

**ETIOLOGY OF FUSARIUM CROWN AND ROOT
ROT OF GRAIN SORGHUM IN SOUTH AFRICA**

BY

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PREFACE

This thesis is a compilation of seven independent manuscripts including a literature review. Each of these chapters was prepared in manuscript format with a view to publishing them in scientific journals. Throughout the study the plant growth (shoot mass, root mass, emergence and root length) and disease impact (crown and root rot, colony forming units (CFU), area under disease progress curve (AUDPC), were used for assessing the main treatment effects.

The first chapter is a literature review of management of *Fusarium* soil borne diseases in graminaceous crops. Main topics discussed include the impact of *Fusarium* root rot pathogen on the host plant, isolation and identification of *Fusarium* spp., determining the pathogenicity of *Fusarium* spp., influence of soil characteristics on crown and root rot pathogen, genetic sources of resistance, crown and root rot disease management strategies, integrated crop management systems and a conclusion with suggestions for the use of a combination of management strategies in order to develop disease management system.

In chapter 2, the *Fusarium* spp. associated with crown and root rot disease of grain sorghum were determined. The occurrence and distribution of *Fusarium* spp. was established through the isolation of causal fungi from sorghum roots and field soils, from three areas / provinces in South Africa i.e, Cedara in Kwazulu-Natal, Bethlehem in the Free State province and Potchestroom in the North West province. *Fusarium* population densities in soils and sorghum roots were determined and *Fusarium* isolates recovered from field soils and sorghum roots were characterized and identified.

Chapter 3 investigated the effect of soilborne pathogens and *Fusarium* spp. on sorghum crown and root rot. The effect of soilborne pathogens on host plant growth and development was assessed. The effect of *Fusarium* spp. isolated from sorghum roots and field soils on pre- and post-emergent sorghum was assessed. The variation in pathogenicity of *Fusarium* spp. on sorghum genotypes was evaluated.

Chapter 4, assessed the resistance of sorghum genotypes to crown and root rot of *Fusarium* spp. Glasshouse inoculation techniques and resistance of sorghum genotypes to crown and root rot pathogen were evaluated. The standardization of the inoculum concentrations to incite crown and root rot in sorghum was studied.

Chapter 5 evaluated the resistance to *Fusarium* crown and root rot in selected sorghum genotypes. *Fusarium* spp. were tested for virulence on pre- and post-emergence damping-off of sorghum. Age related resistance, availability of sources of partial of resistance in sorghum genotypes under investigation and the existence of induced systemic resistance in sorghum in the presence of the pathogen and an inducer (antagonist) were evaluated.

Chapter 6 investigated the role of edaphic factors on *Fusarium* crown and root rot disease of sorghum. The study assessed the effect of soil characteristics such as soil type, soil moisture, soil fertility and pH and soil additives/amendments on *Fusarium* crown and root rot pathogen, development and survival on selected sorghum genotypes.

Commercially produced seeds are treated with fungicides to protect the seed from fungal infection by soilborne pathogens and limited the growth of seedborne fungal pathogen after planting. Chapter 7 examined the efficacy of seed treatment fungicides and bio-control agents on *Fusarium* crown and root rot of grain sorghum. Selected fungicides and a commercial bio-control agent were evaluated for their

efficacy on *Fusarium* crown and root rot pathogen of grain sorghum on selected sorghum genotypes.

The present study will hopefully contribute to a better understanding of crown and root rot severity, the factors that favour disease development and their effect on sorghum. Due to the fact that each chapter of this thesis is independent repetition has been unavoidable especially in introductory remarks and references.

GENERAL INTRODUCTION

Grain sorghum [*Sorghum bicolor* (L.) Moench] may be the world's fifth most important cereal, but it is the second most important feed grain in the United States and an important food source for the poor in many parts of the world. Since 1948-1950, the area planted to sorghum worldwide increased by 71%, and yields have increased by 160% (FAO and ICRISAT, 1996; Anonymous, 2000; FAO, 2002). Recent world sorghum statistics (1989-1999) have suggested yield increase rather than an increase in area under sorghum production has contributed to an annual gain of 5 million metric tonnes.

The major sorghum producers are the United States, Argentina, Mexico and Australia (Anonymous 2000; FAO 2002). In 2001, over 58.1 million tonnes of sorghum were harvested worldwide from about 42.6 million ha of land with an average yield of 1,364 kg /ha (FAO, 2002). In South Africa, sorghum is produced both by commercial and small-scale farmers mainly for traditional food and the production of opaque beer (Wenzel *et al.*, 1997).

Sorghum is a major cereal food for Africa, Asia and is used for livestock feed in developed countries (Tarr, 1962; Martin, 1970). Although it is used mainly for human consumption and animal feed, its utilization in alcohol production and especially in the brewing industry is increasing (Hall *et al.*, 2000). In the early 1980's global sorghum production for food and feed was estimated at 39% and 54% respectively (FAO and ICRISAT, 1996). Projections to 2005 suggested a 16 and 15% increase in utilization of sorghum for animal feed and food respectively. Because of its stress tolerance and performance stability sorghum, can be grown over more

diverse environments than maize. The yield advantage for maize normally occurs when available water exceeds 550-600 mm annually (Krieg *et al.*, 1990).

Soil is a medium for plant growth as well as a habitat for many *Fusarium* spp. which occur in a variety of soil types in many regions of the world (Burgess, 1981; Burgess *et al.*, 1988) and *Fusarium* spp. have been isolated from soil in temperate, tropical, desert, arctic and alpine regions of the world (Gordon 1954; Gordon 1960; Kommedahl, Windels and Lang 1975). The majority of *Fusarium* spp. are recovered near the soil surface from plant debris in cultivated or range soils (Smith 1967). *Fusarium* spp. are associated with plant roots, either as parasites or saprophytes and are known to exist as discrete propagules in the form of chlamydospores and resistant conidia in soil and soil debris (Burgess, 1981).

