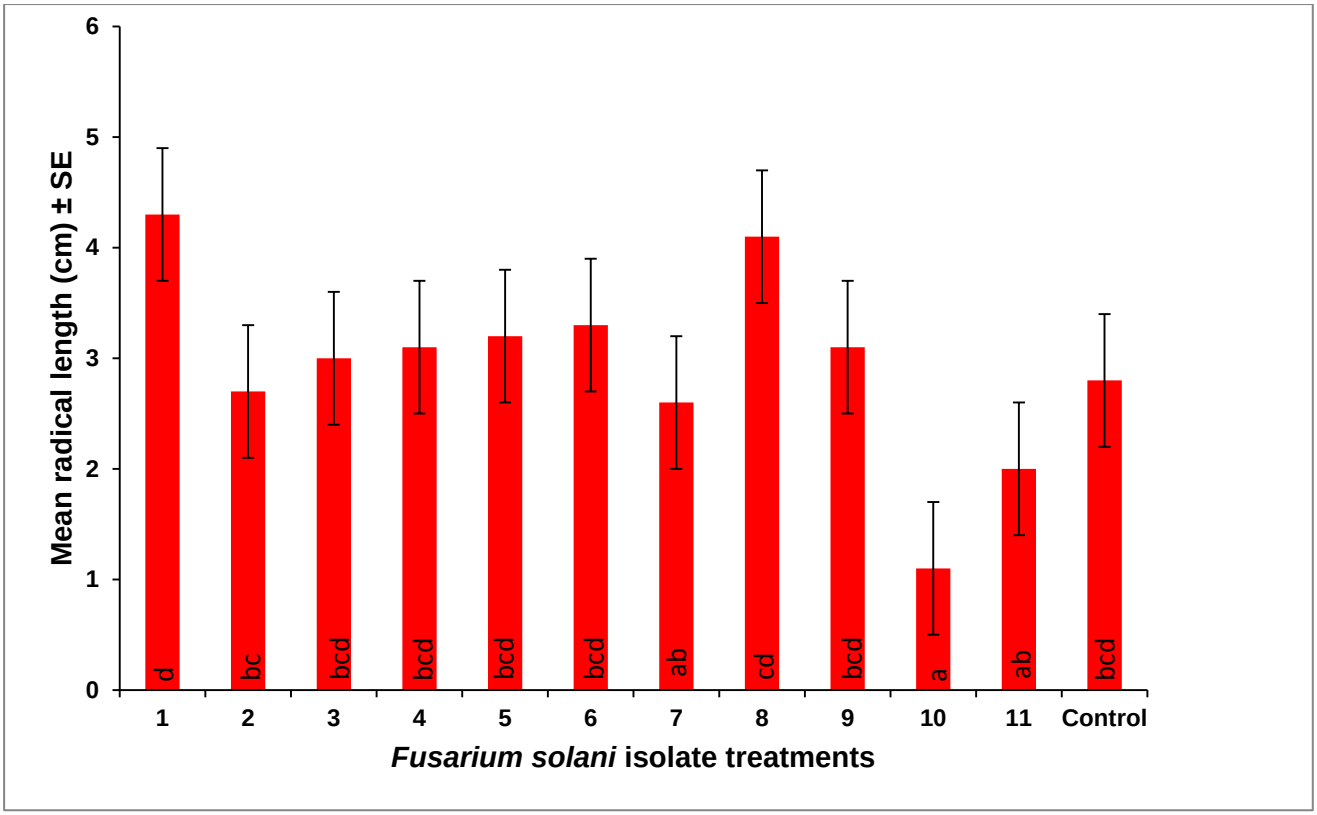

Figure 2.4: The effect of *Fusarium solani* isolates on mean radical lengths of maize seedlings in a glasshouse trial.

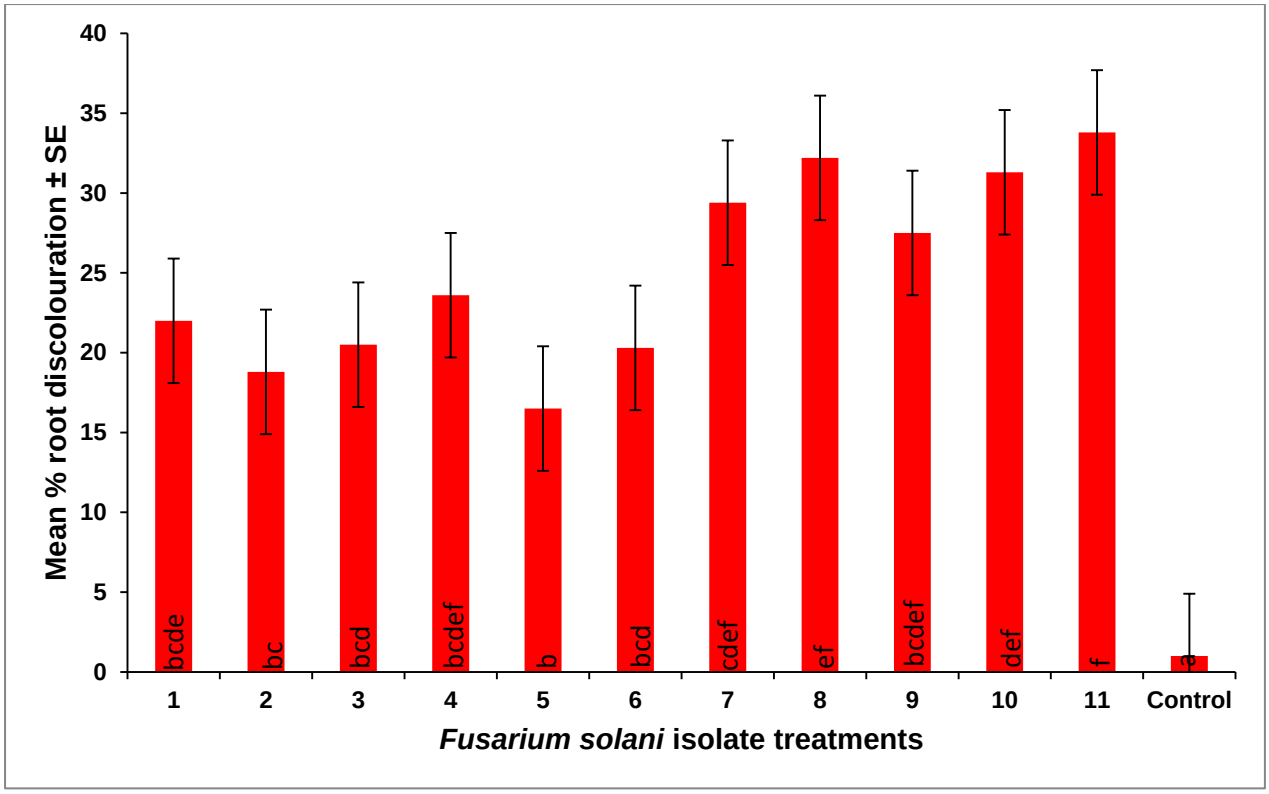

Figure 2.5: The effect of *Fusarium solani* isolates on mean percentage root discolouration of maize in a glasshouse trial.

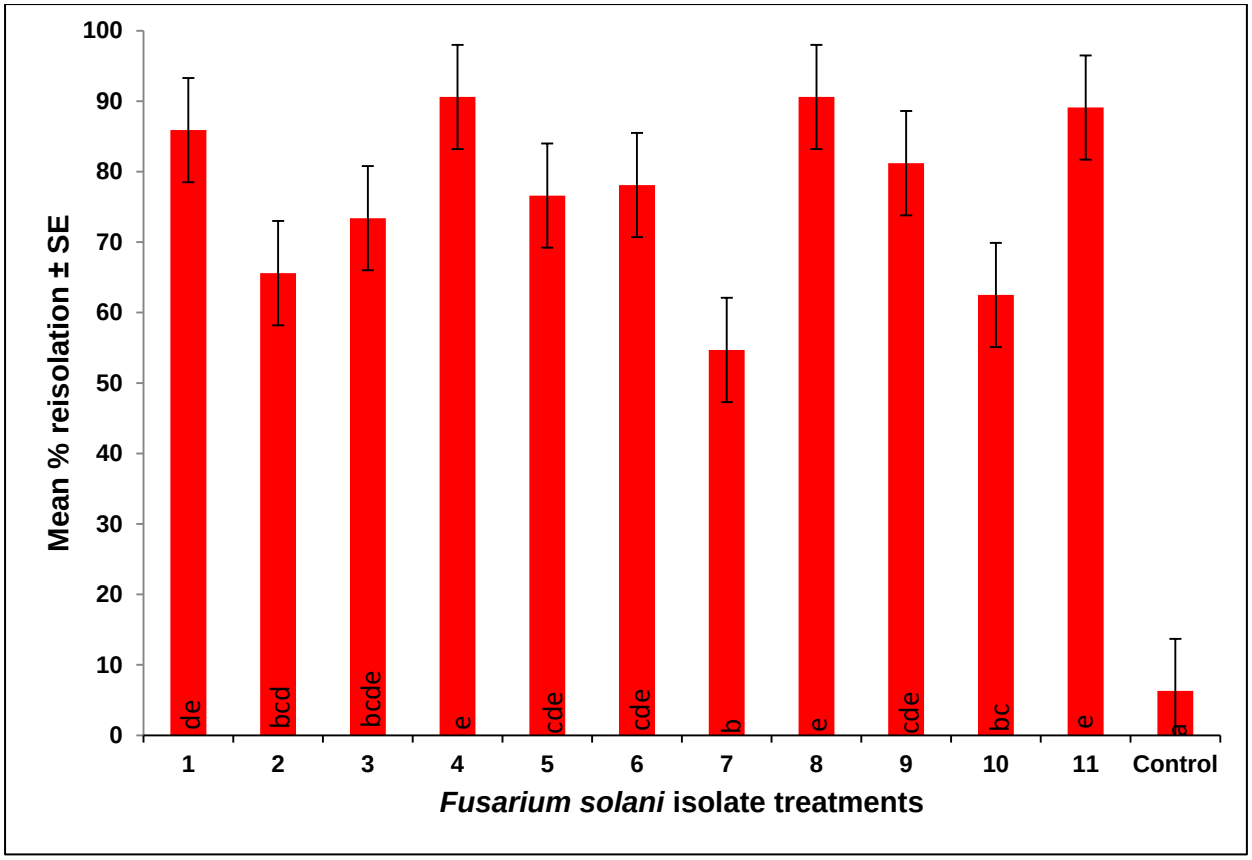

Figure 2.6: Mean percentage reisolation of *Fusarium solani* isolated from the roots of maize seedlings in a glasshouse trial.

Table 2.4: Results of the factorial analyses of variance for parameters evaluated for the screening of maize seedlings against different *Fusarium verticillioides* isolates in a glasshouse trial (n = 8).

Effects	Df	F– values								
		Length (cm)				Mass (g)		%		
		Root	Coleoptile	Mesocotyl	Radical	Wet	Dry	Discolouration	Emergence	Reisolation
Isolate	10,132	4.7**	0.4	0.9	0.5	2.2*	2.7*	5.6***	1.6	8.1***
Line	1, 132	3.1	0.1	0.0	3.1	13.9**	16.5***	0.7	10.8*	2.1
Sterilised/Unsterilised soil	1, 132	0.7	2.0	5.9*	7.7*	0.4	0.0	4.4*	10.9*	2.8
Isolate x Line	10, 132	1.9	0.1	0.7	0.3	0.6	0.7	0.5	1.1	1.5
Isolate x Sterilised/Unsterilised soil	10, 132	2.3*	1.1	0.3	0.3	0.3	0.4	0.3	0.9	1.4
Line x Sterilised/Unsterilised soil	1, 132	1.7	2.0	0.5	0.0	2.3	1.6	5.0*	0.5	0.7
Isolate x Line x Sterilised/Unsterilised soil	10, 132	1.0	0.7	0.6	0.8	0.3	0.4	0.5	1.4	0.8
CV %		14.1	42.5	29.9	44.6	30.3	27.3	16.7	5.7	14.9

Significance is indicated by *; *P < 0.05, **P < 0.001, *** P < 0.0001

Table 2.4.1: The effect of *Fusarium verticillioides* isolates and soil on average root length (cm) of maize seedlings in a glasshouse trial.

***Fusarium verticillioides* isolate treatments**	**Mean root length ± SE (n = 8)**		**Average**
	Soil		
	Sterilised	**Unsterilised**	
1	14.2 ± 1.5 ef	14.1 ± 2.0 ef	14.2a
2	11.7 ± 1.6 cdef	14.4 ± 1.6 f	13.1a
3	11.7 ± 1.6 cdef	18.6 ± 0.8 g	15.1a
4	11.0 ± 2.1 bcdef	11.6 ± 1.4 bcdef	11.3a
5	11.6 ± 1.2 bcdef	12.0 ± 1.7 cdef	11.8b
6	10.8 ± 1.3 bcdef	7.8 ± 0.7 ab	9.4b
7	9.5 ± 1.5 abc	10.8 ± 1.3 bcdef	10.2b
8	10.6 ± 0.8 abcde	10.2 ± 1.6 abcd	10.4b
9	11.6 ± 0.9 bcdef	8.9 ± 1.4 abc	10.3b
10	9.9 ± 0.8 abc	6.8 ± 1.2 a	8.4b
Control	11.4 ± 1.1 bcdef	13.8 ± 1.6 def	12.6a
Average	11.29A	11.76A	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 2.4.2: The effect of maize lines and soil on average percentage root discolouration in the *Fusarium verticillioides* glasshouse trial.

Line	Mean percentage root discolouration ± SE (n= 8)		Average
	Soil		
	Sterilised	Unsterilised	
I 137 TN W	34.8 ± 2.9 b	34.4 ± 3.0 b	34.6a
V 0617 YZ	26.4 ± 2.8 a	38.3 ± 3.2 b	32.3a
Average	30.6A	36.4B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

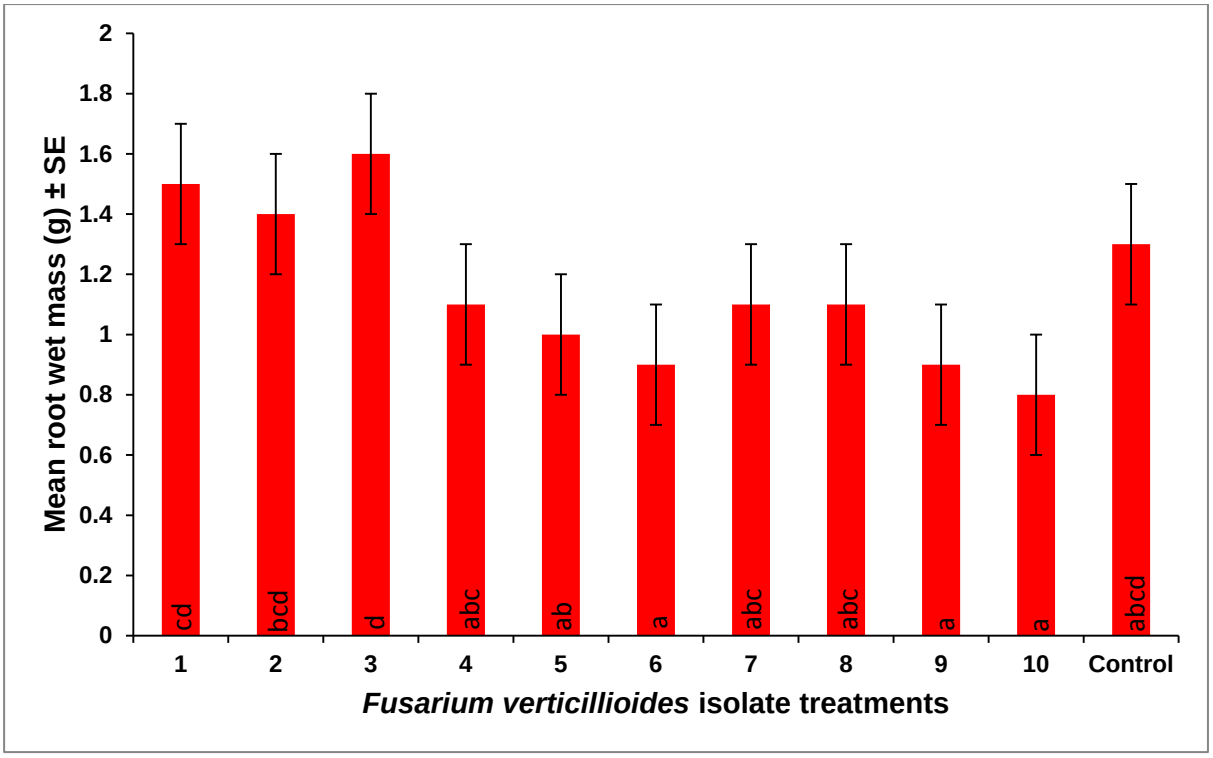

Figure 2.7: Effect of *Fusarium verticillioides* isolates on mean root wet mass of maize seedlings in a glasshouse trial.

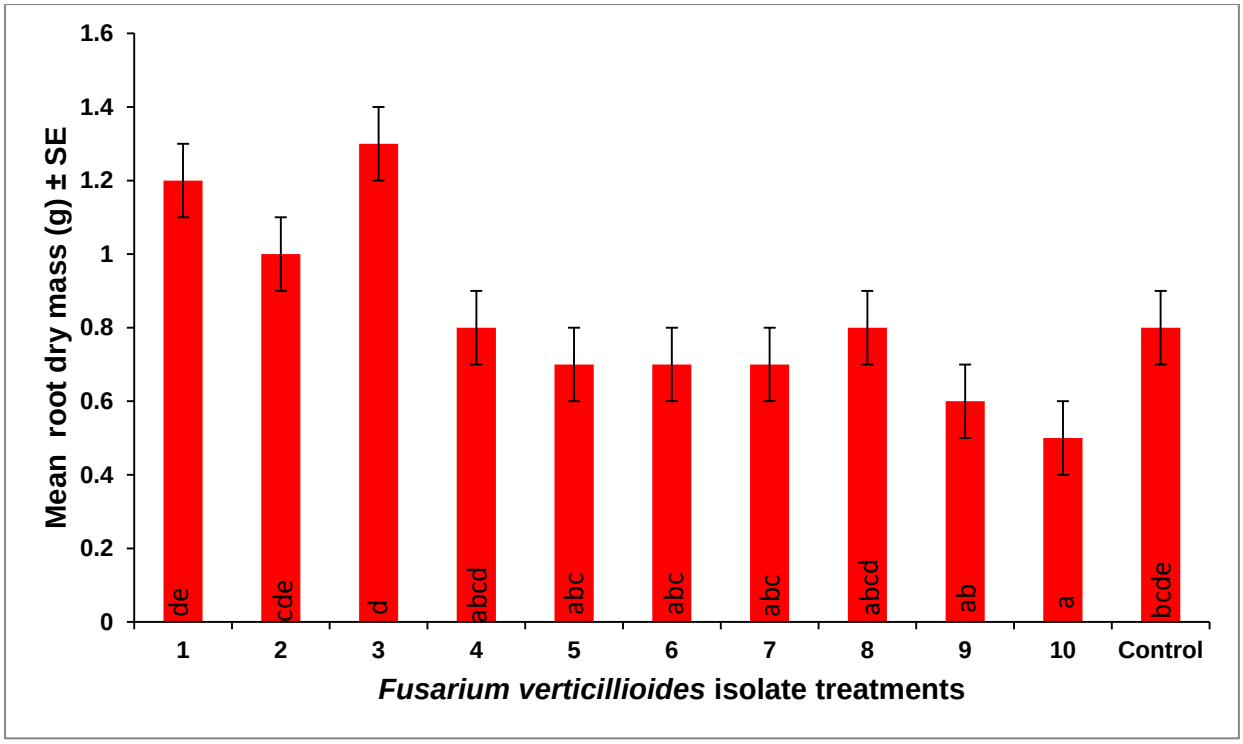

Figure 2.8: The effect of *Fusarium verticillioides* isolates on mean root dry mass of seedlings in a glasshouse trial.