Fusarium thapsinum is a pathogen in tropical and temperate regions of the world. It incites seed rots, seedling blights and root and stalk rots of numerous crops including maize, millet, sudangrass and sorghum. Other *Fusarium* species reported to colonize root and stalk tissue of sorghum include *F. equiseti*, *F. graminearum*, *F. moniliforme*, *F. napiforme*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. semitectum*, *F. solani*, *F. subglutinans* and *F. tricinctum* (Clafin 2000). Root and stalk rots are some of the most prevalent diseases of sorghum (Reed *et al.*, 1983) and breeding for resistance to these diseases remains a research priority in most sorghum programs (Tuinstra *et al.*, 2002).

Etiology of the *Fusarium* root and stalk rot complex depends on host genotype, pathogen and environmental conditions. Disease incidence is sporadic from season to season within regions or at a single location. Variables such as soil type, fertility, drainage, cultural practices, temperature, moisture, insects other

diseases and host genotype resistance may singly or in combination influence disease severity (Claflin 2000).

Based on the above and a review of available literature, the objectives of this thesis were identified. The role of *Fusarium* spp. as a production constraint was established. Their distribution and population densities within the key areas was determined and the species were isolated, characterized and identified. Their pathogenicity on sorghum was quantified. Inoculation techniques and inoculum concentration for assessing resistance of sorghum genotypes to crown and root rot pathogen were determined. Genetic resistance of sorghum genotypes and pathogen variation were evaluated. The effects of soil characteristics on the development and severity of *Fusarium* crown and root rot were analysed. Finally the efficacy of seed treatment fungicides and bio-control agents in the control of crown and root rot of sorghum was investigated.

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CHAPTER 1

MANAGEMENT OF *FUSARIUM* SOILBORNE DISEASES IN GRAMINACEOUS CROPS

1.0 INTRODUCTION

Grain sorghum (*Sorghum bicolor* (L.) Moench) is a drought tolerant crop that is cultivated in arid and semi-arid tropics where climates are too dry and hot for other cereals. It is a major cereal food for Africa and Asia and is used for livestock feed in developed countries (Tarr, 1962; Martin, 1970). The total world sorghum production in 1981 was 72 million metric tonnes (FAO, 1981; FAO and ICRISAT, 1996) placing it fifth after wheat, maize (*Zea mays* L.), rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.). In sub-Saharan Africa, sorghum is the second most important cereal following maize (*Zea mays* L.) (FAO, 1995). The USA is the leading producer with 16 % of world production. Other major producing countries are India, Argentina, Mexico, Nigeria and Sudan (Tarr, 1962).

The population of sub-Saharan Africa has grown faster than the rate of food production since 1970. Consequently, per capita food production has declined by about 1.3% a year (FAO, 1991a). According to FAO (1988) the food deficit could be met by a 26% increase in the area cultivated and a 74% increase in yield per unit area through crop intensification. The latter category includes yield increases associated with diminished losses. In many regions of the world, including developed countries, the cultivation of dense stands of uniform, commercial hybrids or improved varieties of grain crops has encouraged disease outbreaks (Agricultural Statistics and Planning, 2002). In developing countries, where traditional methods using land race varieties of sorghum prevail, there is little evidence that pathogen variability has threatened production of traditional sorghum. This is due in part to methods of cultivation, sparse stands, intra-field crop mixtures and low levels of soil fertility (Agricultural Statistics and Planning, 2002).

Grain sorghum is very important to Botswana's national economy because of erratic climatic conditions and low soil fertility in the country. In 1997, the total area under grain sorghum production as a traditional crop was 154,000 ha, followed by maize (*Zea mays* L.) (117,000 ha), pulses (Legumes) (77,000 ha), millet (*Pennisetum* spp) (11,000 ha), sunflower (*Helianthus annus* L.) (1,000 ha), groundnuts (*Arachis hypogaea* L.) (5,000 ha), and other crops (3,000 ha) (Agricultural Statistics and Planning, 2002). The area of the crops harvested was recorded as follows: sorghum (64,000 ha), maize (*Zea mays* L.) (79,000 ha), millet (*Pennisetum* spp.) (8,000 ha), pulses (Legumes) (34,000 ha), sunflower (*Helianthus annus* L.) (1,000 ha), groundnuts

(*Arachis hypogaea* L.) (4,000 ha), and other crops (1,000 ha). These figures are indicative of a need to address the difference between the total area under crop production and total area harvested. Part of the solution may include encouraging healthy root systems that will enable plants to grow quickly and evenly, reducing the risk of water deficits during drought periods and increasing resilience to stress.

This review examines the impact of crown and root rots on sorghum including the causal organisms, their host range, predisposing factors, mechanism of infection and economic importance of the disease. Selection for environmentally stable resistance in several environments under a maximum array of agro-ecological conditions and soil characteristics is reviewed. This served as a basis for the development of effective disease management strategies.

2.0 IMPACT OF THE CAUSAL COMPLEX ON CROWN AND ROOT ROT.

2.1 Fusarium crown and root rot symptoms.

Fusarium spp. are soilborne pathogens of numerous agricultural crops and ornamental plants at both seedling and adult growth stages. They cause a variety of diseases including root, crown and stem rots, deterioration of subterranean storage organs such as bulbs, corms and tubers, vascular wilts and post-harvest decays (Booth, 1977; Farr *et al.*, 1989). *Fusarium* spp. are associated with sorghum from seed to senescence and can live in sorghum plants with no obvious symptoms. The absence of symptoms should however, not be equated with the absence of *Fusarium* spp. (Leslie, 2000). *Fusarium* spp. have been reported to infect sorghum rootlets where they remain latent in symptomless plants until maturity. Once plants reach maturity, necrosis of roots takes place inducing rotting, lodging, depriving the plant of water and resulting in plant death (Giorda, Martinez and Chulze, 1995).