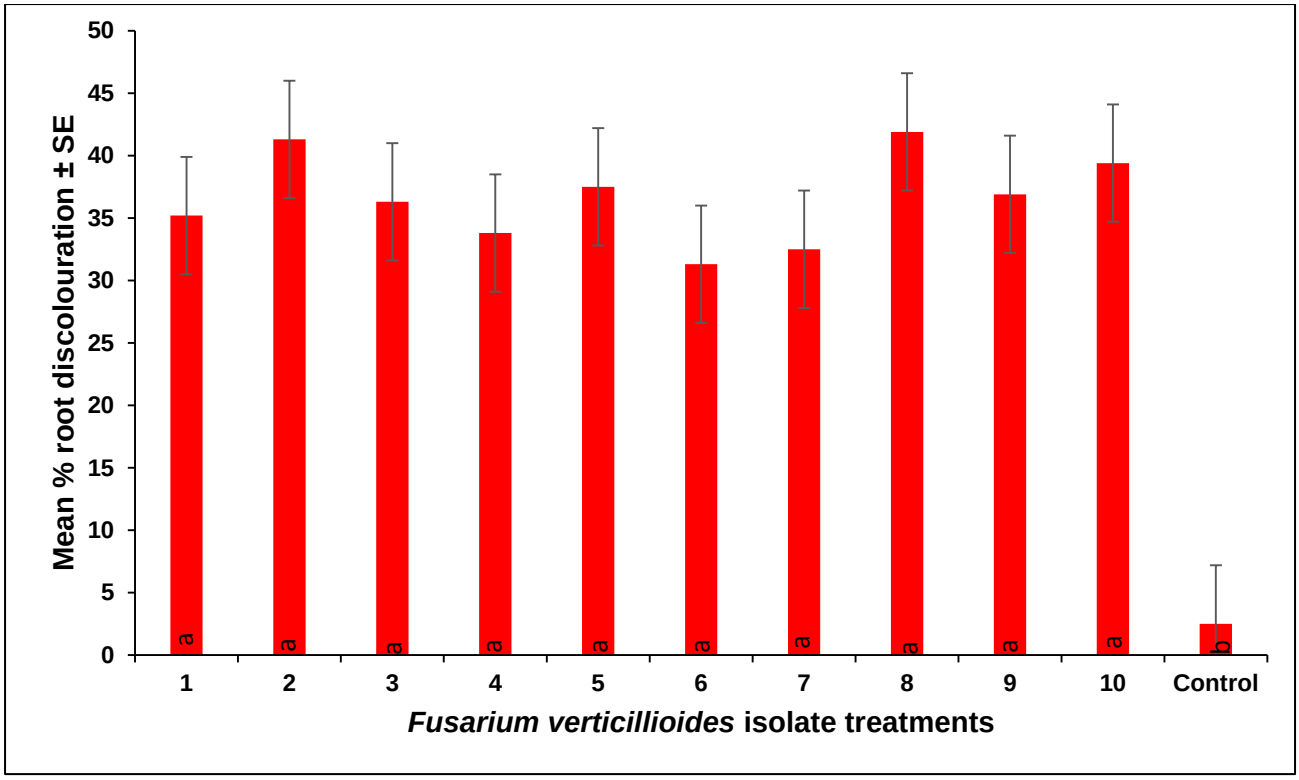

Figure 2.9: The effect of *Fusarium verticillioides* isolates on mean percentage root discolouration of maize seedlings in a glasshouse trial.

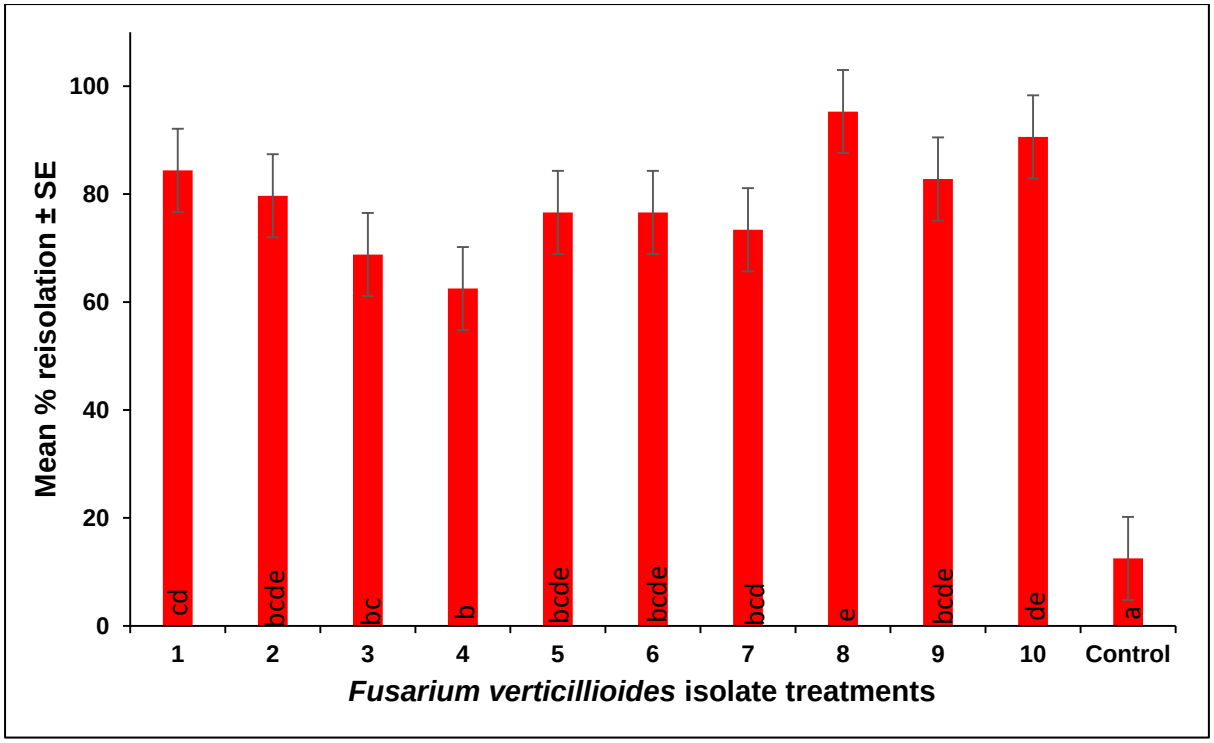

Figure 2.10: Mean percentage reisolation of *Fusarium verticillioides* isolated from the roots of maize seedlings in a glasshouse trial.

Table 2.5: Results of the screening of maize seedlings against *Fusarium oxysporum* isolates in a glasshouse trial showing the factorial analyses of variance for parameters evaluated (n = 8).

Effects	Df	F– values								
		Length (cm)				Mass (g)		%		
		Root	Coleoptile	Mesocotyl	Radical	Wet	Dry	Discolouration	Emergence	Reisolation
Isolate	9, 120	4.4***	1.1	2.6*	3.4*	6.0***	5.9***	4.0**	4.4***	12.1***
Line	1, 120	0.3	6.2*	0.4	0.1	0.0	0.4	0.1	2.1	0.0
Sterilised/Unsterilised soil	1, 120	1.5	1.4	0.1	0.4	0.0	0.1	15.2**	0.9	6.4*
Isolate x Line	9, 120	1.1	1.4	0.6	0.9	1.1	0.7	0.9	1.4	0.8
Isolate x Sterilised/Unsterilised soil	9, 120	0.7	1.4	1.5	1.0	1.3	2.1*	2.2*	0.3	2.7*
Line x Sterilised/Unsterilised soil	1, 120	5.1*	3.7	3.9*	4.7*	1.4	0.6	0.1	0.3	0.4
Isolate x Line x Sterilised/Unsterilised soil	9, 120	0.9	0.5	0.3	0.5	0.3	0.5	0.7	0.3	1.5
CV %		11.2	17.7	6.9	31.3	28.2	24.8	5.9	10.6	14.3

Significance is indicated by *; *P < 0.05, **P < 0.001, *** P < 0.0001

Table 2.5.1: The effect of maize lines and soil on the average root length (cm) of maize seedlings in the *Fusarium oxysporum* glasshouse trial.

Line	Mean root length (cm) ± SE (n = 8)		Average
	Soil		
	Sterilised	Unsterilised	
I 137 TN W	9.8 ± 0.8ab	9.0 ± 0.5ab	9.4a
V 0617 YZ	8.4 ± 1.0a	11.1 ± 0.9b	9.8b
Average	9.1 A	10.1B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 2.5.2: The effect of maize lines and soil on the average radical length (cm) of maize seedlings in the *Fusarium oxysporum* glasshouse trial.

Line	**Mean radical length (cm) ± SE (n = 8)**		**Average**
	Soil		
	Sterilised	**Unsterilised**	
I 137 TN W	3.9 ± 0.5 b	2.5 ± 0.3a	3.2a
V 0617 YZ	3.0 ± 0.6ab	3.7 ± 0.6ab	3.4a
Average	3.5 A	3.1 A	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 2.5.3: The effect of *Fusarium oxysporum* isolates and soil on the average root dry mass (g) of maize seedlings.

***Fusarium oxysporum* isolate treatments**	**Mean root dry mass ± SE (n = 8)**		**Average**
	Soil		
	Sterilised	**Unsterilsed**	
1	1.5 ± 0.4 f	0.7 ± 0.2 abcde	1.1ab
2	0.4 ± 0.2 abc	0.7 ± 0.1 abcdef	0.6bc
3	0.6 ± 0.1 abcde	1.0 ± 0.2 cdef	0.8bc
4	0.3 ± 0.1 abc	0.7 ± 0.1 abcde	0.5bc
5	0.4 ± 0.1 abc	0.5 ± 0.1 abcd	0.4bc
6	0.1 ± 0.0 a	0.5 ± 0.2 abcd	0.3b
7	0.9 ± 0.2 bcdef	1.3 ± 0.3 ef	1.1ab
8	2.3 ± 0.8 g	1.2 ± 0.4 def	1.8a
9	0.9 ± 0.2 bcdef	0.4 ± 0.1 abcd	0.7bc
Control	0.3 ± 0.1 abc	0.2 ± 0.1 ab	0.3b
Average	0.8A	0.7A	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 2.5.4: The effect of *Fusarium oxysporum* isolates and soil on average percentage root discolouration of maize seedlings.

***Fusarium oxysporum* isolate treatments**	**Mean percentage root discolouration ± SE (n = 8)**		**Average**
	Soil		
	Sterilised	**Unsterilised**	
1	11.3 ± 5.2 ab	42.5 ± 4.5 e	26.9a
2	12.5 ± 6.7 abc	25.0 ± 6.3 bcd	18.8a
3	11.3 ± 6.1 ab	26.3 ± 5.0 cd	18.8a
4	13.8 ± 5.3 abc	35.0 ± 5.3 de	24.4a
5	20.3 ± 7.3 bc	19.4 ± 7.8 bc	19.8a
6	12.5 ± 3.1 abc	18.8 ± 6.1 bc	15.6ab
7	14.2 ± 3.2 abc	13.8 ± 6.0 abc	10.0ab
8	17.2 ± 2.4 bc	20.6 ± 5.3 bcd	18.9a
9	13.4 ± 4.1 abc	15.6 ± 3.5 bc	14.5ab
Control	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0b
Average	12.6A	21.7B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 2.5.5: The effect of isolates and soil on the average percentage reisolation of *Fusarium oxysporum* from the roots of maize seedlings planted in a glasshouse.

***Fusarium oxysporum* isolate treatments**	**Mean percentage reisolation ± SE (n = 8)**		**Average**
	Soil		
	Sterilised	**Unsterilised**	
1	75.0 ± 14.2 b	100.0 ± 0.0 b	87.5a
2	46.9 ± 16.7 ab	90.6 ± 9.4 b	68.8ab
3	59.4 ± 12.4 b	75.0 ± 10.6 b	67.2ab
4	53.1 ± 12.9 ab	84.4 ± 10.5 b	68.8ab
5	65.6 ± 11.5 b	65.6 ± 10.5 b	65.6ab
6	56.3 ± 12.3 b	96.9 ± 3.1 b	76.6a
7	90.6 ± 9.4 b	56.3 ± 14.0 b	73.4ab
8	100.0 ± 0.0 b	90.6 ± 9.3 b	95.3a
9	78.1 ± 14.5 b	84.4 ± 10.5 b	81.3a
Control	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0b
Average	62.5A	74.4B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

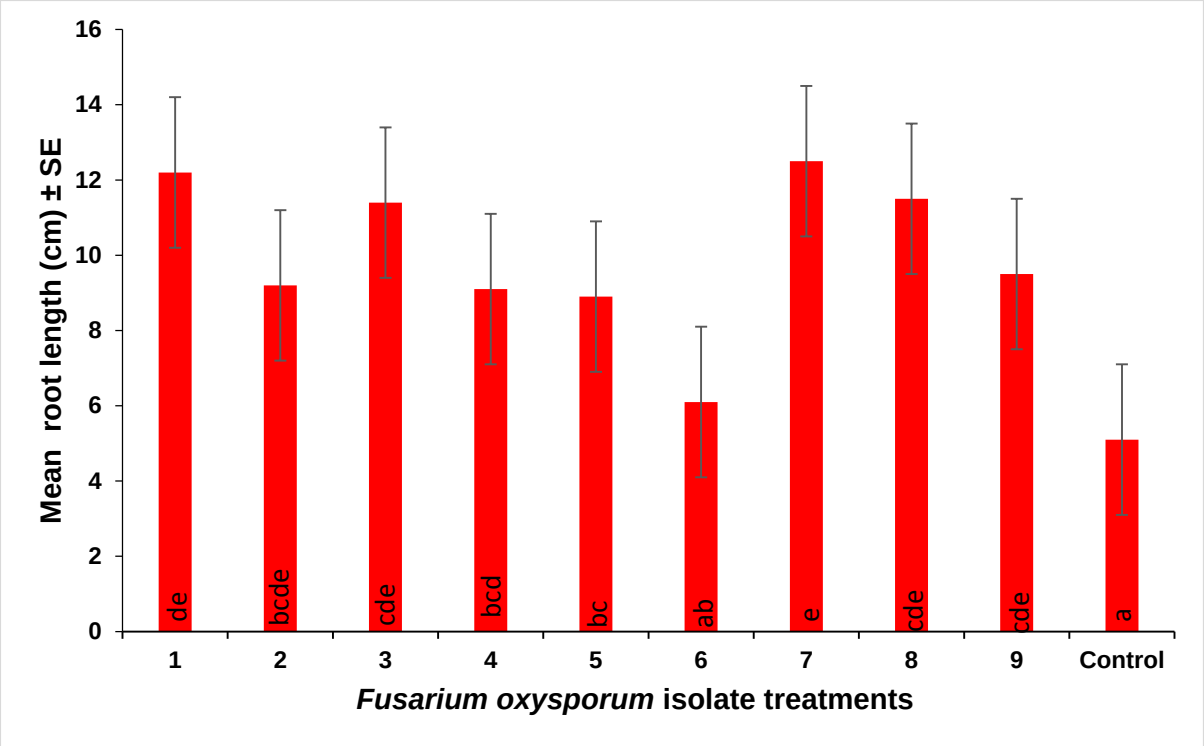

Figure 2.11: The effect of *Fusarium oxysporum* isolates on mean root length of maize seedlings in a glasshouse trial.

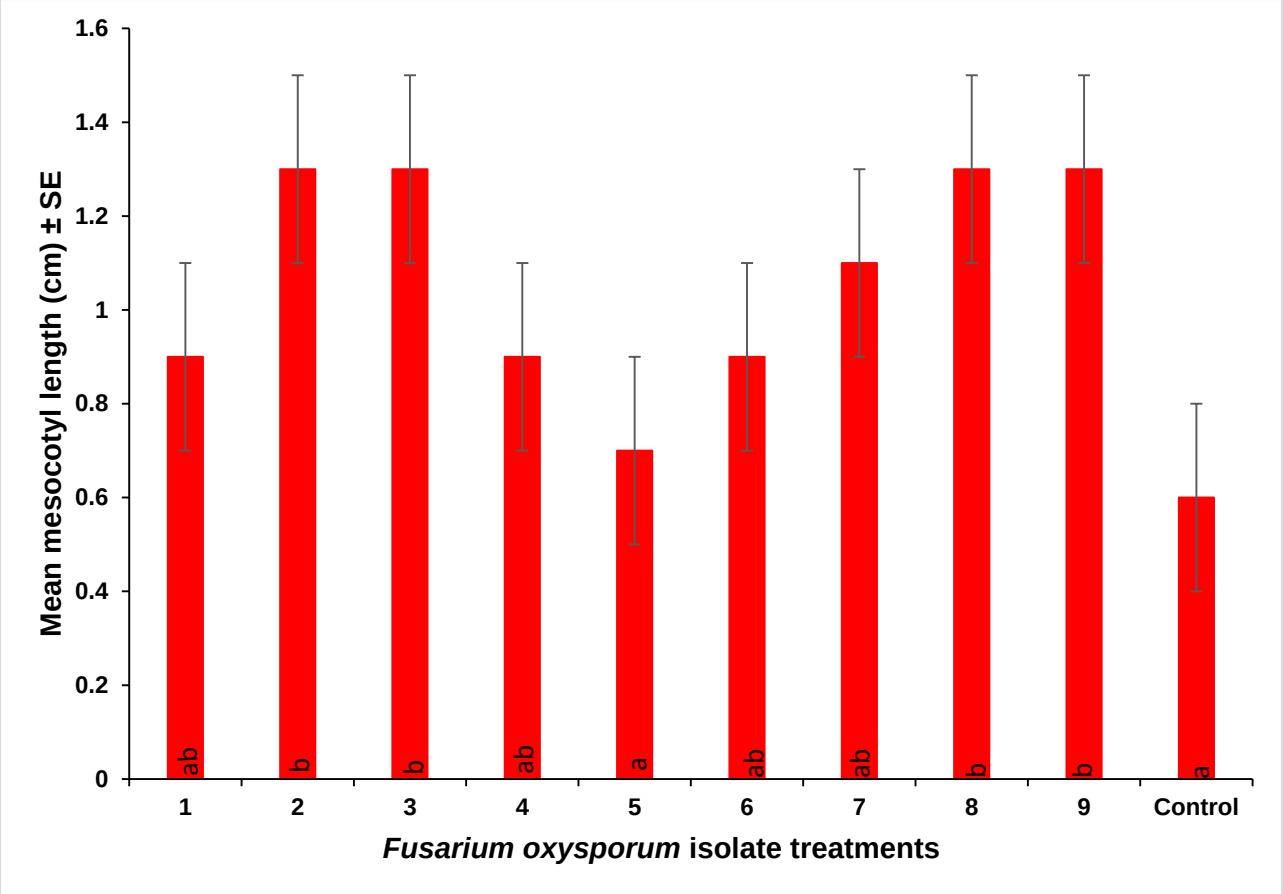

Figure 2.12: The effect of *Fusarium oxysporum* isolates on mean mesocotyl length of maize seedlings in a glasshouse trial.