Fusarium spp. causes lesions of variable sizes ranging from small, circular spots to elongated streaks. Root lesions may be purple, tan, or red depending on genotype. Infected sorghum seedlings display dark red discolouration of the cortex of roots often observed early in the season (Clafin, 2000). Lesions may be present in internal and external root tissues and the fungus may spread to stalk tissue during the growing season. Symptoms are more prevalent in tissue in which injuries or insect damage has occurred (Frezzi, 1975). Aerial symptoms include wilting, chlorosis and necrosis of

leaves and apices (Kvartskhava, 1957; Tamietti and Matta, 1989; Davis, Marshall and Valencia, 1993; Dutky and Wolkow, 1994; Holcomb and Reed, 1994). A damaged root system results in mortality due to reduced water and nutrient uptake in addition to loss of anchorage and lodging (Claflin and Giorda, 2002).

The most frequently observed symptom of the *Fusarium* crown and root rot syndrome involves yellowing of leaves, progressing to tan and subsequent withering. Roots display cortical decay distal to the crown and discolouration of the vascular stalk proximal to the crown. Crowns show pockets of necrosis that vary in size and overall plants are stunted and declining (Wang and Jeffers, 2000). *Fusarium* spp. affects plants by clogging the root system and depriving plants of soil water and nutrients (Frezzi, 1975). *Fusarium* stalk rot of sorghum reduces seed filling and causes lodging which is a serious problem under environmental conditions that favour disease development (Giorda, *et al.*, 1995).

Symptoms observed on host plants may vary among *Fusarium* isolates. Many isolates induce strong chlorotic symptoms while some produce a transient or less definitive response. Others consistently colonize the plant surface without inducing chlorosis. Symptom expression probably is related to environment and host genotype rather than the pathogen genotype present in a specific area (Katan, Shlevin and Katan, 1997).

2.2 *Fusarium* spp. associated with crown and root rot.

Fusarium spp. are the most important fungi associated with root and stalk rot of sorghum and these include *F. culmorum* Sacc., *F. equiseti* (Cda.) Sacc., *F. oxysporum* Schlecht, *F. scirpi* Lambotte & Fantr., *F. solani* (Mart.) Appel et Wr. and *F. tricinctum* Cda. However, pathogenicity has not been proven (Zummo, 1983). Most species belong to the section *Liseola* [teleomorph: *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura] (Giorda, *et al.*, 1995). Seeds and seedlings can be infected by several species of *Fusarium* particularly *F. Verticillioides*, (synonym *F. moniliforme* Sheldon) *F. proliferatum*, *F. subglutinans* (Wollenw. & Reinking) and *F. graminearum* (Schw.). These species can be seedborne (Kabeere, Hampton and Hill, 1997; Dodd and White, 1999) or survive in the soil or crop residues (Nyvall and Kommedahl, 1970).

Giorda *et al.* (1995) examined sorghum plants with root and stalk rot at harvest. *Fusarium* spp. were identified and classified using the characters suggested by (Nelson, Toussoun and Marasas, 1983). *Fusarium* spp. associated with stalk rot symptoms were *F. moniliforme* Sheldon emend. Snyder & Hansen, *F. proliferatum* Matsushima, *F. semitectum* Berk. & Rav. *F. scirpi* Lambotte & Faut, and *F. nygamai* Burgess & Trimboli. Disease symptoms are not usually expressed during vegetative growth of sorghum but rather under photosynthetic stress as the plants approach maturity (Giorda *et al.*, 1995). Lodging of plants normally occurs after anthesis and can occur from the crown at soil level up to the peduncle due to stalk infection. Glasshouse trials have demonstrated that *Fusarium* stalk rot of sorghum is associated with drought stress between flowering and mid-dough stages (Trimboli and Burgess, 1983).

Fusarium oxysporum is an important component of soil microflora throughout the world. All strains of *F. oxysporum* are saprophytic and able to grow and survive for long periods on organic matter in the soil. Some strains, are however, pathogenic (Nelson, Toussoun and Cook, 1981), being responsible for vascular wilt diseases on many plants of economic importance (Edel *et al.*, 1995).

2.3 Economic Importance.

Damage caused by *Fusarium* spp. can result in severe economic losses to sorghum growers. Giorda *et al.* (1995), Claflin (2000) and Claflin and Giorda (2002) suggested that losses caused by *Fusarium* spp. vary from 5-10% but may approach 100% in a localized area. They attributed yield reductions directly to poor filling of kernels and to weakened or lodged peduncles or indirectly to lodging and stalk breakage that hinder harvesting operations. Another form of loss is due to mouldy grain unfit for consumption (Claflin, 2000). Yield losses of up to 80% in sorghum fields in Argentina were reported (Frezzi, 1975; Maunder, 1984). Yield reductions in grain sorghum caused by *Fusarium* spp. depends on host growth and development, environmental conditions, edaphic factors (nutrition and drainage) and changes in crop production management systems. These may influence the distribution and composition of the pathogen population as well as the growth and development of the host plant (Giorda, *et al.*, 1995; Claflin and Giorda, 2002).

In addition to the damage to plants caused by *Fusarium* spp., some species produce toxic metabolites. For example *F. thapsinum* Klittich, Leslie Marasas produces moniliformin which is toxic to poultry (Kriek *et al.*, 1977). *F. Verticillioides* and *F. proliferatum* are the most prolific fumonisin producers. Among the fumonisins produced, fumonisin B₁ is known to cause leukoencephalomalacia in horses (Marasas *et al.*, 1988; Kellerman *et al.*, 1990), pulmonary edema in swine (Harrison *et al.*, 1990), liver cancer in rats (Gelderblom *et al.*, 1991) and oesophagal cancer in humans (Rheeder *et al.*, 1992). Furthermore, some species produce fusarins (Weibe and Bjeldanes, 1981), fusaric acid, (Bacon *et al.*, 1996) and gibberelic acid (Cerdeira-Olmedo, Fernandez and Avalo, 1994).