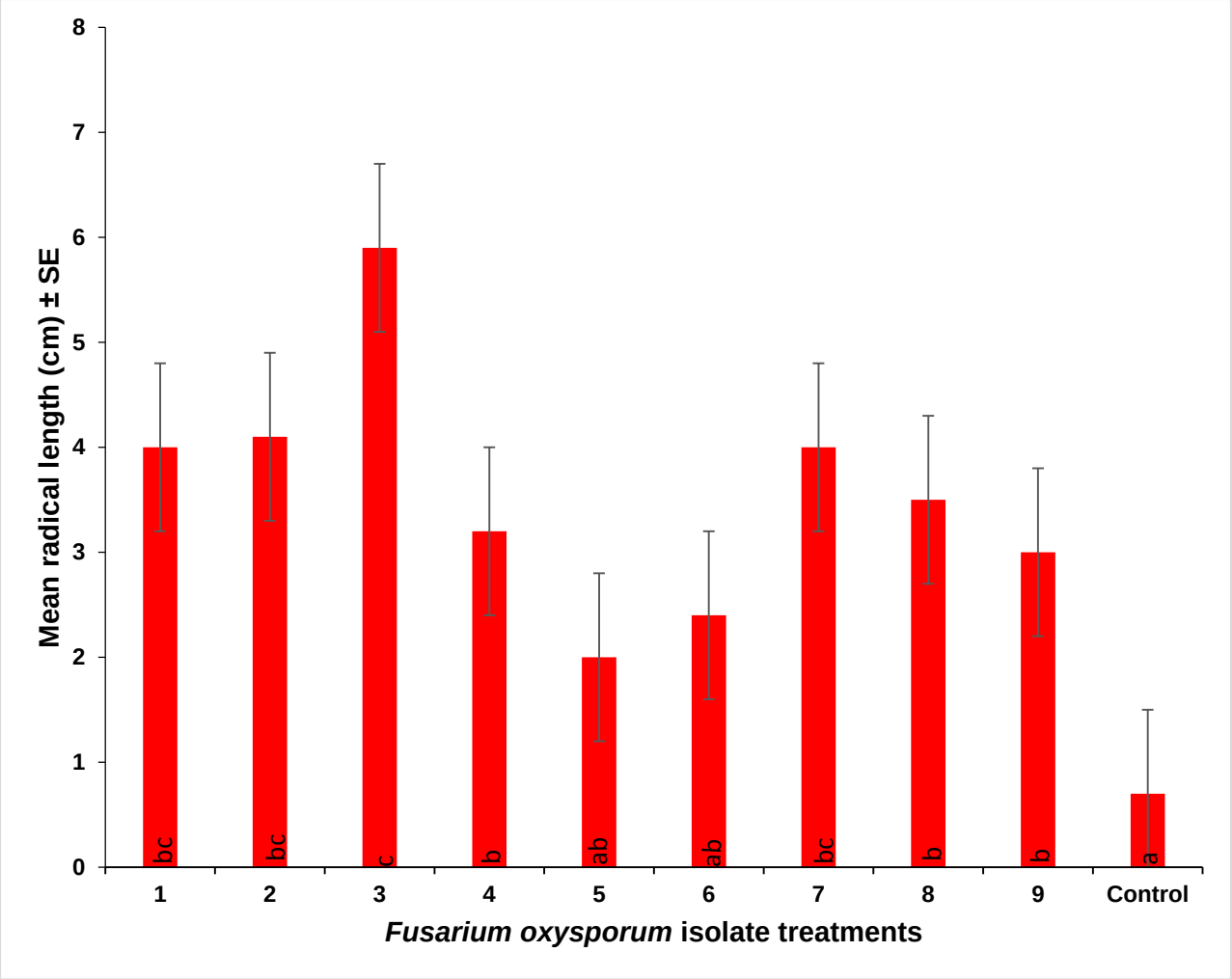

Figure 2.13: The effect of *Fusarium oxysporum* isolates on mean radical length for maize seedlings in a glasshouse trial.

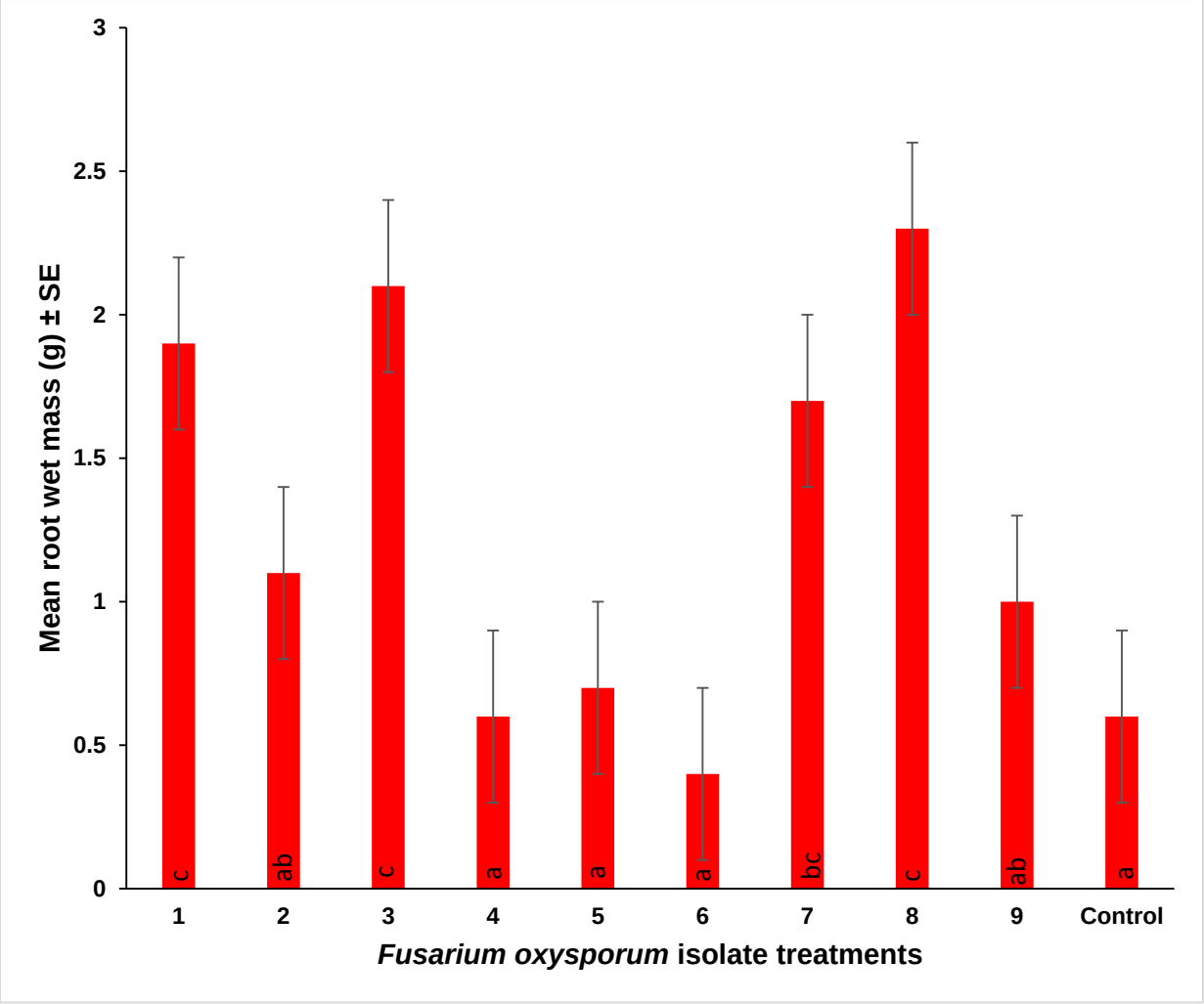

Figure 2.14: Mean root wet mass for maize seedlings inoculated with *Fusarium oxysporum* isolates in a glasshouse trial.

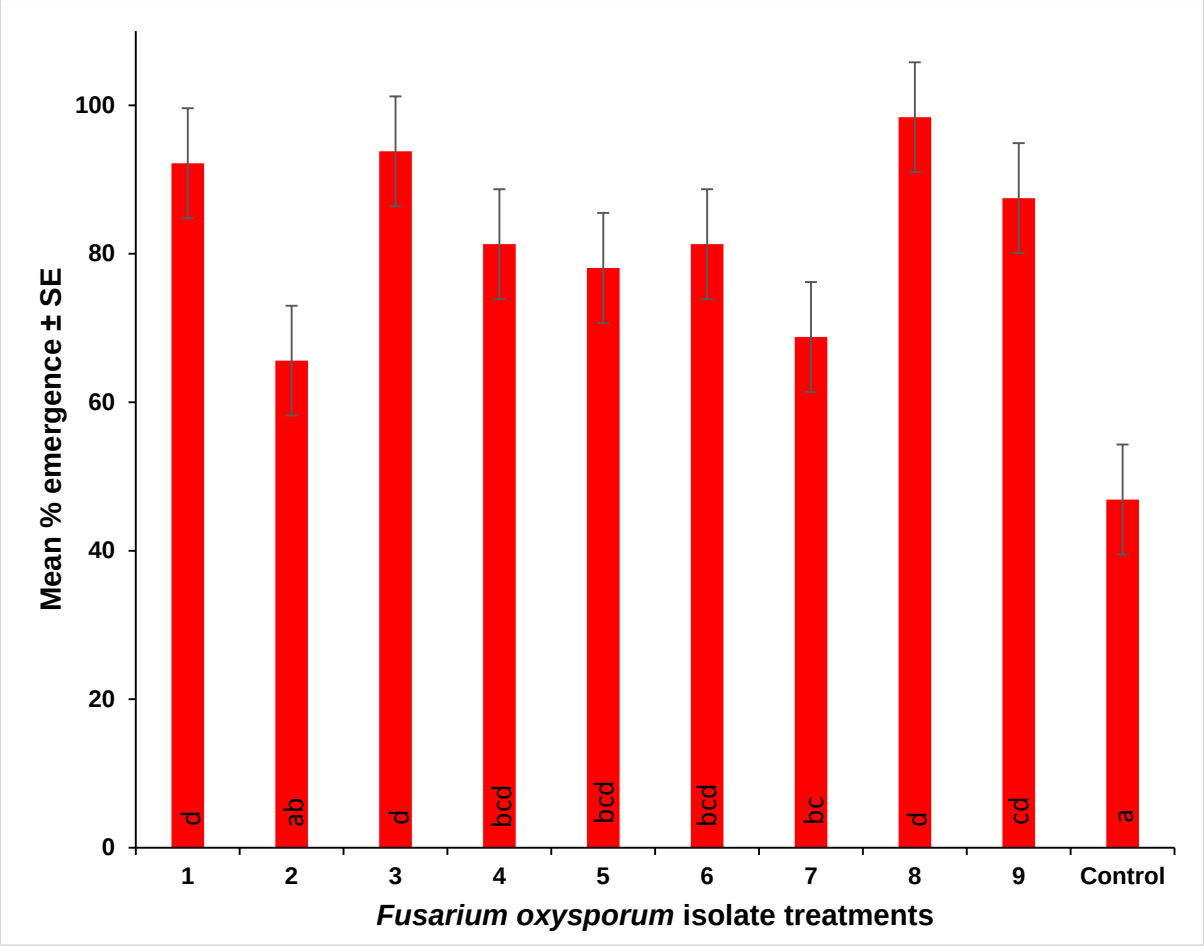

Figure 2.15: Mean percentage emergence of maize seeds inoculated with *Fusarium oxysporum* isolates in a glasshouse trial.

Chapter 3

Trichoderma species associated with maize seedling roots in South Africa

3.1 Abstract

Maize is grown worldwide and is an important staple crop of millions of people. In South Africa 2.68 million ha of agricultural land is under maize production with an estimated annual yield of 13.029 million tons. *Trichoderma* spp. is the most frequently isolated fungal species from agricultural soils worldwide and play an important role in the production of maize due to their ability to recycle nutrients and promote plant health and development. The diversity of *Trichoderma* spp. found in South African agricultural soils is unknown. Samples of 38 maize seedling fields across South Africa were sampled in the 2012/2013 production season. A number of *Trichoderma* spp. isolates were found to be associated with maize seedling roots. DNA based sequence comparisons and 70 isolates were used in growth studies to identify the species involved. Isolates were identified using the ITS gene region and nBLAST analyses were done on the TrichoBLAST and MycoBank databases. Three species were identified namely *T. asperellum, T. afro-harzianum* and *T. gamsii*. Ten selected isolates were screened using soil inoculation in a glasshouse, on two maize lines, *viz.* I 137 TN W and V0617 YZ to determine if the isolates could promote seedling growth. The parameters evaluated were root, mesocotyl and coleoptile length, wet and dry root mass as well as percentage root discolouration, seed emergence and reisolation of the inoculated fungus from seedling roots. Significant differences ($P < 0.05$) were found when comparing the coleoptile length of maize seedlings inoculated with *T. gamsii* compared to the other two *Trichoderma* spp. and also showed significant differences ($P < 0.05$) when the radical length and wet seedling masses were compared. Results of this study indicated that *T. gamsii* is a common root coloniser of maize in South Africa and that *T. gamsii* promotes maize seedling growth as does *T. afro-harzianum*.

Key words: Maize seedlings, *T. asperellum, T. afro-harzianum* and *T. gamsii*

3.2 Introduction

Maize is grown worldwide and serves as a staple diet to millions of people (SAGIS, 2014). In South Africa maize is planted on 2.68 million ha of agricultural soil, with an estimated yield of 13.029 million tons (SAGIS, 2014). Thus, South African farmers are highly dependent on seasonal rain for crop production under dryland conditions. These crops are also subjected to harsh extreme climatic conditions, pest damage and disease epidemics during the planting and growing season which often result in reduced yield (Martin *et al*., 2000).

Certain species of soil- and seed borne fungi also play an important role in the production of maize due to their ability to recycle nutrients and promote plant health and development (Newton *et al*., 2003). One of these groups of fungi is the genus *Trichoderma* which are found worldwide in agricultural soils (Killham, 1994). The genus *Trichoderma* was first described in 1794 by Persoon and later in more detail by Rifai in 1969 (Rifai, 1969). It is a highly competitive genus that is also referred to as indirect antagonists due to their interactions with other microorganisms (Dix and Webster, 1995). Species demarcation of *Trichoderma* spp. based on morphological characters is limited by overlap in characters (Siddiquee *et al*., 2007). Kubicek *et al*. (2001) reported that morphological identification was highly prone to error and that roughly 50 % of the *Trichoderma* spp. obtained by morphological identification alone were wrongly identified.

The ecological role of *Trichoderma* spp. spans from pathogen to symbiont (Windham *et al*., 1989; Woo *et al*., 2006). Various species have the ability to utilise a variety of nutrient sources including man-made chemicals and toxins (Woo *et al*., 2006). *Trichoderma* species are also ecologically successful due to their ability to colonise a wide range of substrates under adverse conditions. This is apparent from the vast number of reports in literature reporting on *Trichoderma* as a biocontrol agent, a substitute for chemical pesticides and a major role player in integrated disease management systems (Cavalcante *et al*., 2008). More than 50 different *Trichoderma* based agricultural products, registered in many different

countries (Woo *et al*., 2006). *Trichoderma harzianum* is a well-studied biocontrol agent used as a seed coat dressing and systemic based product on maize (Pal *et al*., 2013). A study conducted by Suhaida and Nurainizzati (2013) used *Fusarium verticillioides*, *Fusarium solani* from maize ears showing ear rot symptoms and used *T. harzianum* isolates to suppress these ear rot disease species in glasshouse trials, and found that *Trichoderma* are a more aggressive root coliniser which outcompeted the other species before it can release its mycotoxins indicating that *T. harzianum* was successful in protecting the maize plant from the pathogens infecting the plant (Suhaida and Nurainizzati, 2013).

The aim of this study was to determine the biodiversity of *Trichoderma* spp. in localised areas of the South African maize producing region.

3.3 Materials and Methods

3.3.1 Field survey

Maize seedlings were sampled within three weeks after emergence from 38 commercial fields in the maize producing area of South Africa during the 2012/13 production season. The fields were all planted with maize hybrid seeds treated with fungicides prior to planting. Ten seedlings were randomly sampled from each field and placed in a brown paper bag (25 X 50 cm) and the GPS coordinates of each field were recorded. Each paper bag was sealed and transported in a cooler box to the ARC-GCI laboratories in Potchefstroom, South Africa. Each maize seedling was washed with distilled water until no visible soil particles were left. The roots were cut into 1 mm pieces and sterilised using a 1 % sodium hypochlorite mixture for 1 minute (Lamprecht, 2007). The roots were washed again with distilled water and filter paper was placed on top of the roots to dry. Fifty root pieces from each sample were randomly selected and plated out, four root pieces per Petri dish, on half strength potato dextrose agar (Bilolab, Merck Pty. Ltd), preparing it as described by Leslie and Summerell's, (2006). These Petri dishes were incubated in a growth chamber for five days at 25 °C. Petri dishes with *Trichoderma* spp. like growth from root pieces were selected and the colonies

were single spored and placed on water agar and left to grow for 24 hours (Leslie and Summerell's, 2006). The single spored cultures were then placed onto half strength potato dextrose agar and incubated for two weeks. Isolates were grouped according to their visual morphological characteristics (Seaby, 1996) and numbered according to the locality and colony morphology. These isolates were accessioned and identified by the South African National Collection of Fungi at the ARC – PPRI, Pretoria, South Africa.

3.3.2 Growth studies

Isolates were grown on potato dextrose agar (PDA), as well as synthetic low nutrient agar (SNA) (Nirenberg, 1976) and colony morphology was documented. Growth rates were determined by placing a plug of actively growing mycelium on a PDA and a SNA plate and the average growth in millimetres from the plug were calculated after 3 days for isolates at 35 and 40 °C respectively. Three plates per isolate, per type of medium were used.

3.3.3 DNA extraction, amplification and sequencing

All isolates obtained were grown on PDA at 25 ºC for 3 days. DNA was isolated using the DNAeasy® Plant Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's specifications. Extracted DNA was used as template in PCR reactions to amplify a part of the intertranscribed spacer region (ITS) using the primer set ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3) and ITS 4 (5'- TCCTCCGCTTATTGATATGC -3') (White *et al*., 1990). The PCR reaction consisted of 1x Excel Taq reaction buffer with $MgCl_2$, dNTPs (250 µM each), primers (0.2 µM each), and template DNA (25 ng) and Excel Taq polymerase (0.25 U). The PCR reaction, for the ITS gene region was amplified by initial denaturation at 94 °C for 2 min. This was followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and elongation at 72 °C for 1 min and elongation step at 72 °C for 5 min. The resulting amplicons were purified with a QIAquick PCR Purification kit (QIAGEN, Germany) and subjected to automated DNA sequencing using the same primers that were used for the PCR amplifications and the ABI PRISMTM Dye Terminator Cycle

Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, (Applied Biosystems, UK) and Applied Biosystems' 3730 DNA Analyser. Raw sequence trace files were analysed with BioEdit. All of the sequences generated in this study have been deposited in GenBank (Table 2). The partial sequence data for the ITS gene region was compared against the TrichoBLAST (www.isth.info/tools/blast/index.php) and MycoBank (www.mycobank.org) databases.

3.3.4 Glasshouse trials

Ten *Trichoderma* spp. isolates were selected (*T. gamsii* 14671, *T. asperellum* (Samuels, Lieckf. and Nirenberg) 14675, *T. gamsii* 13823, *T. asperellum* 14667, *T. gamsii* 13824, *T. afro-harzianum* 13818, *T. afroharzianum* 14669, *T. gamsii* 14678, *T. gamsii* 13817, *T. asperellum* 14677) to evaluate their effect on maize seedling growth in glasshouse trials. Each isolate was plated out on half strength PDA to develop an actively growing colony which was then again plated onto 52 half strength PDA Petri dishes and kept in an incubation chamber for two weeks at 25 °C. A total of 520 consol jars with a volume of one litre, were half filled with a mixture of small bird seed (Nel *et al*., 2006) and 50 ml water was added. These jars were autoclaved after 24 hours, for 20 minutes at 121 °C, left to cool down and autoclaved again for 20 minutes. After they cooled down, 52 jars for each isolate were inoculated with the respective *Trichoderma* isolates, and sealed. The jars were kept in a culture room at 26 °C for 50 days to allow the *Trichoderma* isolates to grow. The inoculum was then removed from the jars and transferred to brown paper bags and air dried (Dumroese *et al*., 1998), after which each set of inoculum was milled using a Cyclotec 1093 Foss mill to grind the inoculum to a fine powder. After each isolate was milled the miller was sterilised with a 70 % ethanol suspension, prior to the milling of the next isolate to prevent contamination.

The glasshouse trial was planted in a randomized complete design with two maize lines, I 137 TN W and V 0617 YZ, 10 treatments (*Trichoderma* isolates) and 8 replicates per

treatment. A total of 320 five litre pots were planted with four seeds of two different maize lines respectively. Milled inoculum of each *Trichoderma* isolate, was added to the soil at a rate of 150 g per pot and mixed into the soil (Nel *et al*., 2006). There also was an uninoculated control. The glasshouse temperature was 26 °C during the day and 18 °C at night with natural day and night lengths. The plants were removed from the soil in a cautious manner, three weeks after emergence. The seedlings were washed with distilled water and the primary and secondary root discolouration were visually assessed and recorded using the root disease index (RDI) based on an adjusted scale of 0 - 4 were 0 is no symptoms, 1 = 1– 25 % rot, 2 = 26 – 49 % rot, 3 = 50 – 74 % rot, and 4 = 75 – 100 % rot (Soonthornpoct *et al*., 2000). The coleoptile, mesocotyl, radical and root lengths were measured and wet root mass was determined. The wet maize seedling roots were left to dry at 105 °C for 24 hours (Srobarova and Eged, 2005) and the dry mass was determined. Root pieces (1 mm) were cut from the inoculated and uninoculated maize seedlings and sterilised with a 3.5 % sodium hypochlorite mixture for 1 minute. The root pieces were washed with distilled water and filter paper was placed on the roots to dry. Four root pieces from each plant in the glasshouse trial were plated out on half strength PDA in Petri dishes kept at 25 °C in an incubation chamber. The percentage reisolation of *Trichoderma* spp. from the roots was determined after five days.

The data was analysed by means of a factorial analysis of variance (ANOVA), followed by Fisher's protected least significant difference (LSD) test using Statistica® 12th edition (StatSoft Inc., Tulsa, UK).

3.4 Results

3.4.1 Field survey

Trichoderma spp. were isolated from 38 different maize seedling samples in the maize producing region of South Africa. The different species associated with maize seedling roots are provided in Table 3.1. Three different species of *Trichoderma* were identified namely *T.*

asperellum, *T. afroharzianum* and *T. gamsii* with *T. gamsii* being the most commonly isolated species from maize seedling roots. Figure 3.1 indicates the different localities and regions in which the *Trichoderma* spp. isolates were collected. The maize seedlings collected from these maize fields showed no root discolouration.

3.4.2 Growth studies and DNA comparison

Growth rates of *Trichoderma* spp. colonies were measured and average growth rate computed after 3 days of growth. Demarcating the growth rates in 10 mm intervals ie. 0-10; 11-20; 21-30; 31-40 separated the 50 isolates into 14 morpho-groups. A total of 38 representative isolates from each of the morpho-groups were then selected for future characterisation based on sequence data for the ITS gene region (Schoch *et al*., 2012). The nBLAST searches on TrichoBLAST (www.isth.info/tools/blast/index.php) and MycoBank (www.mycobank.org) databases for the ITS gene region identified the isolates as three *Trichoderma* spp., namely *T. asperellum, T. harzianum* and *T. gamsii* (Table 3.1).

3.4.3 Glasshouse trial

There was a significant line x soil interaction in terms of *Trichoderma* spp. percentage reisolation from maize seedling roots (Table 3.2). The mean percentage *Trichoderma* spp. reisolation from maize seedling roots was significantly higher for line V 0617 YZ planted in sterilised soil compared to the same line in unsterilised soil and line I 137 TN W in both sterilised and unsterilised soil (Table 3.2.1). Mean coleoptile length of maize seedlings from soil inoculated with *T. gamsii* (13817) on line I 137 TN W was significantly longer than mean coleoptile length of maize seedlings from soil inoculated with *T. gamsii* (14671), *T. gamsii* (14678), *T. afroharzianum* (14669), *T. asperellum* (14677) and the uninoculated control (Table 3.2.2). On line V 0617 YZ, *T. gamsii* (14678) had a significantly longer mean seedling coleoptile length than isolates *T. gamsii* (14671), *T. gamsii* (13817), *T. asperellum* (14677) and *T. afroharzianum* (13818). There was a significant differential reaction for *T. gamsii* (13817) which had a significantly longer mean seedling coleoptile length on line I 137 TN W

than on line V 0617 YZ. *Trichoderma gamsii* (14678) had a significantly longer mean seedling coleoptile length on line V 0617 YZ compared to line I 137 TN W (Table 3.2.2). Mean maize seedling radical length was significantly longer in the sterilised soil compared to unsterilised soil ($F_{1,132} = 4.6$; $P < 0.05$).

The percentage reisolation for all the *Trichoderma* spp. isolates were significantly higher than the percentage reisolation for isolate *T. gamsii* (13817) which in turn was significantly higher than the uninoculated control (Figure 3.2). No significant interactions for isolate x soil, and isolate x line x soil were obtained.

3.5 Discussion

Trichoderma species are soile borne fungi with a cosmopolitan distribution (Okoth *et al*., 2011). *Trichoderma* spp. isolates were sampled throughout the South African maize producing region and were most commonly found in the KwaZulu /Natal province which has a relatively high annual rainfall. *Trichoderma* spp. has a preference to soils with temperatures ranging from 15 °C to 30 °C and prefer soils with up to 35 % soil moisture (Devi and Paul, 2008) (Figure 3.1). This may explain the higher incidence of *Trichoderma* spp. isolates found in KwaZulu/Natal area compared to other parts of the maize producing area.

Most of the *Trichoderma* spp. identified in this study are known for their direct antagonism against other soil-borne plant pathogens (Jaklitsch *et al*., 2006; Hajieghari, 2010: Okoth *et al*., 2011). Results of this present study showed that three *Trichoderma* spp. are primarily associated with maize seedling roots in South Africa. These *Trichoderma* spp. include *T. gamsii*, *T. asperellum* and *T. afro-harzianum*. *Trichoderma harzianum* has been previously reported to be successful biocontrol agents against pathogens like *F. proliferatum* (Matsush.) Nirenberg, *F. subglutinans* (Wollenw. and Reinking) P. E. Nelson, Toussoin and Marasas, *F. culmorum* (W. G. Sm.) Sacc, and *F. graminearum* that cause ear rot on maize (Okoth *et al*., 2011; Matarese *et al*., 2012; Suhaida and Nurainizzati, 2013).

The occurrence of *T. gamsii* in soil has been reported from Russia, U.S.A., Italy, Australia, and three previous isolates from South Africa (Jaklitsch *et al*., 2006). These three South African isolates originated from Kwa-Zulu Natal (soil in a *Eucalyptus* spp. plantation), Western Cape Province (soil under *Leucodendron* spp.) and the Cape Province (soil under a *Protea* spp.) (Jaklitsch *et al*., 2006). In this study, *T. gamsii* has been found in association with maize seedlings roots from various localities and can therefore be seen as a first report of *T. gamsii* in maize seedling roots in South Africa.

Significant *Trichoderma* spp. isolate x line interactions observed for coleoptile length indicated that *T. gamsii* (13817) had a higher mean coleoptile length on maize line I 137 TN W which differed significantly from the same isolate on line V 0617 YZ. *Trichoderma* spp. have the ability to promote maize plant growth (Shoresh and Harman 2008; Vinale *et al*., 2008; Matarese *et al*., 2012). This and findings by Harman *et al*. (2004) and Hajieghrari (2010) that *Trichoderma* have a range of mechanisms to promote plant growth supports the finding's in this study, where there were significant differences in coleoptile and radical length. A significant interaction was observed when isolates were reisolated from maize lines planted in sterile or unsterile soils. Line I 137 TN W showed no reisolation differences between the sterilised and unsterilized soils while line V 0617 YZ had a higher percent reisolation from roots grown in the sterilised soil when compared to the unsterilised soil and this can be due to the absence of competing microflora in the soil (Windham *et al*., 1989; Harman *et al*., 2004; Woo *et al*., 2006; Hajieghrari, 2010). Findings in this study indicated that although there were differences between isolates they were still highly effective maize seedling root colonisers.

Previous studies conducted on maize seedlings generally used *Trichoderma harzianum* as a biocontrol agent (Shoresh and Harman 2008; Harman *et al*., 2004; Nzanza *et al*., 2011; Okoth *et al*., 2011) and studied their effect on the maize seedling emergence and root length and found that maize seeds treated with the biocontrol agents have a higher emergence rate, longer roots and greater seedling root mass. Hajieghari (2010) also conducted a study

with different *Trichoderma* spp. isolates and found that isolates of *T. harzianum* influenced the mass of maize seedlings. In this present study *Trichoderma* spp. isolates were compared to one another and the average maize seedling root mass were greater even if there were no significant differences between isolates. Observations during the present study also found that seedling roots from which the different *Trichoderma* spp. isolates were isolated from had no discolouration. This can be confirmed by Vinale *et al*. (2008) who conducted a study on *Trichoderma* plant pathogen interactions, that *Trichoderma* spp. in the soil control competitive pathogenic fungi by means of chemical mechanisms such as use of cell wall degrading enzymes, stimulating root growth and improved plant health.

3.6 Conclusion

Although the dominant distribution of *T. gamsii* has been suggested in Australia, North America and Europe, primarily from soil, *Eucalyptus nitens* stems and *Ricinus communis* stems (Jaklitch *et al*., 2006), the results of this study suggest that South Africa should be included as a biodiversity hotspot for this fungus and that *T. gamsii* should be considered as a first report on maize seedling roots. Based on information gathered during this study, *T. gamsii* is associated with maize seedling roots and proved to effectively colonise maize seedling roots and this association has been shown to be beneficial to the maize seedlings as *T. gamsii* significantly increased mesocotyl length. The application of different *Trichoderma gamsii* isolates to the soil seemed to have positively influenced maize seedlings of line I 137 TN W. Many *Trichoderma* spp. have been reported to be used as biocontrol agents, but in this study findings did not show the significance of *Trichoderma* spp. as growth enhancers or potential biocontrol agents. These *Trichoderma* spp. isolates need to be tested in soils that are highly conducive to maize seedling blights to establish their full potential in improving emergence and seedling growth or to act as biocontrol agents.

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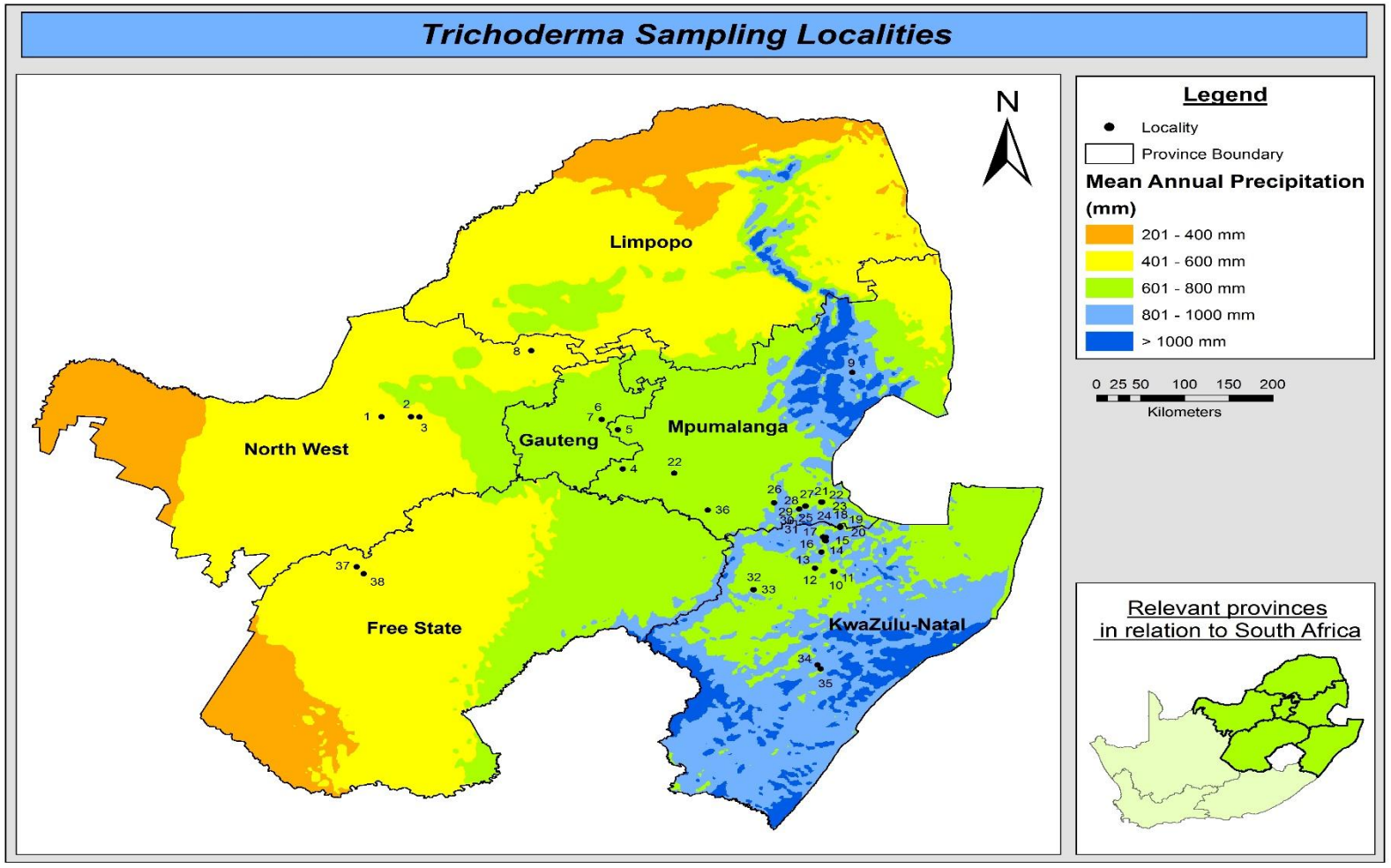

Figure 3.1: *Trichoderma* spp. isolate distribution from fields in the maize producing region of South Africa.

Table 3.1: Distribution of *Trichoderma* spp. isolates from maize seedlings collected from South Africa's maize producing region.

PPRI numbers	Locality	Morpho group	BLAST result (TrichoBLAST)	BLAST result (MycoBank)
13817	-26.512S 26.19619E	2	*Trichoderma gamsii*	*Trichoderma gamsii*
13818	-25.9894S 26.49728E	6	*T. afroharzianum*	*T. harzianum*
13819	-25.9929S 26.58508E	2	*T.gamsii*	*T.gamsii*
13820	-26.6342S 28.65953E	9	*T. asperellum*	*T. asperellum*
13821	-26.1501S 28.60908E	1	*T. gamsii*	*T. gamsii*
13823	-26.0244S 28.44283E	2	*T. gamsii*	*T. gamsii*
13824	-26.0244S 28.44283E	1	*T. gamsii*	*T. gamsii*
13825	-25.1749S 27.72367E	9	*T. asperellum*	*T. asperellum*
14667	-25.4426S 31.00222E	3	*T. asperellum*	*T. asperellum*
14668	-27.8955S 30.81569E	2	*T. gamsii*	*T. gamsii*
14669	-27.8962S 30.80911E	5	*T. harzianum*	*T. afro-harzianum*
14670	-27.858S 30.61989E	9	*T. harzianum*	*T. afro-harzianum*
14671	-27.6601S 30.68725E	2	*T. gamsii*	*T. gamsii*
14672	-27.5167S 30.722E	2	*T. gamsii*	*T. gamsii*
14673	-27.522S 30.73144E	1	*T. gamsii*	*T. gamsii*
14674	-27.4735S 30.70483E	1	*T. gamsii*	*T. gamsii*
14675	-27.4766S 30.73181E	4	*T. asperellum*	*T. asperelloides*
14676	-27.3553S 30.87703E	1	*T. gamsii*	*T. gamsii*
14677	-27.3463S30.87964E	9	*T. asperellum*	*T. asperelloides*
14678	-27.3454S 30.87781E	8	*T. gamsii*	*T. gamsii*
14679	-27.0431S 30.68533E	9	*T. asperellum*	*T. asperelloides*
14680	-26.6879S 29.18258E	6	*T. harzianum*	*T. afro-harzianum*
14681	-27.0424S 30.68986E	1	*T. gamsii*	*T. gamsii*
14682	-27.0423S 30.69033E	2	*T. gamsii*	*T. gamsii*

14683	-27.0429S 30.69444E	1	*T. asperellum*	*T. asperelloides*
16759	-27.0525S 30.20256E	2	*T. asperellum*	*T. asperellum*
16760	-27.0929S 30.52631E	3	*T. asperellum*	*T. asperellum*
16761	-27.0916S 30.52611E	2	*T. asperellum*	*T. asperellum*
16762	-27.0923S 30.52325E	1	*T. gamsii*	*T. gamsii*
16763	-27.0918S 30.52217E	7	*T. asperellum*	*T. asperellum*
16764	-27.1299S 30.45994E		*T. asperellum*	*T. asperellum*
16767	-28.1228S 29.99072E	1	*T. gamsii*	*T. gamsii*
16768	-28.1238S 29.99494E	7	*T. asperellum*	*T. asperellum*
16769	-29.0514S 30.64906E	3	*T. gamsii*	*T. gamsii*
16770	-29.1025S 30.67767E	7	*T. asperellum*	*T. asperellum*
17304	-27.8414S 25.94308E	1	*T. gamsii*	*T. gamsii*
17305	-27.9278S 26.01564E	7	*T. asperellum*	*T. asperellum*

Table 3.2: Results of the screening of maize seedlings against *Trichoderma* isolates in a glasshouse trial showing factorial analyses of variance for parameters evaluated (n = 8).

Effects	Df	F- values								
		Length (cm)				Mass (g)		%		
		Root	Coleoptile	Mesocotyl	Radical	Wet	Dry	Discolouration	Emergence	Reisolation
Isolate	10, 132	0.9	0.7	1.7	1.4	0.6	0.7	0.7	0.86	33.9***
Line	1, 132	0.4	0.4	1.3	3.6	0.3	0.0	0.5	0.3	0.6
Sterilised/Unsterilised soil	1, 132	0.2	2.9	3.1	4.6*	2.0	1.3	0.5	0.3	4.3*
Isolate x Line	10, 132	1.1	2.1*	1.2	2.3	0.8	1.2	0.6	0.9	0.2
Isolate x Sterilised/Unsterilised soil	10, 132	1.1	0.8	1.6	0.8	0.4	0.4	1.0	0.3	0.3
Line x Sterilised/Unsterilised soil	1, 132	1.6	2.4	0.5	0.2	0.0	0.0	0.5	0.0	4.3*
Isolate x Line x Sterilised/Unsterilised soil	10, 132	0.9	0.7	0.7	0.3	0.5	0.6	1.1	0.4	1.1
CV %		11.7	22.6	11.9	30.2	30.1	35.4	196.4	2.9	3.3

Significance is indicated by *; *P < 0.05, **P < 0.001, *** P < 0.0001

Table 3.2.1: The effect of *Trichoderma* spp. isolates and maize lines on average coleoptile length (cm) of seedlings planted in a glasshouse.