2.4 Host range.

Fusarium spp. have a wide host range and are known to occur in both temperate and tropical areas (Giorda, *et al.*, 1995; Clafin, 2000; Clafin and Giorda, 2002). *Fusarium* spp. incite seed rots, seedling blight, root and stalk rot, and grain mould of numerous crops including maize (*Zea mays* L.), millet (*Pennisetum* spp.), sudan grass, sorghum (*Sorghum bicolor* (L.) Moench) (Tarr, 1962; Partridge *et al.*, 1984; Frederiksen and Rosenow, 1986; Nirenberg *et al.*, 1998; Kulda, Tsai and Schardl, 1999), soybean (*Glycine maximum* (L.) Merr. and sunflower (*Helianthus annus* L.) (Sydenham *et al.*, 1993) and rice and sugarcane (Booth, 1971). *F. moniliforme* (*sensu lato*) (teleomorph *Gibberella fujikuroi*) is an important pathogen in a complex of several fungi associated with root and stalk rots of maize (*Zea mays* L.) and sorghum (Leslie *et al.*, 1990). In particular, *F. moniliforme* (*sensu lato*) is known to occur in at least 31 families of plants (Booth, 1971). The host range of *F. moniliforme sensu lato* was further extended to 11,000 species of plants (Bacon *et al.*, 1996). Strains of *Fusarium* spp. can cause an extraordinarily broad range of plant diseases. The most important are crown and root rots, stalk rots, head and grain blights and vascular wilt diseases (Nelson *et al.*, 1981; Summerell *et al.*, 2001). Lesser known diseases such as malformation disease in mango (Ploetz, 2001) and bakane disease in rice are also caused by *Fusarium* spp. and can have important local economic impact.

Host preference can be an important tool in identifying *Fusarium* spp. Isolates from maize for example, are usually *F. Verticillioides* while those from grain sorghum

are more commonly *F. thapsinum* (Leslie and Marasas, 2002). Grain sorghum has been found to be a host of several other *Fusarium* spp. including *F. andiyazi* (Marasas *et al.* 2001), *F. equiseti* (Cda.) Sacc, *F. graminearum*, *F. Verticillioides*, *F. napiforme* (Marasas *et al.*, 1987), *F. nygamai* Burgess and Trimboli, *F. oxysporum*, *F. proliferatum*, (Matsushima), *F. semitectum* Berk. and Rav., *F. solani* (Mart.) Appel & Wollenw. emend. Synyder & Hansen, *F. subglutinans* Wollenweber and Reinking, *F. thapsinum* Klittich *et al.* (1997) and *F. tricinctum* (Corda) Sacc (Leslie, 2000). Different mating populations of *Fusarium* spp. differ in the frequency with which they colonise maize and sorghum, in the mycotoxins they produce and their relative aggressiveness to sorghum (Leslie and Plattner, 1991).

2.5 Predisposing factors and infection mechanisms.

Predisposition of sorghum to *Fusarium* crown and root rot infection by insects, nematodes, and other diseases is economically important (Nelson *et al.*, 1983; Claflin and Giorda, 2002). Pathogens enter the roots and stalks through natural wounds or through injuries from machinery, insects or other causes. Infection results in plugged rootlets, preventing translocation of water and nutrients (Pande and Karunakar, 1992).

A positive correlation between longitudinal and transversal stalk rot of sorghum and green bug (*Schizaphis graminum* Rond) was recorded with higher correlations on later planting. These correlations indicate the importance of green bug in predisposing sorghum plants to *Fusarium* stalk rot (Giorda *et al.* 1995). Damage generally occurs between flowering and mid-dough stages. *Fusarium* stalk rot of sorghum is associated with drought stress between flowering and mid-dough stages (Trimboli and Burgess, 1983). Micro-nutrient deficiency predisposes plants to crown rot disease of wheat (*Triticum* spp. L) caused by *F. graminearum* (Grewal, Graham and Rengel, 1996).

A photosynthetic stress translocation balance concept was developed to explain the predisposition of maize to diplodia and gibberella stalk rot and of sorghum to charcoal rot and *Fusarium* stalk rot. According to this hypothesis, plants are predisposed to rot as the root cells senesce because of a reduction in carbohydrates required to maintain basic metabolic functions such as resistance (Dodd, 1980a, 1980b).

3.0 IMPACT OF ENVIRONMENTAL FACTORS ON CROWN AND ROOT ROT.

3.1 Influence of climate.

Temperature and humidity are the main climatic factors influencing development of *Fusarium* crown and root rot diseases of cereals. The influence of these climatic factors is not dependent on other environmental and host factors (Doohan, Brennan and Cooke, 2003). Interactions between environmental factors, host plant and pathogen population will determine the occurrence of disease. For example, *Fusarium* spp. found in the tropics are quite diverse in terms of numbers, distribution, host range, and virulence as compared to their counterparts in temperate regions (Gordon, 1960). The problem of *Fusarium* diseases in the tropics is compounded by high temperatures and humidity which often lead to more rapid colonization of infected plants and secondary infection by fungi and bacteria (Summerell, Salleh and Leslie, 2003).