***Trichoderma* spp. PPRI isolates treatments**	**Mean coleoptile length (cm) ± SE (n = 8)**		**Average**
	Lines		
	I 137 TN W	**V 0617 YZ**	
***T. asperellum* (14675)**	1.5 ± 0.2 abcd	1.4 ± 0.2 abcd	1.5bcd
***T. asperellum* (14667)**	1.5 ± 0.2 abcd	0.9 ± 0.3 a	1.2ab
***T. asperellum* (14677)**	1.3 ± 0.1 abc	1.0 ± 0.3 ab	1.1a
***T. afro-harzianum* (13818)**	1.6 ± 0.3 abcd	1.0 ± 0.3 ab	1.3abc
***T. afro-harzianum* (14669)**	1.1 ± 0.3 ab	1.8 ± 0.4 bcd	1.4abc
***T. gamsii* (14671)**	1.3 ± 0.1 abc	1.1 ± 0.1 ab	1.2ab
***T. gamsii* (13823)**	1.4 ± 0.2 abcd	1.5 ± 0.2 abcd	1.4abc
***T. gamsii* (13824)**	1.7 ± 0.1 abcd	1.6 ± 0.2 abcd	1.7d
***T. gamsii* (14678)**	1.0 ± 0.3 ab	1.9 ± 0.4 cd	1.5bcd
***T. gamsii* (13817)**	2.1 ± 0.5 d	1.0 ± 0.3 ab	1.6d
Control	1.2 ± 0.2 abc	1.6 ± 0.4 abcd	1.5bcd
Average	1.4A	1.3B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 3.2.2.: The effect of maize lines and soil on average percentage reisolation of *Trichoderma* spp. isolates from the roots of maize seedlings planted in a glasshouse.

Line	Mean percentage reisolation ± SE (n = 8)		Average
	Soil		
	Sterilised	Unsterilised	
I 137 TN W	77.8 ± 4.8 b	77.8 ± 4.0 b	77.8a
V 0617 YZ	85.2 ± 4.3 a	74.4 ± 4.3 b	79.8b
Average	81.5A	76.1B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

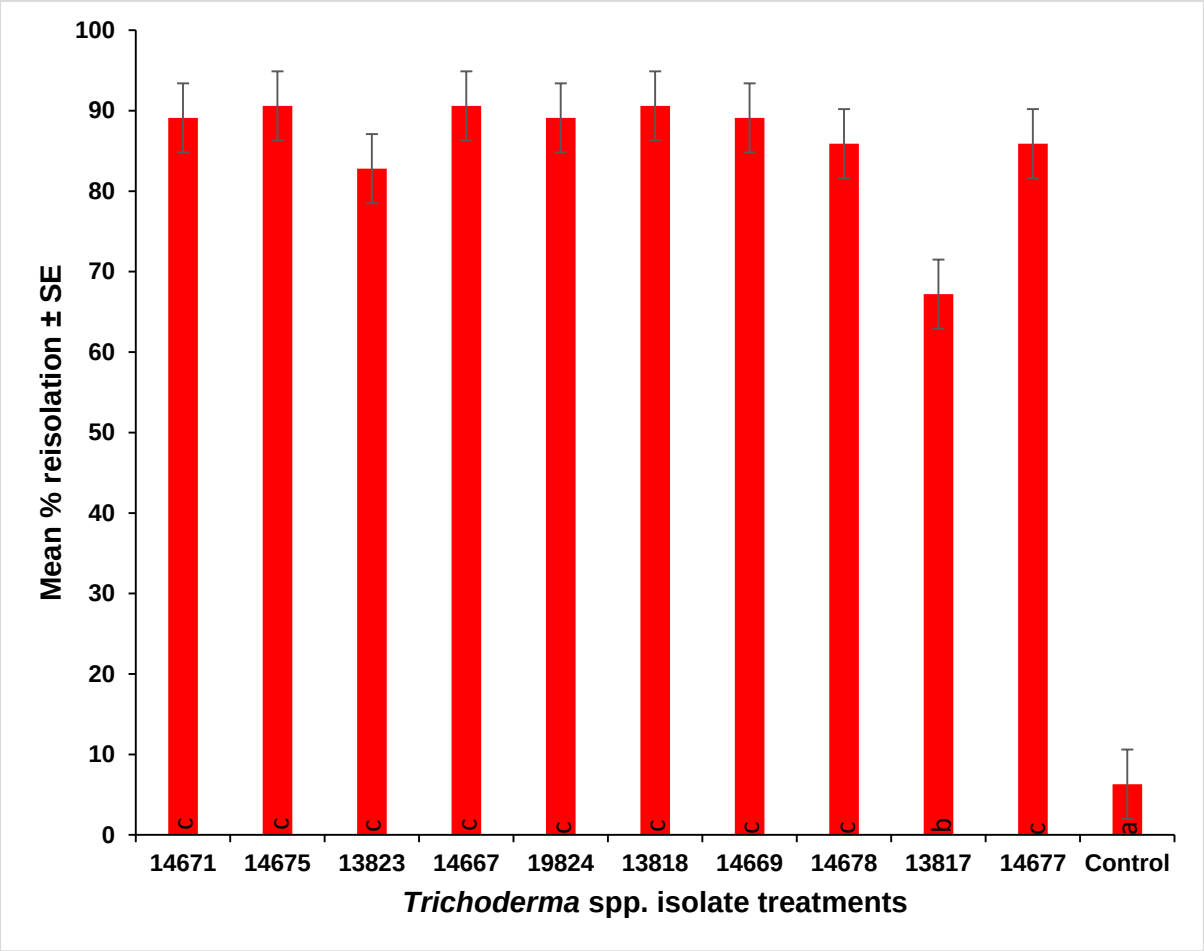

Figure 3.2: Mean percentage reisolation of *Trichoderma* spp. isolated from the roots of maize seedlings in a glasshouse trial.

Chapter 4

The efficacy of fungicide seed treatments for the control of maize seedling blights

4.1 Abstract

Seedling blight is a widespread disease and can be a significant problem in localised maize fields throughout the world. Seedling blight consists of a number of different seed- and soil-borne fungi acting as a disease complex which varies over time. It negatively affects crop stand and seedling vigour under favourable conditions for the disease to occur. The disease is most prevalent in cool soils with temperatures below 13 °C. Seedling blight of maize can be controlled effectively by fungicide seed treatment applications. For the past decade, the fungicide seed treatments N-trichtoromethylthio~cyclohexene–1,2–dicarboximide, fludioxonil/mefenoxam as well as tetramethylthiuram disulfide, were the only seed treatments registered for use on maize in South Africa. New fungicidal active ingredients are constantly developed and tested for efficacy against seedling blight. Nine field trials were planted with two maize hybrids namely; DKC 77 – 77 BR and DKC 80 – 30 R, in Potchefstroom (early and late season), Ermelo, Douglas, Jacobsdal, Vaalharts, Ventersdorp, Makhathini and Greytown in South Africa. Each trial consisted of five different seed treatments namely Celest, an experimental product applied at a single dose, an experimental product applied at a triple dose, an experimental combined fungicide product, Baytan and an untreated control which were replicated four times in a randomised complete block design. The aim of the study was to determine the efficacy of seed treatments for the control of maize seedling blight. There were significant interactions for locality x hybrid in terms of root length and locality x treatment for dry root mass ($P < 0.05$). Root discolouration differed significantly between the respective seed treatments ($P < 0.001$). The experimental product applied at a triple dose had the lowest mean average root discolouration but did not differ significantly from other seed treatments but did differ from the untreated control. The

dry mass of seedlings from fungicide treated seed was greater and root discolouration reduced compared to that of the untreated control treatment.

Key words: Maize seedling blight, disease complex, fungicide seed treatments

4.2 Introduction

Seedling blight of maize is globally a widespread disease and can be a major economic problem in localised areas (Dodd and White, 2002). The disease is caused by a complex of different fungal pathogens, which are soil- or seed borne and infect maize seedlings prior to or after emergence (Govender, 2008: Solorzano and Malvick, 2011). The incidence of seedling blight, that may continue to cause root rot of maize, is enhanced by various environmental factors such as strong winds, drought stress, shallow soil and tillage practices that result in soil compaction (Dodd, 1980). The disease is most prevalent when seeds are planted early in the season while soils are still cold and temperatures are below 13 °C (Dodd and White, 2002). Above-ground symptoms are poor maize plant stands and uneven seedling growth, with some seedlings that may be stunted and chlorotic (Dodd, 1980). Seeds can rot below the soil and die prior to emergence and the roots of seedlings can be discoloured with dark brown to black sunken lesions on the surface (Sumner and Bell, 1983).

Chemical control of fungal infections provides effective control of diseases, but the type of fungicide used on maize constantly changes due to new products being developed (Solorzano and Malvick, 2011). The ideal seed treatment should be highly effective against a wide variety of pathogens and should be safe to the seed and the environment (Saleem *et al*., 2012). Aveling *et al*. (2012) reported that commercially produced maize seed is almost universally treated with a fungicide prior to sale, to protect the seed from fungal infections after planting, which reduces the risk of seedling blight and seed borne diseases (Soloranzano and Malvick, 2011). However, to develop a seed treatment to control all the potential seedling blight pathogens is very complex, and the rate of fungicide application as well as efficacy against soil- and seed borne pathogens are made more complex due to differing environmental conditions (Saleem *et al*., 2012).

Various fungicides are registered for use on maize seed globally, but only a few are registered for use in South Africa (Van Zyl, 2011). In the period 2003 to 2011, no new

fungicides, except for the biocontrol agent, *Trichoderma harzianum* as a seed treatment, was registered in South Africa to add to the already existing N-trichtoromethylthio~cyclohexene–1, 2–dicarboximide, fludioxonil/mefenoxam as well as tetramethylthiuram disulfide (Van Zyl, 2011). The continued use of the same fungicide as a seed treatment may select for species with decreased sensitivity and may result in a shift in species composition on maize seedlings (Brodes *et al*., 2007). In the United States seeds are treated with formulation mixtures of fludioxonil and mefenoxam (Maxim XL), captab in combination with metalaxyl, captab with fludioxonil which are broad spectrum fungicides (Munkvold and O'Mara, 2002) which in combination increase the spectrum of pathogenic fungi that can be controlled.

The objective of this study was to determine the efficacy of fungicide seed treatments for control of seedling blights in selected maize fields in the maize producing region of South Africa.

4.3 Materials and methods

Trials were planted at nine localities identified in a previous survey, where seedling blight symptoms were the most prevalent (see Chapter 2). Details of the field trial localities and planting dates at each locality are provided in Table 4.1. Trial fields were prepared and maintained using normal farming practices for each region.

Each field trial was planted as a randomized complete block design with 4 replicates. Trials were planted with an inter-row spacing of 100 cm and an intra-row spacing of 30 cm, equivalent to ± 33 300 plants ha^{-1}. Each row was 5 m long. Seed of two maize hybrids, DKC 77 – 77 BR and DKC 80 – 30 R were treated prior to planting with five selected fungicides namely; Celest, experimental treatment (single dose), experimental treatment (triple dose), an experimental combined product, Baytan and an untreated control. Infections by fungi that occurred naturally in the soil were screened for efficacy of these treatments.

Seedlings were removed from the soil 35 days after planting and washed with distilled water until no soil particles were visible. Primary and secondary root and coleoptile discolouration were visually assessed and recorded using the root disease index (RDI). Disease ratings were based on an adjusted scale of 0 - 4 where 0 is no symptoms, 1 = 1-25 % rot, 2 = 26 – 49 % rot, 3 = 50 – 74 % rot, and 4 = 75 – 100 % rot (Soonthornpoct *et al*., 2000). The coleoptile, mesocotyl, radical and root lengths were measured and wet root mass were also quantified. The maize seedling roots were dried at 105 °C for 24 hours (Srobarova and Eged, 2005) and the root dry mass determined.

The data was analysed by means of a factorial analysis of variance (ANOVA) and significant differences were determined using Fisher's protected least significant difference (LSD) test using Statistica® 12th edition (StatSoft Inc., Tulsa, UK).

4.4 Results

There was a significant locality x fungicide seed treatment interaction on mean maize seedling dry root mass (Table 4.3.1). Potchefstroom early and late planted experiments, Ermelo and Ventersdorp had no significant maize seedling root dry mass differences over all treatments. The mean maize seedling root dry mass of the untreated control did not differ significantly except for Jacobsdal (irrigation), Vaalharts (irrigation) and Greytown which were significantly higher in mass. At Douglas (irrigation) there were no significant effects on mean maize seedling root dry mass for all fungicide seed treatments when compared to the untreated control. At Jacobsdal (irrigation) the experimental product at single dose fungicide seed treatments had significantly higher mean dry maize seedling root mass than all other fungicide seed treatments, except for Baytan (Table 4.3.1). In turn, the Baytan fungicide seed treatment had a significantly higher mean maize seedling dry root mass than Celest seed fungicide treatment and the untreated control but did not differ significantly from the other seed treatments tested at Jacobsdal. At Vaalharts (irrigation) the experimental fungicide seed treatments product at triple dose differed significantly from all other seed

fungicide treatments and the untreated control, except for the Baytan fungicide seed treatment. The maize seedling dry root mass of the Baytan fungicide seed treatment differed significantly from the other fungicide seed treatments and the untreated control but not from the experimental fungicide seed treatment product at single dose and triple dose (Table 4.3.1). At Greytown the experimental combined fungicide seed treatment product differed significantly from all other seed treatments as well as the untreated control but did not differ significantly from the Celest fungicide seed treatment (Table 4.3.1). At Makhathini the Baytan fungicide seed treatment significantly increased maize seedling dry root mass compared to the Celest fungicide seed treatment but did not differ from the experimental fungicide seed treatments and the untreated control (Table 4.3.1).

A significant locality x maize cultivar interaction was observed for mean maize seedling root length (Table 4.3.2). For cultivar DKC 77 – 77 BR, Douglas (irrigation) had the longest maize seedling mean root length compared to all other locality by cultivar interactions. Jacobsdal (irrigated) and Vaalharts (irrigated), although significantly lower than Douglas (irrigation), differed significantly from one another for DKC 77 – 77 BR but did not differ significantly from DKC 80 – 30 R which in turn did not differ significantly from DKC 80 – 30 R planted at Douglas. Potchefstroom early and late planted, Makhathini and Greytown localities did not differ significantly in terms of maize seedling root length for both hybrids DKC 77 – 77 BR and DKC 80 – 30 R. The mean maize seedling root length at Ermelo was only significantly greater than Ventersdorp, however, the hybrids DKC 77 – 77 BR and DKC 80 – 30 R did not differ significantly (Table 4.3.2).

A significant locality x cultivar interaction was obtained for maize seedling mean radical length (Table 4.3.3). For cultivar DKC 77 – 77 BR, Douglas (irrigation) had the longest seedling mean radical length compared to all other locality by cultivar interactions except for both hybrids, DKC 77 – 77 BR and DKC 80 - 30 R at Vaalharts (irrigation) (Table 4.3.3). DKC 77 – 77 BR at Jacobsdal, Vaalharts (irrigation) and DKC 80 – 30 R at Jacobsdal and Douglas (irrigation) did not differ significantly from each other in terms of mean radical

length, but were significantly shorter than Douglas DKC 77 – 77 BR. Potchefstroom early and late planted and Ermelo did not differ significantly regarding maize seedling mean radical length over localities and for both hybrids. Mean radical length of cultivar DKC 80 – 30 R at Makhathini and both hybrids at Greytown did not differ significantly from mean radical length in the Potchefstroom early and late planted trials and Ermelo trials.DKC 77 – 77 BR did not differ significantly from cultivar DKC 80 – 30 R planted at Makhathini but had a significantly lower mean seedling radical length than DKC 80 – 30 R planted early at Potchefstroom. Ventersdorp had the lowest seedling radical length for both hybrids when compared to all other localities (Table 4.3.3).

Mean coleoptile length of maize seedlings differed significantly over localities (Figure 4.1). Potchefstroom early season, Ermelo and Greytown had significantly longer maize seedling mean coleoptile lengths than all other localities. Mean seedling coleoptile length in the Vaalharts (irrigation) trial, was significantly shorter than in the Potchefstroom early season planted trial. Mean maize seedling coleoptile length in the Ermelo and Greytown trials did, however, not differ significantly from those of the Jacobsdal (irrigation) and Makhathini trials, which in turn did not differ significantly from Douglas (irrigation). Maize seedlings in the Ventersdorp trial had the lowest mean maize seedling coleoptile length followed by Potchefstroom late season which did not differ significantly from those in the Douglas (Irrigation) trial (Figure 4.1).

Mean mesocotyl length of seedlings differed significantly over localities (Figure 4.2). Maize seedlings planted at Potchefstroom early season and Greytown had significantly longer mesocotyls than all other localities, followed by Vaalharts (irrigation), Ermelo and Douglas. Mean maize seedling mesocotyl length at Ventersdorp was significantly shorter than at all other localities followed by Potchefstroom late season, Jacobsdal (irrigation) and Makhathini, which did not differ significantly.

There was a significant difference in seedling mean wet root mass over localities (Figure 4.3), with Vaalharts (irrigation) having the highest maize seedling mean root wet mass followed by Jacobsdal (irrigation) and Greytown which did not differ significantly from one another. Ventersdorp and Ermelo had the lowest significant seedling mean root wet mass over all localities, but did not differ significantly from the Potchefstroom late season trial. The seedling mean wet root mass from maize planted at Potchefstroom (late season) did not differ significantly from maize from Douglas and Makhathini but was significantly lower than in the Potchefstroom early season planted maize (Figure 4.3).

Percentage seedling root discolouration differed significantly over localities with Potchefstroom early season being significantly lower than Potchefstroom late season, Douglas (irrigation) and Ventersdorp but did not differ significantly from Ermelo, Jacobsdal (irrigation), Vaalharst (irrigation), Makhathini and Greytown. Ventersdorp had the highest mean percentage maize seedling root discolouration but did not differ significantly from Potchefstroom late season, Ermelo and Douglas (irrigation).

Percentage seedling root discolouration was significantly reduced by all fungicide seed treatments when compared to the untreated control (Figure 4.5). The seed treatments did not differ significantly from one another in terms of mean percentage maize seedling root discolouration.

Cultivar had a significant effect on coleoptile length, mean maize seedling root wet and dry mass (Table 4.3). The mean coleoptile length of DKC 80 – 30 R was significantly longer than DKC 77-77 BR ($F_{1,\ 324} = 4.8$; $P < 0.05$), mean maize seedling root wet mass of DKC 80 – 30 R was significantly greater than DKC 77 – 77 BR ($F_{1,\ 324} = 5.2$; $P < 0.05$) and similarly mean maize seedling root dry mass of DKC 80 – 30 R was significantly greater than DKC 77 – 77 BR ($F_{1,\ 324} = 8.2$; $P < 0.01$).