The composition of fungal populations is likely to vary with time of year and the environmental conditions associated with a particular field. Yield losses from *Fusarium* depend on the growth stage of the host plant, environmental conditions and variables such as fertility, soil drainage, cultural practices, insects, nematodes and disease (Young and Kucharek, 1977; Windels and Kommedahl, 1984; Claflin and Giorda, 2002). *Fusarium* stalk rot of sorghum was observed in later growth stages under wet, cool conditions following hot dry weather (Odvody and Dunkle, 1979; Zummo, 1984). Avoidance of planting in cold wet soil has been shown to reduce *Fusarium* root rot. Low soil temperatures retard seed germination, providing the pathogen with a better chance to infect seed. The use of plump, heavy clean seeds results in strong seedlings which are more resistant to root rot pathogens and there is a need to avoid planting scabby seeds infected with *Fusarium*. Some seeds which appear normal on inspection may carry spores of the seedling blight fungi on or under the seed coat. These spores will germinate with the seed and infect new seedlings causing blight (Stack and McMullen, 1999).

Development of technologies that are more environmentally acceptable and contribute to soilborne disease management could be effective with a combination of control agents. Combination of methyl bromide, metham sodium and soil solarisation

were effective against *Fusarium oxysporum* f. sp. *bacilici*, *F. oxysporum* f. sp. *melonis*, *F. oxysporum* *racidis-lycopersici* and *F. oxysporum* f. sp. *vasifectum* (Eshel *et al.*, 2000).

3.2 Soil characteristics.

Soils harbour large populations of non-pathogenic *F. oxysporum* and both pathogenic and non-pathogenic strains are able to persist through saprophytic growth on organic matter in soil (Burgess, 1981). Soils naturally suppressive to Fusarium wilt of numerous crops caused by pathogenic formae speciales of *F. oxysporum* have been reported in several regions of the world (Toussoun, 1975; Alabouvette, 1986; Louvet, 1989; Couteaudier and Alabouvette, 1992). The physical characteristics of soil are said to be involved in its suppressiveness (Stotzky, 1966; Cook and Baker, 1983; Alabouvette, 1986; Amir and Alabouvette, 1993). Generally, wilt suppressive soils tend to have high clay and organic matter content, which support a larger, diverse population of antagonistic bacteria and actinomycetes (Toussoun, 1975; Scher and Baker, 1982; Alabouvette, 1986; Louvet, 1989; Couteaudier and Alabouvette, 1992).

Suppressive soils prevent disease development even though the pathogen and susceptible hosts are present. These soils have several characteristics in common, including their general physical characteristics (high pH, organic matter and clay content) (Toussoun, 1975; Scher and Baker, 1982). Suppression does not result in inhibition of chlamydospore germination or reduced saprophytic growth of the pathogen (Larkin, Hopkins and Martin, 1993). Because of these unique aspects, suppression may involve different organisms, mechanisms or interactions that differ from other wilt suppressive soils. Furthermore, biological components from this soil may be effective under soil conditions normally more conducive to disease development (Larkin *et al.*, 1993).

Nitrogen is essential in plants for the production of proteins, certain phytoalexins and also influences the amount of cellulose in cell walls and thus mechanical strength (Vos and Franking, 1997). Manipulation of nitrogen fertilization can increase host resistance, alter virulence or growth of the pathogen and induce biological control through micro-floral interactions or a combination of these factors. Nitrogen application can reduce severity of *F. graminearum* in wheat seedlings in pots

in the glasshouse (Rowaished, 1981). Free amino acids and proteins were higher than in untreated plants which suggest that N influences disease resistance by maintaining plant tissues in a juvenile stage (Rowaished, 1981). Maize stalk rot due to *F. graminearum* (*Gibberella zeae*), *F. moniliforme* (*G. fujikuroi*) and charcoal rot (*M. phaseolina*) was unaffected by nitrogen but stalk rot was found to increase significantly with plant density (Matinez and Senigagliesi, 1983). Adequate nitrogen and potassium levels appear to reduce the severity of Fusarium root rot but excessive levels can increase the severity of the disease in wheat and barley (Stack and McMullen, 1999).

Excessive nitrogen enhances vegetative growth which uses excess moisture resulting in increased moisture stress. An integrated system which includes pulses can overcome this problem by providing a dense ground cover thus reducing moisture extraction while adding nitrogen to the soil (Simpfendorfer, Wildermuth and Slatter, 2005). Sesame wilt caused by *F. oxysporum* f. sp. *sesami* under glasshouse and field conditions is less following the application of zinc and copper followed by cobalt and boron (Abd-El-Moneem, 1996). Peroxidase activity increased most following copper application and zinc, boron, cobalt, manganese, nickel and molybdenum in order of priority had the same effect (Abd-El-Moneem, 1996). Wheat genotypes that are efficient at extracting zinc are more resistant to crown and root rot caused by *F. graminearum* (Schw.) Group 1 (Grewal *et al.*, 1996).

A close relationship between *Fusarium* diseases and soil pH has been observed. Soils suppressive to *Fusarium* wilt have a higher pH than the non-suppressive soils (Kobayashi and Komada, 1995). *Fusarium* wilt of cucumber was almost completely suppressed at pH 8.0 while root rot of common bean (*Phaseolus vulgaris* L.) was suppressed at pH 4.0 (Fravel, Stosz and Larkin, 1995). Population densities of *Fusarium* f. sp. *erythroli* were significantly greater or tended to be greater in Hawaiian clay at pH 5.0 and Galestown gravel loamy sand at pH 5.8 when compared to Hatbalo loamy sand which had a pH of 4.4 and red clay subsoil with pH 4.4 (Fravel *et al.*, 1995). In sorghum, mesocotyl discoloration increased with decreasing pH while secondary root discoloration increased greatly at pH less than 4.6 (McLaren, 2004).

Osmotic or temperature stress can affect disease development by inducing or priming the host defence mechanisms prior to pathogen attack (Conrath, Pieterse and Maunch-Mani, 2002). Manipulation of soil moisture conditions in controlling *Fusarium* diseases, such as the use of soil solarization to adjust soil temperature and

moisture has been successfully applied for the control of numerous soilborne pathogens (Katan, 1981).

Fusarium infection may begin in the rootlets of sorghum under optimal soil moisture conditions and remain latent in symptomless plants until plant maturity when root necrosis occurs especially under environmental conditions that favour disease development (Frezzi, 1975). The incidence of maize stalk rot caused by *F. moniliforme* at the end of the season increased when field grown plants were exposed to mild water stress. The effect of moisture stress during pre-tassel, post-pollination and grain-filling stages of development increased maize stalk rot incidence to 60.3, 25.3 and 7.7 % respectively, while the non-stressed control was only 24.7 % (Schneider and Perdery, 1983).

Cool moist conditions following a period of hot, dry weather or stress conditions that occur from bloom until the hard dough stages of growth tend to favour disease development on sorghum (Claflin, 2000). Drought and hail affect the amount of stalk rot caused by *F. moniliforme* in corn (Christensen and Schneider, 1950; Littlefield, 1964; Palmer and Kommendahl, 1969). Preventative measures such as a proper irrigation schedule (Summer and Hook, 1985) can reduce stalk rot severity. *F. moniliforme* is known to survive at low water potential (Woods and Duniway, 1984) and *F. graminearum* is known to actively cause root rot at stress-related nitrogen levels (Leslie, 1986; 1987). Maintaining moisture and nitrogen at optimum levels may also reduce both pathogens to low levels (Woods and Duniway, 1984).

Infection and systemic colonization of roots by *G. fujikuroi* increase significantly following mild early season water stress (Schneider and Perdery, 1983). Grass weeds are alternate hosts of *F. pseudograminearum* and can facilitate pathogen survival. Grass weeds reduce water storage over summer and winter fallow and weed control during crop and fallow is thus critical (Simpfendorfer *et al.*, 2005). Yield loss to Fusarium crown rot is a function of both initial inoculum and water use of break crops. Crops such as chickpeas and field peas have shallow root depth and use less water, therefore crops following these break crops benefit from this water saving and suffer less moisture stress resulting in less crown rot infection (Simpfendorfer *et al.*, 2005).

4.0 GENETIC SOURCES OF RESISTANCE.

Sources of resistance to *Fusarium* crown and root rot exists in various crops in breeder lines, landraces, wild relatives and germplasm collections. They need to be exploited by means of conventional breeding or biotechnological techniques (Duncan and de Milliano, 1995). Breeding for quantitative resistance traits, may be accelerated by better methods of gene identification and transfer that will almost certainly involve biotechnology.

4.1 Host plant resistance.

Resistance is generally expressed as either morphological or physiological. Both play an important role in disease resistance in wheat and barley. Morphological resistance includes characteristics such as genotypes with long grain filling which are susceptible compared to tall genotypes that have rapid grain fill which contributes to resistance to *Fusarium* infection (Miedaner, 1997; Mesterhazy, 1995). Physiological resistance includes resistance to initial infection, resistance to colonization within the spike, kernel size and number retention, yield tolerance and the decomposition of mycotoxins (Mesterhazy, 1995).

Host plant resistance is the preferred method of control and is the backbone of integrated disease management strategies. For this reason major research efforts have focused on development of resistant sorghum cultivars (Bandyopadhyay *et al.*, 2000).

Characteristics associated with quantitative resistance to *Fusarium* stalk rot in sorghum include lodging resistance, non-senescence and green bug resistance (Giorda and Martinez, 1995). Non-senescence and lodging resistance are considered post-flowering expressions and are the most important plant characters related to stalk lodging and post floral drought resistance. However, moderate resistance was not related to known genes for resistance and appeared to be quantitative (Henzell *et al.*, 1984). The use of sorghum cultivars resistant to both biotic and abiotic stresses is generally considered the most effective and environmentally sound approach for addressing stalk rot diseases. Development of sorghum hybrids that are resistant to *Fusarium* stalk rot has been difficult because the disease is caused by a complex of pathogens and disease incidence is exacerbated by environmental stress (Bramel-Cox

et al., 1988). Fusarium stalk rot of sorghum was increased 13.5 fold in the presence of stress compared with 2.8% in the absence of stress (Mughogho and Pande, 1984).

Progress has been made in identifying sorghum genotypes resistant to Fusarium stalk rot (Reed, Partridge and Nordquist, 1983; Bramel–Cox *et al.*, 1988; Tefarra *et al.*, 2001). The best sources of Fusarium stalk rot resistance were identified in sorghum genotype SC599 and its hybrid derivatives (Bramel-Cox *et al.*, 1988; Tefarra *et al.*, 2001). Mean disease reactions among hybrids indicate a high degree of resistance to *F. proliferatum* infection on sorghum genotypes SC1158 and Tx2737, hybrid SC599 (Tesfaye, Claflin and Tuinstra, 2004).

Sources of genetic resistance have been identified to *M. phaseolina* and *F. thapsinum*, and resistance genes have been deployed in some commercial sorghum varieties and hybrids (Tuinstra *et al.*, 2002). A gene coding for a rice chitinase was incorporated into an elite sorghum inbred line Tx430 by a biolistic transformation protocol expressed in T2 and T3 generation plants. When T2 and T3 generation plants were inoculated with conidia of *F. thapsinum* the development of stalk rot symptoms was significantly reduced in transgenic plants with a higher expression of rice chitinase compared to plants with low chitinase (Zhu *et al.*, 1999). Commercial wheat durum and barley varieties vary in susceptibility to root rot caused by *Fusarium* spp., but none of the commercial varieties are immune to infection although some tend to have low levels. Many widely grown wheat varieties are highly susceptible (Stack and McMullen, 1999).