4.5 Discussion

The efficacy of fungicide seed treatments for control of maize seedling blight were evaluated on two maize hybrids, at nine localities using various plant parameters as indicators. The trials were located at various sites representing the different environmental conditions found in the South African maize producing region. The effect of locality was highly significant for all seedling parameters, which include root length, coleoptile length, mesocotyl length, radical length, wet and dry masses of maize seedling roots as well as percentage root discolouration. The pathogenicity (aggressiveness) of the different seedling blight pathogens can vary under different environmental conditions (Munkvold and O'Mara, 2002). There was a significant locality x hybrid interaction observed for maize seedling root length and for radical lengths. Maize hybrids varied in root and radical length at the different localities, this was also observed by McLaren (2002) which screened different sorghum hybrids for resistance to root rot diseases. McLaren (2002) concluded that different localities have different environmental conditions and these different environment conditions influenced root volume and length of the sorghum hybrids. The variation in root and radical length can also be influenced by different agricultural practices, rainfall and soil pH levels (McLaren, 2002). Temperatures during the Ermelo trial were generally low when compared to other localities such as Douglas and Vaalharts. The Douglas trial was regularly irrigated and had higher temperature and in turn also showed that seedlings root and radical lengths were longer when compared to all other localities. This observation indicates that when seeds are planted in soils with higher temperatures they are more likely to repair their cell walls and fewer carbohydrates and proteins are leaked into the soil that stimulates spore growth (Dodd and White, 2000). There were also significant differences observed for the coleoptile and mesocotyl lengths at the different localities. The Ventersdorp locality had the shortest coleoptile and mesocotyl lengths when compared to all other localities. This can be due to the low rainfall observed over the experimental period. Furthermore, the short coleoptile and mesocotyl lengths can also be as a direct effect of the seedling blight disease complex.

Some pathogens involved in the seedling blight complex may show a wide range of pathogenicity under different field conditions (Brodes *et al.,* 2007). These pathogens can cause lesions on the roots of maize while other pathogens can influence coleoptile length, mesocotyl and radical of the maize plant. A combination of the seedling blight pathogens present in a specific soil and different sets of field conditions result in the significant differences found between hybrids and localities. These may be ascribed to the specific hybrid/s not being adapted to the specific environmental conditions where it was planted (Munkvold and O'Mara, 2002: Brodes *et al*., 2007).

The trials planted at the Jacobsdal and Vaalharts localities were regularly irrigated explaining the high wet mass of seedling roots compared to seedling roots from the other trials which were all dry land trials except Douglas which was also irrigated. The efficacy of fungicide seed treatments for control of maize seedling blight disease observed in this study is in accordance with that Solorzano and Malvick (2011); Taye *et al.* (2012) and Broders *et al.* (2007). Rainfall was generally low at all localities during the experimental period. Ermelo had a higher average rainfall when compared to all other localities, but also had a low maximum temperature and low minimum temperature when compared to other localities. The reduction in wet and dry masses of roots at the Ermelo localities can be due to the soil temperatures being low which is also in concordance to literature that low temperatures and high relative humidity increases the incidence and severity of the seedling blight disease complex (Obendorf, 1972; Dodd, 1980; Sharma *et al*., 1993; Dodd and White, 2000; Solorzano and Malvick, 2011 Soil). Results in this study showed that the average mean wet mass and dry mass of roots were significantly lower when compared to the Vaalharts locality and this can be, because the trail was regularly irrigated and the absence of rain did not influence the trial.

Solorzano and Malvick (2011) reported on the effect that different isolates had on maize seedlings treated with a selected seed treatment and found that soil inoculated with the different fungal isolates and seed treatments were an effective way to control fungal

pathogens and that these fungi did not influence seed emergence and seedling dry mass. Solorzano and Malvick (2011) also concluded that maize seedlings treated with a triple seed treatment dose did not have an effect on maize seedling emergence and dry mass. In this present study, the same effect was found but with different seed treatments. Percent seedling root discolouration was significantly reduced by all fungicide seed treatments tested when compared to the untreated control. The fungicide seed treatments did not differ from one another except when compared to the control all the treatments differed significantly. Mao *et al.* (1997) also concluded that fungicide seed treatments significantly decrease the severity of root rot when compared to the same seeds without fungicide seed treatments.

Root discolouration was also observed for the maize seedlings at the different localities. Ventersdorp showed a consistently short maize root, coleoptile, mesocotyl and radical lengths while there were low wet and dry maize seedling root masses recorded but the root discolouration was significantly greater at the Ventersdorp locality. This study emphasises the significance of environmental conditions on the seedling blight disease complex and its effect in maize production.

4.6 Conclusion

Maize seedling blight is a problem in localised areas throughout the world, but can be effectively controlled with fungicide seed treatments. Various seed treatments were evaluated at different localities in the maize producing area of South Africa for efficacy against the seedling blight complex. Results indicate that seed treatments do promote plant growth and help with the effective growth of roots when it was compared to the untreated control. It further indicated that fungicide seed treatments contribute to the control of root discolouration which is caused by seedling blight fungi found in the soil. The experimental product applied at a single dose as well as experimental combined product and Baytan proved to be the most effective seed treatments based on their average higher dry seedling root masses over the various localities compared to the control treatments. However, more

maize hybrids should be screened with different fungicides at different localities so that the correct fungicide can be applied to maize hybrids at a specific locality with its diverse environmental conditions to improve efficacy.

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Table 4.1 Localities and planting dates for field trials to screen fungicide seed treatments for efficacy to control maize seedling blight.

Nearest town	**Province**	**Co-ordinates**	**Planting date**
Potchefstroom	North-West	S 26°43’43.16; E27°04’47.71	15/10/2013
Potchefstroom	North-West	S 26°43’43.16; E 27°04’47.71	05/12/2013
Ermelo	Mpumalanga	S 26°31’32.0; E 29°57’07.2	20/11/2013
Douglas	Northern Cape	S 29°11’02.9; E 23°43’22.8	19/12/2013
Jacobsdal	Northern Cape	S 29°07’28.1; E 24°36’42.5	19/12/2013
Vaalharts irrigation scheme	Northern Cape	S 27°56’38.4; E 24°50’31.8	5/12/2013
Greytown	Kwa-Zulu Natal	S 29°08’05.2; E 30°37’29.7	11/12/2013
Ventersdorp	North West	S 26˚ 49’46.1; E 26˚ 60’20.0	06/12/2013
Makhathini flats	Kwa-Zulu Natal	S 27°23’57.2; E 32°11’00.8	17/12/2013

Table 4.2: Mean climatic conditions at the trial sites for the respective planting dates to sampling of maize seedlings three weeks later.

Localities	**Daily maximum temperature (°C)**	**Daily minimum temperature (°C)**	**Daily rain fall (mm)**	**Total radiation (MJ/m2)**	**Total heat units**	**Mean relative humidity (%)**
Potchefstroom early season	28.3	12.1	2	22.4	10.6	49.2
Potchefstroom late season	28	15.7	6	26.2	11.33	62.5
Ermelo	23.2	12.2	8	17.22	6.53	75.4
Douglas	36.6	17	0.4	24.47	16.35	46.6
Jacobsdal	34.6	17.6	0.6	26.9	15.9	44.9
Vaalharts	32.9	16.6	2.7	26.08	14.2	51.1
Ventersdorp	28.9	15.2	2.7	22.5	11.41	59.9
Makhathini	30.9	19.9	5	26.6	14.6	70.8
Greytown	31.	16.1	2.8	22.1	12.3	63.52

Table 4.3 Results of the screening maize seedlings with different fungicide seed treatments in field trials showing the factorial analyses of variance for parameters evaluated (n = 4).

Effects	Df	F- values						
		Length (cm)				Mass (g)		%
		Root	Coleoptile	Mesocotyl	Radical	Wet	Dry	Discolouration
Locality	8, 324	186.5***	56.5***	47.2***	64.5***	110.1***	155.2***	2.5*
Hybrid	1, 324	0.0	4.8*	0.1	0.1	5.2*	8.2**	2.0
Treatment	5, 324	1.1	0.9	0.1	1.7	1.4	3.9**	41.9***
Locality x Hybrid	8, 324	2.1*	0.6	0.9	2.0*	1.0	0.9	0.9
Locality x Treatment	40, 324	0.7	0.5	0.8	1.0	0.5	1.5*	1.2
Hybrid x Treatment	5, 324	0.5	1.6	2.1	1.4	0.6	0.6	1.2
Locality x Hybrid x Treatment	40, 324	1.3	0.8	1.0	0.7	0.4	1.3	1.3
CV %		1	1.8	2.2	3.1	4.7	3.2	18.3

Significance is indicated by *; *P < 0.05, **P < 0.001, *** P < 0.0001

Table 4.3.1: The effect of locality x treatment interaction on average root dry mass (g) of maize seedlings.

Locality	Mean root dry mass (g) ± SE (n = 4)						Average
	Control	Celest	Experimental product at single dose	Experimental product at triple dose	Experimental combined product	Baytan	
Potchefstroom early planted	12.0 ± 4.0 abcd	10.3 ± 3.8 abc	15.3 ± 5.2 abcdef	6.3 ± 0.9 a	14.4 ± 2.2 abcde	14.6 ± 4.3 abcdef	12.2b
Potchefstroom late planted	11.6 ± 2.1 abc	14.2 ± 2.3 abcde	16.0 ± 1.0 abcdef	13.6 ± 2.2 abcde	13.1 ± 1.7 abcde	14.9 ± 2.7 abcdef	13.9bc
Ermelo	6.2 ± 0.5 a	6.6 ± 0.5 a	6.9 ± 0.5 a	7.4 ± 1.0 ab	6.7 ± 0.4 a	6.0 ± 0.4 a	6.7a
Douglas	16.1 ± 1.9 abcdef	13.3 ± 2.0 abcde	19.2 ± 3.0 cdef	18.5 ± 2.6 cdef	18.1 ± 3.6 bcdef	15.6 ± 2.4 abcdef	16.8cd
Jacobsdal	38.5 ± 2.9 ijk	33.3 ± 4.4 hij	55.8 ± 3.6 mn	41.6 ± 4.0 jkl	41.9 ± 6.4 jkl	45.5 ± 5.0 lm	42.8e
Vaalharts	58.6 ± 6.0 no	55.1 ± 4.0 mn	66.5 ± 3.6 op	77.5 ± 13.9 g	59.0 ± 7.4 no	69.9 ± 5.1 pq	64.4f
Ventersdorp	7.5 ± 2.3 ab	12.0 ± 2.0 abcd	12.0 ± 1.7 abcd	10.3 ± 2.3 abc	12.4 ± 0.9 abcd	8.6 ± 2.0 abc	10.5ab
Makhathini	15.9 ± 1.8 abcdef	14.0 ± 2.5 abcde	23.6 ± 2.3 efgh	14.9 ± 2.5 abcdef	22.5 ± 2.3 defg	25.1 ± 4.3 fgh	19.3d
Greytown	30.6 ± 3.5 ghi	42.3 ± 6.5 ijk	38.4 ± 4.3 ijk	38.1 ± 3.2 ijk	52.1 ± 6.5 lmn	37.3 ± 3.7 ijk	39.8e
Average	21.9A	22.4A	28.2B	25.4AB	26.7B	26.4B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 4.3.2: The effect of locality x hybrid interaction on mean root length (cm) of maize seedlings.

Locality	Mean root length (cm) ± SE (n = 4)		Average
	Hybrid		
	DKC 77 -77 BR	DKC 80 – 30 R	
Potchefstroom early planted	13.4 ± 0.4 c	13.9 ± 0.2 c	13.6a
Potchefstroom late planted	14.3 ± 0.5 c	13.6 ± 0.4 c	13.9ab
Ermelo	11.3 ± 0.3 b	11.6 ± 0.3 b	11.5a
Douglas	24.1 ± 0.7 f	21.7 ± 1.0 de	22.9d
Jacobsdal	22.2 ± 0.4 e	21.4 ± 0.6 de	21.8cd
Vaalharts	20.6 ± 0.4 d	21.4 ± 0.5 de	21.0e
Ventersdorp	5.8 ± 0.7 a	7.2 ± 0.6 a	6.5f
Makhathini	14.8 ± 0.7 c	14.8 ± 0.7 c	14.8b
Greytown	13.8 ± 0.5 c	14.5 ± 0.4 c	14.1ab
Average	15.6A	15.6A	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 4.3.3: The effect of locality x hybrid interactions on mean radical length (mm) of maize seedlings.

Locality	Mean radical length (cm) ± SE (n = 4)		Average
	Hybrid		
	DKC 77 – 77 BR	DKC 80 – 30 R	
Potchefstroom early planted	9.3 ± 0.3 bcd	10.1 ± 0.2 cde	9.7cd
Potchefstroom late planted	9.5 ± 0.3 bcd	9.0 ± 0.3 bc	9.3cd
Ermelo	9.1 ± 0.3 bcd	9.1 ± 0.2 bcd	9.1cd
Douglas	13.3 ± 0.8 i	11.4 ± 0.7 fgh	12.3a
Jacobsdal	11.4 ± 0.4 fgh	11.0 ± 0.4 efg	11.2b
Vaalharts	12.1 ± 0.6 ghi	12.4 ± 0.7 hi	12.2a
Ventersdorp	3.2 ± 0.4 a	4.1 ± 0.4 a	3.7e
Makhathini	8.5 ± 0.5 b	9.1 ± 0.5 bcd	8.8d
Greytown	9.5 ± 0.3 bcd	10.4 ± 0.2 def	9.9c
Average	9.5A	9.6A	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

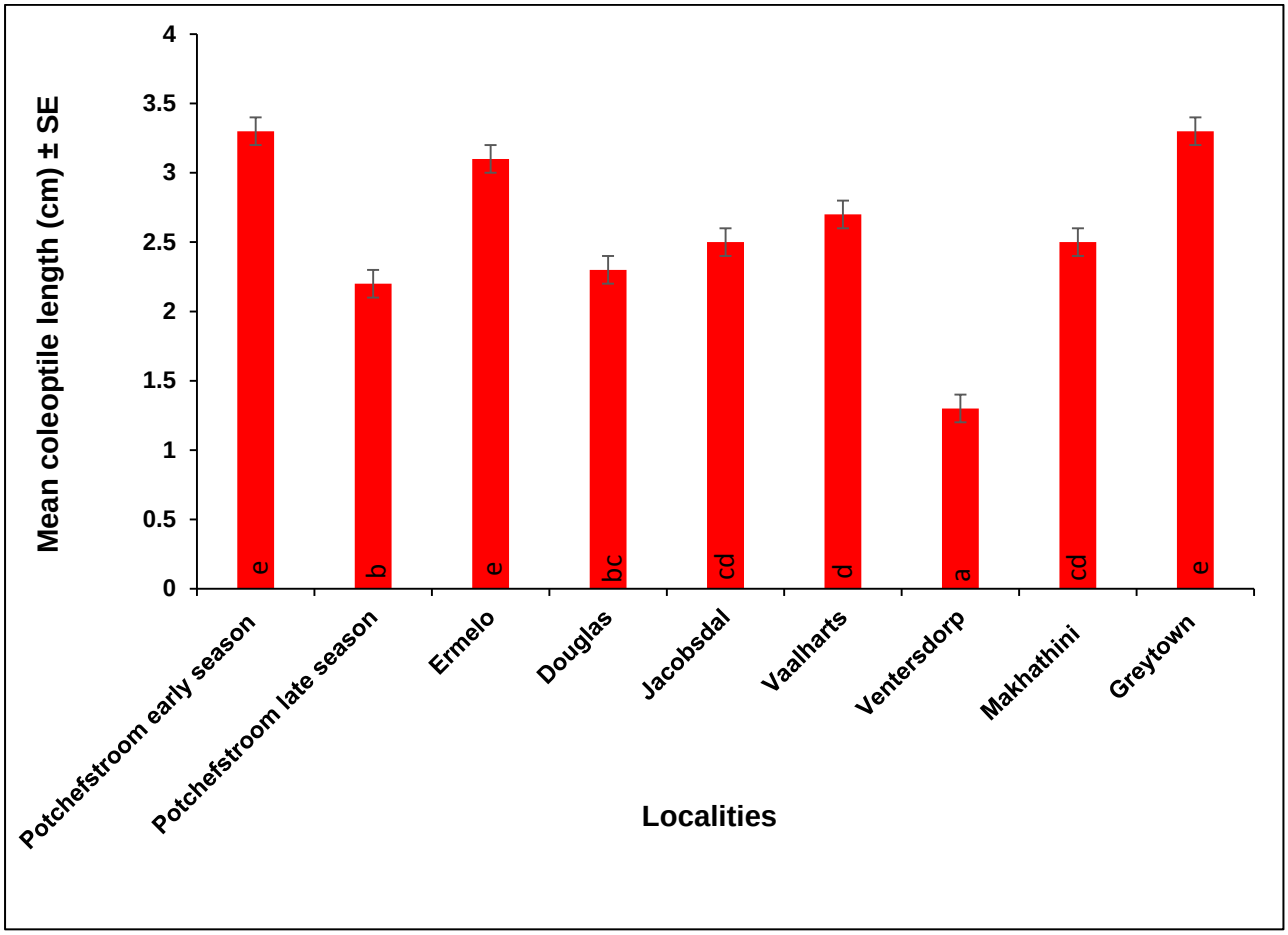

Figure 4.1: Mean coleoptile length of maize seedlings planted at different trial localities.

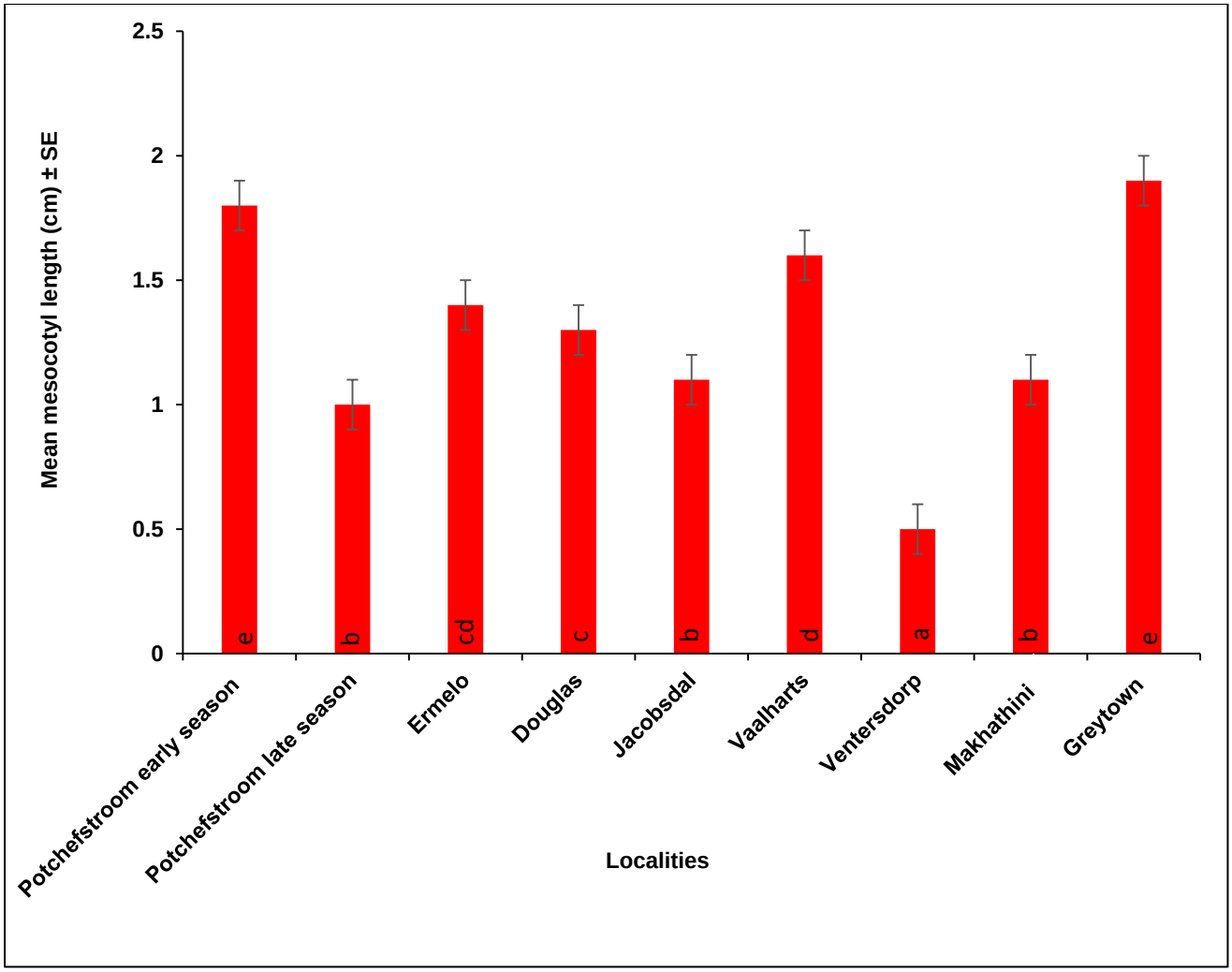

Figure 4.2: Mean mesocotyl length of maize seedlings planted at different trial localities.