Host plant chemicals involved in disease resistance of sorghum genotypes to stalk and root rots have been investigated. Sorghum genotypes with higher sugar content had less infection caused by *M. phaseolina* and *F. moniliforme* (Clarke and Miller, 1980). Sorghum genotypes that are resistant to Fusarium stalk rot contain a greater variety and larger amounts of phenolic acids (Hahn, Faubion and Rooney, 1983; Waniska, Poe and Bandyopadhyay, 1989). Resistance to Fusarium wilt in maize was related to amino acid concentration. Resistant lines and hybrids have low concentrations of free amino acids compared to the high levels found in susceptible genotypes (Brad, Nguyen and Dobrewsin, 1973). The level of amino acids in the exudates of sorghum roots has been associated with stalk and root rot resistance. Many of the major amino acids identified in sorghum root exudates support the *in vitro* growth of *Fusarium* spp. isolated from the rhizosphere and rhizoplane (Odunfa, 1979). These

exudates might contribute to *Fusarium* nutrition resulting in the predominance of these fungi around the roots. Resistance to Fusarium wilt in maize is related to the concentration of amino acids with resistant lines and hybrids having smaller concentrations than susceptible ones (Brad *et al.*, 1973).

4.2 Age related resistance.

Screening for resistance can be done at all plant growth stages but in cases where cultivars differ greatly in terms of maturity, tests should be done at seedling, intermediate and maturity (adult) stages. Wheat seedling resistance to crown rot caused by *F. graminearum* Group 1 was correlated to adult resistance in a number cultivars (Klein *et al.*, 1985). In contrast, resistance to crown rot caused by *F. graminearum* in seedlings and mature wheat plants of the cultivar SST 107 differed significantly with a high degree of tolerance in the mature plant stage, but proved highly susceptible in the seedling stage (Van Wyk *et al.*, 1988). Large scale germplasm screening is critical for the identification of sorghum genotypes with high levels of resistance to crown and root rot. Mitter *et al.* (2006) suggested that the use of seedlings tests can be an efficient method for screening progeny in a breeding program.

Seedling vigour plays an important role in determining the effect of Fusarium root rot on adult resistance in spring wheat and barley plants. Large, heavy seeds produce the most vigorous plants and thereby reduce the effects of root/crown rot. Scabby seeds infected with *Fusarium* result in reduced stands and weaker plants less able to resist the effects of Fusarium crown and root rot at both the seedlings and adult growth stages (Stack and McMullen, 1999).

The performance of 16 wheat cultivars indicated a significant correlation between seedling bioassay and field rankings suggesting that field resistance to Fusarium crown rot in adult plants can be detected using a seedling bioassay (Mitter *et al.*, 2006). Adult plant resistance to Fusarium crown rot was demonstrated in cultivar Kukri using terraces and bulked segregant analyses in which double haploid lines and a resistance locus with polymorphic markers were identified in a dwarfing gene *Rht1* (Wallwork *et al.*, 2004).

4.3 Sources of partial resistance.

Disease data for *Aphanomyces* root rot and *Fusarium* root rot of peas were compared and it was concluded that resistance to both diseases was likely based on several genes or a single gene conferring quantitative and partial resistance (Malvick and Percich, 1999). Combining the available genes conferring partial resistance to *Fusarium* with the desired agronomic characteristics is difficult. Given the lack of highly effective fungicides, effective disease management will likely rely on an integrated disease management system using various control options (McMullen, Jones and Gallenberg, 1997). Partial resistance to seedling blight of wheat occurs in more susceptible cultivars and seedlings can therefore be used to measure partial resistance to crown rot (Wildermuth and McNamara, 1994).

None of the currently available commercial wheat cultivars are immune to *Fusarium* infection but differences in partial resistance or tolerance does occur among different hard red spring wheat cultivars (McMullen and Stack, 1999). *Fusarium* crown rot is a very severe disease of durum crops in South Australia and all varieties are highly susceptible. Bread wheat can also suffer severe crown rot although less frequently (Wallwork, 1997). However, partial resistance is available in the only adapted variety in South Australia Kukri (Wallwork, 1997). Sources of partial resistance to crown rot caused by *F. pseudograminearum* are detected in mature and seedling plants grown in artificially inoculated soil in the field (Wildermuth, McNamara and Quick, 2001).

4.4 Induced Systemic Resistance.

Induced resistance exists when healthy plants occur in a field or glasshouse, despite exposure to a continuous supply of inoculum from diseased plants in close proximity (Duncan and de Milliano, 1995). Induced systemic resistance is the process of resistance dependent on host plant's physical or chemical barriers, activated by biotic and abiotic inducing agents (Kessmann *et al.*, 1994; Kloepper, Tuzun and Kuc, 1992). Selected isolates of non-pathogenic *F. oxysporum* reduced *Fusarium* wilt of watermelon and it was concluded that induced systemic resistance was responsible (Larkin Hopkins and Martin, 1996).

Induced systemic resistance should not be viewed as replacement for chemical control measures but may allow for reduction in the number and dosage of chemical applications (Tally *et al.*, 1999; Romero, Kousik and Ritchie, 2001). Induced systemic

resistance could be deployed early in season to slow growth of the pathogen in rapidly growing host plant tissue and complement the protective activity of the fungicides (Graham and Gottwald, 1999). Compost-mediated induced resistance corresponds with an increase in some enzyme activities such as peroxidases and β -1-3 gluconases considered important in overall host plant defense mechanisms (Benhamou, 1996).

5.0 CROWN AND ROOT ROT MANAGEMENT STRATEGIES.

Most soil pathogens are non-specific in the symptoms they cause yet they can have a significant influence on crop production. These include pathogens such as *Fusarium* spp. which can live on the plant without any obvious symptoms and although not lethal to mature plants, they cause reduction in plant growth and vigour that ultimately leads to reduced yields (Giorda *et al.*, 1995; Leslie, 2000).