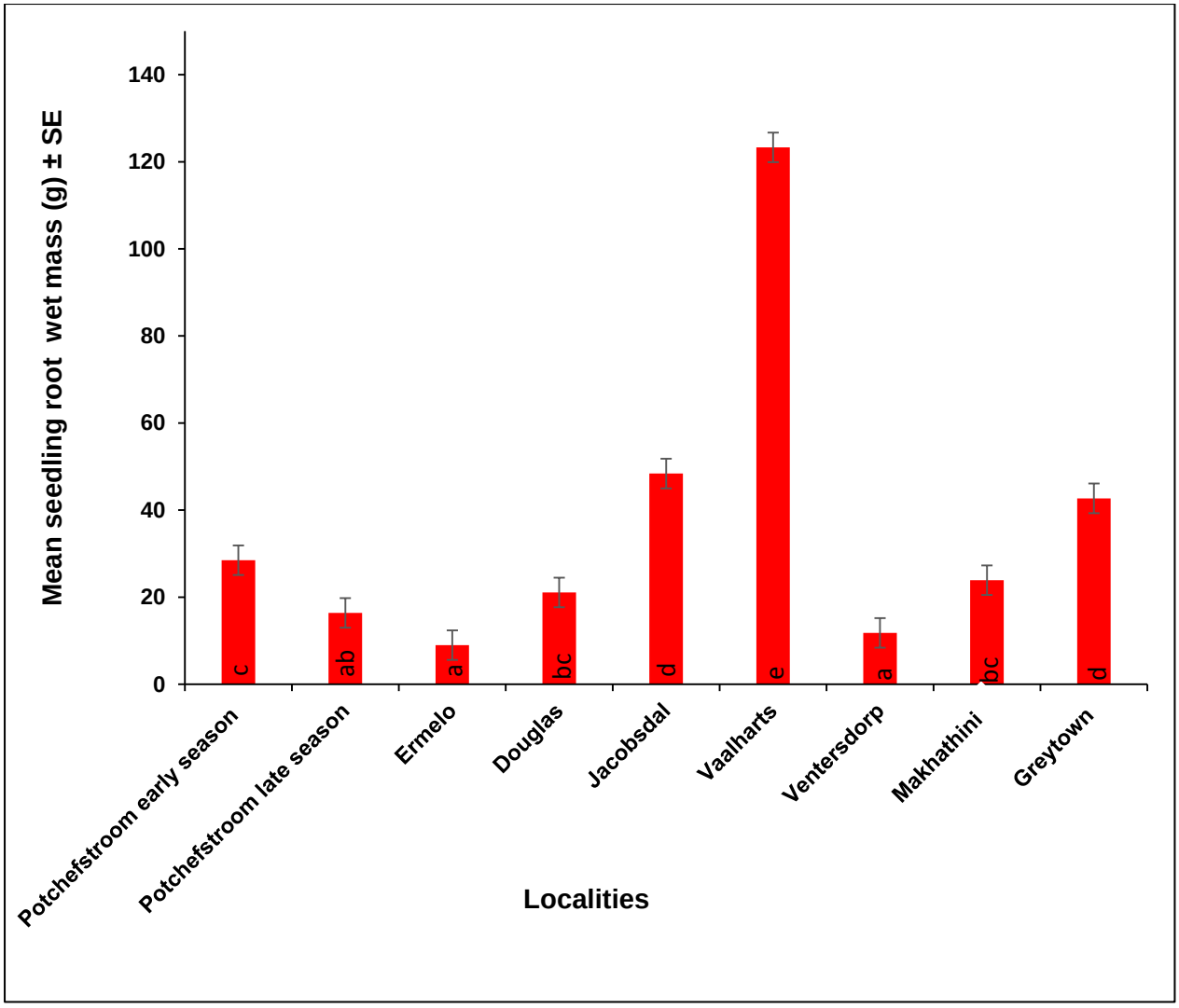

Figure 4.3: Mean root wet mass of maize seedlings planted at different trial localities.

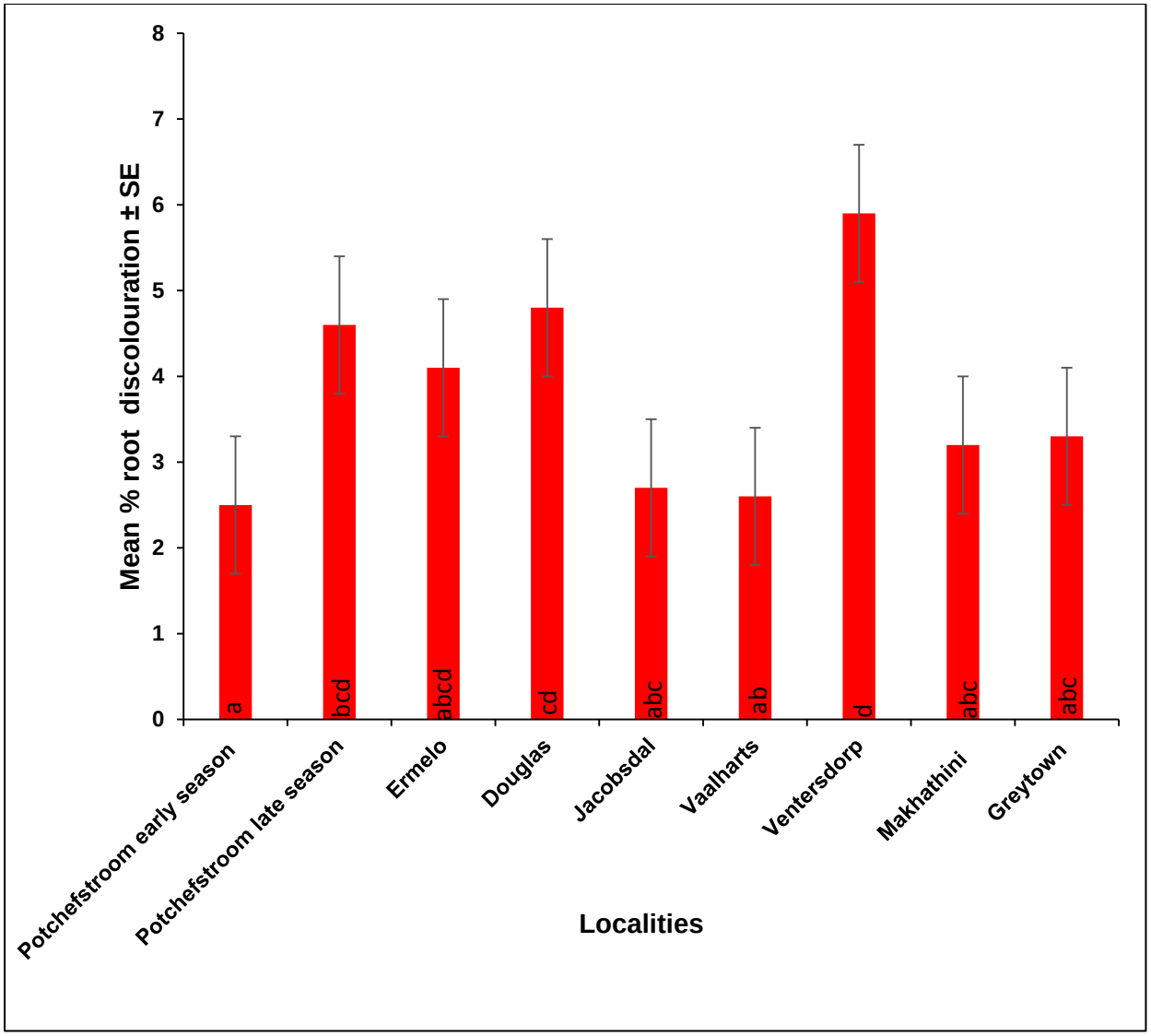

Figure 4.4: Percentage discolouration of maize seedling roots planted at different trial localities.

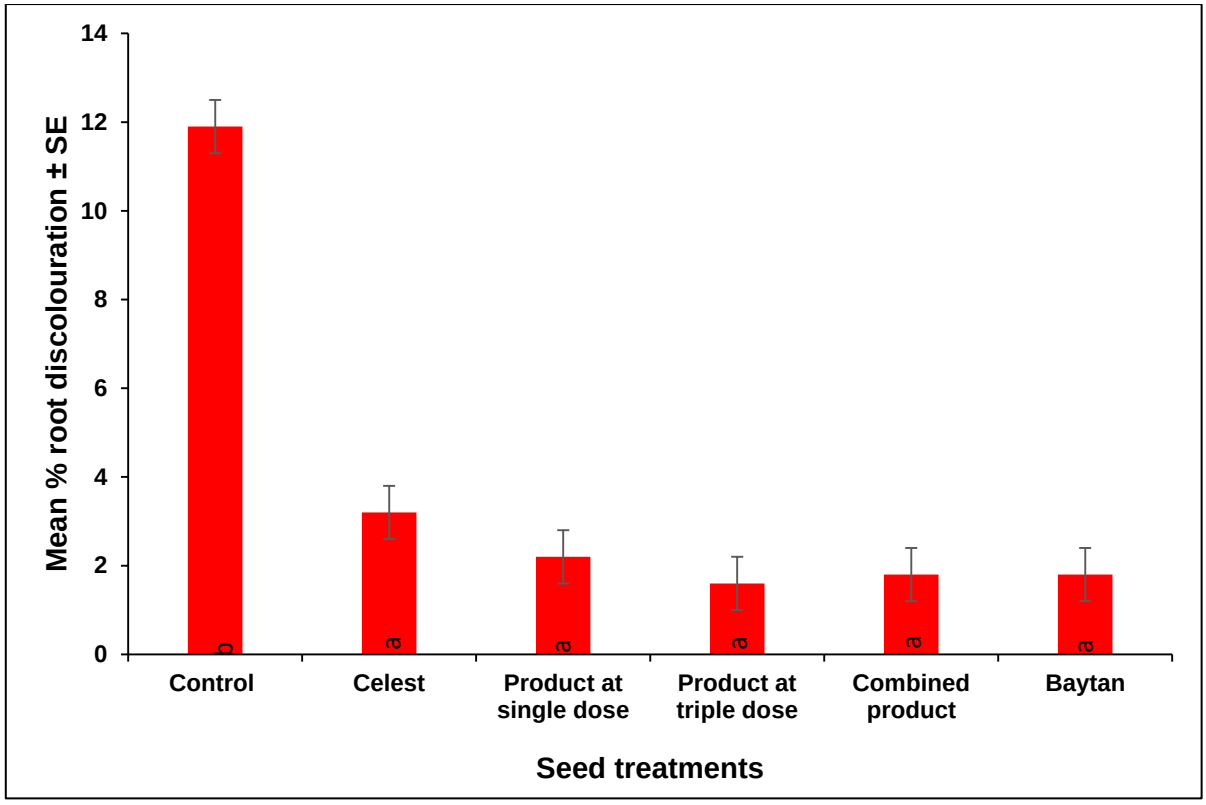

Figure 4.5: The effect of different fungicide seed treatments on mean percentage root discolouration of maize seedling.

Chapter 5

The efficacy of fungicide seed treatments for the control of cob and tassel smut on maize

5.1 Abstract

New fungicide seed treatments are constantly introduced on to the market to protect maize seedlings against soil-borne diseases such as cob and tassel smut caused by *Sphacelotheca reiliana* and various other fungi. Cob and tassel smut reached epidemic proportions in the 1970's and with effective control measures the problem was reduced until it had minimal economic impact. In 2007 an epidemic broke out in the Standerton area and over the last five to six seasons it has spread to various other maize producing areas of South Africa which include Harrismith, Delmas, Ermelo and Middelburg as well as the Underberg/Swartberg area. The primary reason for these outbreaks is the introduction of USA susceptible corn belt hybrids and weather conditions that have been favourable for infection and systemic growth of the pathogen. The first visible signs of infection on the maize plant only appear when ears and tassels are formed late in the season. New fungicide seed treatments were screened for their efficacy against cob and tassel smut maize seedling infections. Five different fungicide seed treatments namely Celest, an experimental treatment applied at a single dose, an experimental treatment applied at a triple dose, an experimental combined fungicide product, Baytan and an untreated control were screened on two maize hybrids DKC 77 – 77 BR and DKC 80 – 30 R at two localities, namely Potchefstroom and Greytown during the 2012/13 and 2013/14 seasons. Trials were planted in a randomised complete block design and replicated four times. Significant season x locality interactions were observed for percentage emergence, percentage infection and percentage yield loss. Treatments showed significant differences for percentage plants infected as well as percentage yield loss. Significant hybrid differences were also observed between seasons and localities with DKC 77 – 77 BR having a higher percentage infection

and yield loss than DKC 80 – 30 R. Baytan controlled head and tassel smut significantly when compared to the untreated control, but did not differ significantly from Celest and the experimental combined fungicide product but also showed the lowest mean yield loss for both hybrids and seasons.

Key words: *Sphacelotheca reiliana*, fungicides, seasons, localities, hybrids

5.2 Introduction

Maize is South Africa's main staple food source and provides food for millions of people with 2.68 million ha under maize production yielding 13.029 million tons during the 2013/14 season (SAGIS, 2014). Fungi play an important role in the cycling of nutrients in maize soils but some soil-borne phytopathogenic fungi are responsible for extremely destructive diseases (Pane *et al.*, 2013). Cob and tassel smut caused by *Sphacelotheca reiliana* (*Ustilago reiliana*) is a disease of maize and sorghum that was first described in 1875 from a sorghum sample from Egypt in 1868 (Reed *et al*., 1927). Cob and tassel smut were first recorded in 1890 on maize from the corn belt region in the United States, which spread to all maize producing areas worldwide (Reed *et al*., 1927). The disease was first reported in South Africa in 1955 near Bloemfontein in the Free State province (Le Roux, 1974). In the 1970's, this disease became a major problem in both seed and commercial maize producing areas in South Africa (Le Roux, 1974). Cob and tassel smut is a devastating disease that can cause up to 80 % yield loss throughout the maize producing areas of the world (Martinez *et al*., 2002). However, new resistant maize hybrids were introduced into South Africa, from the United States, and the cob and tassel smut epidemic declined (Martinez *et al*., 2002). Since 2007 until presently, cob and tassel smut disease started to cause epidemics in the Standerton area of Mpumalanga and has spread to other areas, namely Ermelo, Middelburg and Delmas in Mpumalanga, Harrismith in the Free State and Underberg/ Swartberg in KwaZulu/Natal (Dr. R. Kloppers personal communication).

The first visible signs of infection appear when cobs and tassels are formed late in the season (Ali and Baggett, 1990). The cobs and tassels of the maize plant are infested with black spore masses called smut-sori (Halisky, 1963). These black spore masses overwinter in the soil after the previous season's infections and remain viable for a number of years (Li *et al*., 2008). *Sphacelotheca reiliana* infects maize only via the roots when seedlings start to develop, they dissolve the epidermal cell wall to penetrate the maize seedling roots while they never develop an appressorium at the root surface (Martinez *et al*., 2002). These

spores grow systematically behind the growth point of the maize seedling and will continue growing until physiological maturity is reached by the maize plant (Li *et al*., 2008). In some cases the tassels will not be infected because *S. reiliana* is a systemic pathogen, but the cobs will be infected with a few smut free kernels still intact inside the cob (Halisky, 1963).

Most fungicide seed treatments target soil-borne and seed borne fungal infections and diseases (Ruden, 2013). Fungicides that are used as protectant seed treatments include, captan, fludioxonil, maneb and thiram, and are only effective on the surface of the maize seed. Treatments provide protection against seed surface-borne pathogens and to soil-borne pathogens (Ruden, 2013). Products associated with seed surface protection have a short residual period and control most types of soil-borne pathogens, with the exception of the root rotting organisms that are seed borne (Ruden, 2013). On the other hand systemic fungicide seed treatments are absorbed into the seedling as it emerges and these inhibit or kill the pathogens inside the plant tissue (Ruden, 2013).

The continued evaluation of different fungicide seed treatments can improve the control of infection caused by cob and tassel smut spores in the soil in an attempt to reduce epidemics. In South Africa fungicide seed treatments are available for cob and tassel smut namely N-trichtoromethylthio~cyclohexene–1, 2–dicarboximide, fludioxonil/mefenoxam as well as tetramethylthiuram disulphide (Van Zyl, 2011). New and old seed treatments are constantly being evaluated to determine their efficacy to control cob and tassel smut. In this study different seed treatments were evaluated to determine their efficacy against cob and tassel smut infection on susceptible maize hybrids.

5.3 Materials and Methods

Two field trials were planted in Potchefstroom at ARC-GCI, South Africa (S 26°43' 43.16 E 27°04'47.71) and Greytown, (Pannar Experimental farm) South Africa (S 29°08'05.2 E 30°37'29.7) during the 2012/13 and 2013/14 planting seasons, respectively. Fields were prepared at each locality using local maize production practices relevant to each locality. Trials were planted in a randomized complete block design with each plot/treatment row being five meters in length with one meter row spacing and an intra-row spacing of 30 cm between each seed sowed. Each seed treatment and the untreated control block were replicated four times. The treatments included; two maize hybrids DKC 77 – 77 BR, DKC 80 – 30 R and five different fungicide seed treatments, namely; Celest, experimental treatment at a single dose, experimental treatment at a triple dose, an experimental combined product and Baytan, and an untreated control. The inoculum for the trials was collected during the previous season's infections on screening trials at both localities. The smut-sori were stored in a cold room at 4 ^{o}C in glass jars that were sealed until used for the following season's inoculum. The inoculum was prepared by mixing 50 % sterile sandy soil with 50 % cob and tassel smut spores (Solorzano and Malvick, 2011). The inoculum was placed in brown 50 x 75 cm paper bags and sealed. In each of the inoculated rows, a five gram cob and tassel smut spore mixture was placed on top of each seed at planting, by hand, 2.5 cm into the soil (Matyac and Kommedahl, 1985). For each of the treatments there were two, five meter rows that were inoculated and two, five meter rows that were not inoculated. After one week emergence counts were done and percentage emergence obtained. The trials were rated at the end of the season when the plants reached physiological maturity. Infected cobs and tassels were counted for both the inoculated and the untreated treatments of each trial. Each cob was opened to visually identify the presence of black smut-sori as well as the tassels. Percentage emergence, percentage infection and yield loss were determined as follows:

$$\% \text{ Emergence} = \frac{\text{Seeds emerged}}{\text{Seeds planted}} \text{ X } 100$$

$$\% \text{ Infection} = \frac{\text{Infected plants}}{\text{Total plants in row}} \text{ X } 100$$

$$\% \text{ Yield loss} = \frac{\text{Control} - \text{Observed}}{\text{Control}} \text{ X } 100$$

The data was analysed by means of a factorial analysis of variance (ANOVA) followed by Fisher's protected least significant difference (LSD) test using Statistica® 12th edition (StatSoft Inc., Tulsa, UK)

5.4 Results

There was a significant season x cultivar x treatment interaction for percentage yield loss in the maize cob and tassel smut experiment (Table 5.1). The lowest percentage yield loss of maize infected by cob and tassel smut was observed in the 2012/13 season on cultivar DKC 77 – 77 BR. Baytan fungicide treatment which differed significantly from all other fungicide seed treatments over both cultivars and seasons except for season 2012/13 DKC 80 – 30 R Celest fungicide seed treatment, untreated control in 2013/14 on DKC 77 – 77 BR, experimental treatment at single and triple dose, experimental combined product and Baytan on cultivar DKC 80 – 30 R in season 2013/14 which all significantly reduced yield loss (Table 5.1.1). In 2012/13 on hybrid DKC 77 – 77 BR Celest, experimental treatment at single and triple dose, experimental combined product; 2012/13 DKC 80 – 30 R the untreated control, experimental treatment at single and triple dose, experimental combined fungicide product seed treatment and Baytan; in 2013/14 on hybrid 77 – 77 BR the untreated control, Celest, experimental fungicide seed treatment at single and triple dose, experimental fungicide seed treatment and Baytan; in 2013/14 on hybrid DKC 80 – 30 R the untreated control, Celest and

experimental fungicide seed treatment at single and triple dose did not differ significantly (Table 5.1.1). The untreated control on hybrid DKC 77 – 77 BR in 2012/13 had the highest significant yield loss caused by cob and tassel smut on maize.

The percentage seed emergence at Greytown for hybrid DKC 77 – 77 BR in 2012/13 and 2013/14 and hybrid DKC 80 – 30 R in 2013/14 was significantly higher than at Greytown for DKC 80 – 30 R in 2012/13 and both hybrids over both seasons at Potchefstroom (Table 5.1.2).

A significant season x locality interaction was observed for the percentage cob and tassel smut infected maize plants (Table 5.1.3). Cob and tassel smut infections at Potchefstroom did not differ significantly over the two seasons neither did they differ significantly from percentage cob and tassel smut infection at Greytown over both seasons. However, at Greytown the percentage cob and tassel smut infections were significantly higher in the 2012/13 season than in the 2013/14 season (Table 5.1.3).

Baytan and the experimental fungicide seed treatment at a single dose significantly reduced percentage cob and tassel smut infections on maize plants when compared to all other treatments. The experimental fungicide seed treatment at triple dose and experimental combined fungicide seed treatment had significantly higher percentage cob and tassel smut infection on maize than Baytan and the experimental fungicide seed treatment at single dose, however, it did not differ significantly from the Celest fungicide seed treatment but did differ significantly from the untreated control (Figure 5.1). Celest did not significantly reduce percentage cob and tassel smut infection on maize when compared to the untreated control (Figure 5.1).

Hybrid DKC 77 – 77 BR had a significantly higher percentage cob and tassel smut infection than DKC 80 – 30 R ($F_{1,\ 144} = 6.2$; $P < 0.05$) (Table 5.1). There was a significant locality effect on percentage yield loss caused by cob and tassel smut infections with Potchefstroom

having a significantly higher percentage yield loss than Greytown ($F_{1,\ 144} = 57.4$; $P < 0.001$) (Table 5.1).

5.5 Discussion

All commercial maize hybrids are treated with a fungicide seed treatment before planting. New fungicide seed treatments are constantly being evaluated and made available to the market. This study demonstrates the efficacy of different seed treatments on maize emergence, infection and yield loss caused by cob and tassel smut.

Emergence counts differed over seasons and localities indicating that environmental conditions play a major role in the development of cob and tassel smut epidemics as reported by Wang *et al*. (2007). This also explains the low emergence count observed in the Potchefstroom trials compared to the Greytown trials. Furthermore Li *et al*. (2008) and Wang *et al*. (2007) reported that most of the maize seedlings that do not emerge may be due to the direct influence of the cob and tassel smut inoculum applied onto the maize kernels. Govender (2008) studied different fungicide seed treatments and their influence on seed emergence and reported that seeds treated with fungicides had an increased emergence of 64.5 % when compared to untreated lettuce seeds. There was also no difference between the two seasons regarding emergence for the Potchefstroom trials. In contrast, the trials at Greytown showed a much higher emergence over both seasons. Although Greytown showed a higher emergence percent, its cob and tassel smut infection rate was greater than emergence levels at Potchefstroom during the 2012/13 season but a lower yield loss (13 %) was observed in Greytown than Potchefstroom (34.1 %). Furthermore maize seeds treated with fungicides have an higher emergence percentage and this explains why no significant differences were found for emergence counts of fungicide treated maize seeds (Jin and Tylkowska, 2005).

The two maize hybrids differed significantly from each other during seasons and localities, and can be ascribed to the relative genetic resistance where DKC 77 – 77 BR was shown to

be more susceptible to cob and tassel smut infection than DKC 80 – 30 R as indicated by the results of this study. The hybrids differed significantly regarding cob and tassel smut infection, which can be ascribed to the genetics of a hybrid planted in a given area and the environmental conditions prevailing at planting and during the vegetative growth stage of the maize plants. The combination of different seed treatments should reduce the infection of *S. reiliana* as reported by Bair *et al*. (1994). Cob and tassel smut infections were reduced by the experimental fungicide treatment applied at a single dose and Baytan, and differed significantly from the untreated control, confirming the findings of Solorzano and Malvick (2011) and Bair *et al*. (1994). The fungicide seed treatments, experimental product applied at a triple dose and the experimental combined fungicide product also significantly reduced cob and tassel infection levels, however Celest did not differ significantly, from the untreated control. This implies that the rate of cob and tassel smut infection can be influenced by environmental conditions at a specific locality and the inherent resistance of the hybrid planted (Wang *et al*., 2007). However Wright *et al*. (2006) conducted a fungicide seed treatment study on maize sweet corn and found that treatments like carboxin and thiram failed to reduce the severity of the disease. This implies that although the environment plays an important role in disease severity, the fungicide seed treatments in this study were not effective enough to reduce the infection at Greytown in the 2012/13 season. Cob and tassel smut infections appear to be directly related to yield loss. Different fungicide seed treatments resulted in variation in yield loss due to the efficacy of the treatments (Govender, 2008; Solarzano and Malvick, 2011). This also explains why some fungicide seed treatments have higher yield losses in comparison with others. Baytan showed the most consistent result for head and tassel smut control when compared to other seed treatments. Wright *et al*. (2006) also found that fungicides such as propiconazole and flutriafol + imazalil sulphate had a 70 % higher effective control than seeds treated with carboxin and thiram. This is in concurrence with findings in this study that different fungicide seed treatments have different effects on cob and tassel smut infection and yield loss.

5.6 Conclusion

To conclude it appears that Baytan gave the most consistent control of cob and tassel smut and reduced yield losses in both trials over both seasons. Hybrids were also shown to react differently to certain seed treatments and that the fungicide seed treatment x hybrid interaction needs to be confirmed by screening different seed treatments over a wider range of hybrids. The data also indicated that fungicide seed treatments improved seed emergence, where seeds were inoculated with cob and tassel smut spores compared to the untreated control, which directly influences yield.

5.7 References

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Table 5.1: Results of the screening maize hybrids with different fungicide seed treatments in field trials to control cob and tassel smut showing the factorial analyses of variance for parameters evaluated (n = 4)

Effects	Df	F – values		
		% Emergence	% Plants infected	% Yield loss
Season	1, 144	8.2*	4.0*	4.8*
Locality	1, 144	66.2***	0.1	57.4***
Hybrid	1, 144	1.6	6.2*	7.3*
Treatments	5, 144	0.9	6.4***	3.2*
Season x Locality	1, 144	1.6	4.4*	0.1
Season x Hybrid	1, 144	2.0	0.8	2.2
Locality x Hybrid	1, 144	3.7	0.0	1.5
Season x Treatment	5, 144	1.1	0.5	2.0
Locality x Treatment	5, 144	1.2	1.3	1.6
Hybrid x Treatment	5, 144	1.8	0.8	1.0
Season x Locality x Hybrid	1, 144	4.5*	1.2	3.8
Season x Locality x Treatment	5, 144	0.8	0.9	1.9
Season x Hybrid x Treatment	5, 144	0.2	1.1	5.0**
Locality x Hybrid x Treatment	5, 144	1.1	0.2	1.8
Season x Locality x Hybrid x Treatment	5, 144	0.7	0.1	1.7
CV %		3.7	22.9	21.8

Significance is indicated by *; *P < 0.05, **P < 0.001, *** P < 0.0001

Table 5.1.1: The effect of season, hybrid and treatment on the percentage yield loss caused by *Sphacelotheca. reiliana* on maize.

Season	Treatments	Hybrid		Average
		DKC 77 – 77 BR	DKC 80 – 30 R	
2012/13	**Untreated**	56.8 ± 7.8 g	23.0 ± 12.7 bcdef	39.9a
	Celest	35.5 ± 10.1 ef	5.8 ± 3.4 ab	20.7ab
	experimental treatment at single dose	41.8 ± 15.5 fg	20.4 ± 8.7 bcde	31.3a
	experimental treatment at triple dose	29.3 ± 9.3 def	32.8 ± 10.3 def	31.1a
	experimental combined product	29.3 ± 10.3 def	21.4 ± 7.6 bcde	25.3a
	Baytan	4.5 ± 4.1 a	19.3 ± 5.8 bcde	11.9b
2013/14	**Untreated**	18.9 ± 7. 9 abcde	30.7 ± 9.8 def	24.8a
	Celest	21.6 ± 1.7 bcde	30.8 ± 9.1 def	26.2a
	experimental treatment at single dose	24.8 ± 6.2 cdef	15.5 ± 4.7 abcd	20.1ab
	experimental treatment at triple dose	21.2 ± 8.1 bcde	14.9 ± 5.9 abcd	18.0ab
	experimental combined product	21.5 ± 5.7 bcde	9.8 ± 3.2 abc	15.7ab
	Baytan	24.3 ± 8.1 bcdef	10.3 ± 4.2 abc	17.3ab
Average		27.5A	19.6B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 5.1.2: The effect of season, hybrid and locality on the percentage emergence caused by *Sphacelotheca reiliana* infections on maize in field trials.

Season	Hybrid	Locality		Average
		Potchefstroom	Greytown	
2012/13	DKC 77 – 77 BR	70.2 ± 4.7 a	94.6 ± 0.9 b	82.4bc
	DKC 80 – 30 R	72.2 ± 5.4 a	78.9 ± 3.3 a	75.5a
2013/14	DKC 77 – 77 BR	74.4 ± 3.8 a	96.1 ± 1.2 b	85.2c
	DKC 80 – 30 R	74.3 ± 3.0 a	96.9 ± 0.6 b	85.6c
Average		72.8A	91.6B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

Table 5.1.3: The effect of season and locality on the percentage *Sphacelotheca reiliana* infected maize plants.

Season	Locality		Average
	Potchefstroom	Greytown	
2012/13	11.6 ± 2.2 ab	16.4 ± 2.5 b	14.0a
2013/14	11.9 ± 1.7 ab	8.5 ± 1.6 a	10.2b
Average	11.7A	12.4B	

Means within the same column followed by the same lower case letter do not differ significantly at P = 0.05 (LSD).

Means within the same row followed by the same upper case letter do not differ significantly at P = 0.05 (LSD).

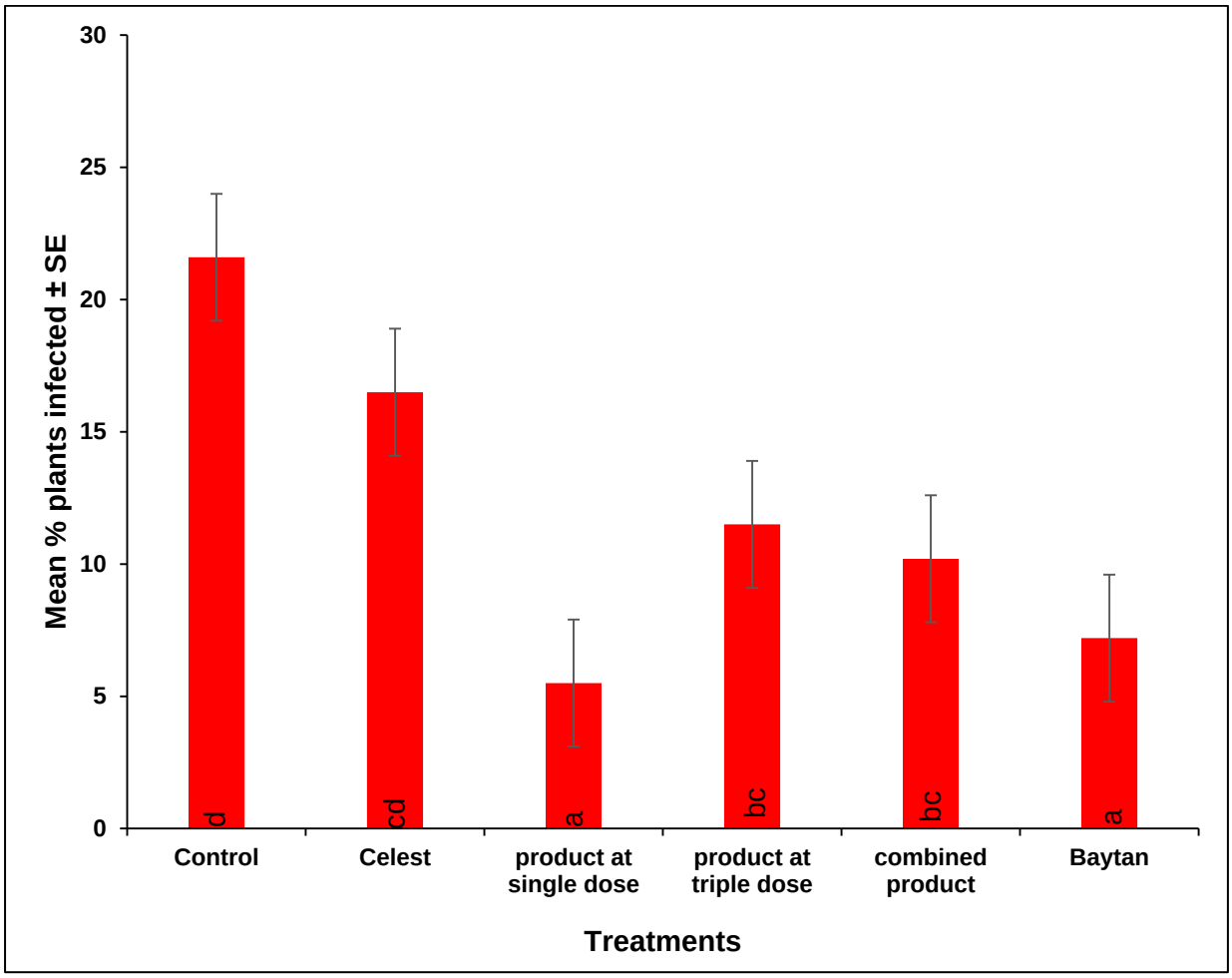

Figure 5.1: Mean percentage *Sphacelotheca reiliana* infected maize plants for the respective maize seed treatments.

Chapter 6

Conclusion

Seedling blight of maize has significantly influenced field crop stands and seedling vigour over various localised localities and seasons worldwide (Dodd, 1980). The seedling blight disease is caused by a range of environmental conditions, on farm practices and fungal pathogens. Pathogens, which include species such as *Pythium* spp., *Fusarium* spp. and *Rhizoctonia* spp., all contribute to the disease in which the disease can be seen as a complex due to different fungi found in the soil that can influence the plant under susceptible conditions (Berjak and Villiers, 1972). Maize (*Zea mays* L.) which serves as the staple food for millions of people is constantly being infected by various fungal pathogens at any given time. The aim of the first chapter was to give an overview of soil- and seed borne fungi and how these different fungi contribute to the seedling blight complex as well as cob and tassel smut which has become an epidemic in localised areas since 2007 in South Africa.

The aim of chapter 2 was to look at the status of maize seedling blight in South Africa. A total of 101 maize fields were surveyed throughout the maize producing region of South Africa to confirm the status of the disease complex. Results indicated that only 15 % of the maize seedling samples had more than 50 % root discolouration that severely influence seedling stand and vigour. This indicates that seedling blight is only a problem in localised areas of the maize producing region of South Africa. In this study results indicated that more than one fungal species can be associated with maize seedling roots, because of a diverse 70 different fungal species being isolated from seedling roots. The full influence of all the fungi on the seedling blight complex are not yet fully understand, only the most frequently isolated root fungi were used to determine their pathogenicity in glasshouse trials. These were *Aspergillus niger*, *Fusarium solani*, *Fusarium verticillioides* and *Fusarium oxysporum*. The results from the glasshouse trials imply that different isolates from different geographical

areas had different effects on maize seedlings, and influence maize lines differently. Overall, the study indicated that although various fungi were isolated from maize seedling roots, it does not mean that they are pathogenic towards the seedlings.

Trichoderma spp. was one of the fungal species commonly isolated, and the aim of chapter three was to determine the different *Trichoderma* spp. associated with maize seedling roots throughout South Africa. *Trichoderma* spp. are widely known for their biocontrol properties and plant growth promoting abilities and are there for advantageous fungi found in soils. In the survey 38 different maize seedlings were sampled and the most frequently isolated *Trichoderma* species were; *T. asperellum*, *T. afro-harzianum* and *T. gamsii*. *Trichoderma gamsii* was the most isolated spp. and were previously reported to occur in Australia, North America and Europe (Jaklitch *et al*., 2006). Isolates were screened in glasshouse trials to determine their potential plant enhancing abilities. *Trichoderma gamsii* proved to have plant growth enhancing abilities which suggests that South Africa should also be considered as a biodiversity hotspot for this fungus.

In South Africa only a few seed treatments are registered for the control of maize seedling blights, while new treatments are continually making their way onto the market. The aim of chapter four was to determine the efficacy of fungicide seed treatments for the control of maize seedling blight. Old and new treatments which included Celest, experimental product at a single dose, experimental product at a triple dose, experimental combined product and Baytan were screened in 9 field trials over four provinces. Results from this study indicated that these seed treatments are effective against maize seedling blights. The most effective being the experimental product at single dose as it had the highest dry mass percentage compared to the control and Baytan, which had the mean lowest percentage root discolouration observed, making seed treatment an important part of disease management.

Cob and tassel smut reached epidemics soon after it was found in Bloemfontein Free State in the 1970's. This epidemic was brought under control with adequate management

practices, which included seed treatments and hybrid resistance. The disease has since been under control but managed to reach epidemic proportions once again back in 2007 in localised areas in the maize producing area of South Africa. The aim of this study was to determine the efficacy of fungicide seed treatments for the control of cob and tassel smut. Five seed treatments were used; namely Celest, experimental product at a single dose, experimental product at a triple dose, experimental combined product and Baytan and compared to an untreated control. Four field trials were planted over two seasons and two localities which were Potchefstroom North-West Province and Greytown KwaZulu/Natal province. The seed treatment proved to be effective against inoculation with cob and tassel smut spores in comparison to the untreated control. Baytan had the mean lowest percentage yield loss over two seasons and localities making it an effective fungicide seed treatment against the cob and tassel smut disease caused by *Sphacelotheca reiliana*.

More isolates should be screened in the glasshouse to determine their influence on maize seedlings. As well as to determine if the species that were isolated from maize seedling roots are pathogenic and if so what their role in the seedling blight diseases complex are. The most pathogenic isolates should also be screened against fungicide seed treatment to determine the efficacy of seed treatments in glasshouse studies.

6.1 References

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