Several disease management strategies can be utilized to address pathogen problems on sorghum caused by *Fusarium* spp. Cultural control methods, manipulation of planting dates, sanitation and destruction of infected plants are commonly used to manage crown and root rot. Other common strategies include exploitation of host plant resistance and chemical control. Basic information on disease occurrence and distribution, yield loss assessments, host-pathogen-environment interaction, physiological races (pathotypes) and ecology of the pathogen is paramount to the implementation of any disease management strategy (Duncan and de Milliano, 1995; Fredericksen, 1986).

5.1 Intercropping and cultural control.

The use of resistant / tolerant sorghum genotypes and intercropping as components of an integrated pest management programme is widely used for disease management and yield increase (Karikari, Chaba and Molosiwa, 1999). Intercropping barley and pea increased land equivalent ratio to values exceeding 1.0 indicating the advantage of intercropping in forage biomass and protein yield (Chen *et al.*, 2004). Productivity of a maize and bean intercropping system was evaluated in terms of crop yield and growth and the results demonstrated a total land equivalent ratios for yield and growth ranging between 1.06 to 1.58 and 1.38 to 1.86 respectively (Tsubo *et al.*, 2003).

Intercropping is a viable means of intensifying crop production, under unfertilized conditions and under biotic and abiotic stresses. In the dryland regions of northern Ethiopia intercropping sorghum with cowpea in an optimal temporal and spatial rearrangement draws multiple benefits (Reda, Verkleij and Ernst, 2005). Intercropping on both winter and summer cereal crops reduces *Fusarium* crown rot on wheat (Simpfendorfer *et al.*, 2005). Sowing between previous cereal rows decreased the crown rot severity with an average of (51%) and incidence of (45%) crown rot in following cereal crops (Simpfendorfer *et al.*, 2005).

Yield of Bambara groundnuts in intercropped sorghum at populations of 75:25% was more than 75% of the sole crop implying that sorghum did not depress the growth of Bambara groundnuts (Karikari *et al.*, 1999). In contrast, yields in the intercrop Bambara groundnuts under millet and maize were less than 75% of the sole crop yields, meaning pearl millet and maize depressed the growth of Bambara groundnuts (Karikari *et al.*, 1999). Intercropping of pigeon pea and finger millet or pigeon pea and sorghum in a 2:2 row arrangement gave higher total land equivalent ratio (LER) values than 2:1 or 3:1 and this was found to be an optimal row arrangement (Rubaihayo, Osiru and Okware, 2000).

5.2 Tillage systems.

Tillage treatments have an impact on the activity, ecology and population dynamics of micro-organisms in soil and can also affect the development of plant pathogens (Steinkellner and Langer, 2004). The effects of tillage practices may however, be masked by the endophytic and seedborne nature of *Fusarium* spp. and their wide host range thus facilitating inoculum dissemination from adjacent host plant fields (Booth and Waterson, 1964a, 1964b; Smelzer, 1959)

Growing sorghum under eco-fallow conditions could considerably reduce the presence and occurrence of *Fusarium* root and stalk rot and also increase yields when compared to conventional tillage systems (Clafin, 2000). Minimum tillage or no-till has been shown to reduce the level of *Fusarium* root rot because the abundant fungal spores from surface residue are not incorporated into the root zone depth. Reduced tillage reduces soil erosion, conserves energy, soil moisture and increases crop yields. Many crop pathogens however, survive on previous crop residues making disease more

problematic under reduced tillage. Therefore, for reduced tillage to be effective additional control measures such as chemical control, biological control, host plant resistance and cultural control in particular crop rotation are needed (Bockus and Shroyer, 1998).

Incorporation of straw and crop residues into soil improves the physical properties of soil and also stimulates soil micro-organisms antagonistic to many root rot pathogens (Stack and McMullen, 1999). Removal of infected plant debris by incorporating crop residues into soil before planting can reduce disease pressure. Tillage can also reduce inoculum in the field for maize ear rot infection by negatively influencing the survival of *Fusarium* spp. provided that nearby fields, are free of inoculum (Cotton and Munkvold, 1998).

Mouldboard plough treatment has resulted in lower number of *Fusarium* spp. than in chisel plough and rotary tiller treatments. This is attributed to the tillage depths which seem to play a decisive role for the presence of the *Fusarium* spp. Steinkellner and Langer (2004) found that the deeper the tillage the lower the isolation frequency and diversity of total colony forming units and *Fusarium* spp. from winter wheat and maize plots. Similarly, Nyvall and Kommedahl (1970) found *F. moniliforme* survival to be poorer on surface corn stubble compared with stubble buried to 30 cm. However, Skoglund and Brown (1988) recovered *F. moniliforme* and *F. subglutinans* in equal numbers from buried and surface stubble. Alternating tillage practices had no effect on corn ear rot caused by *F. moniliforme*, *F. subglutinans* and *F. graminearum* (Flett, McLaren and Wehner, 1998).

Levels of *Fusarium* crown rot and common root rot were higher where wheat stubble was retained than where it was removed. Where there was no tillage, the incidence of crown rot was significantly higher (32.2%) than where the stubble was removed (4.7%). Disc tillage showed no difference in disease level between stubble treatments (Wildermuth *et al.*, 1997). *F. graminearum* survives mainly on infected stubble after harvest (Wearing and Burgess, 1977; Dodman and Wildermuth, 1987). In Australia, stubble burning was used to control *Fusarium* crown rot of wheat but it is no longer recommended because stubble is necessary for soil conservation and water retention (Burgess *et al.*, 1993).

Wheat stubble burning followed by rotation with sunflower reduced the number of plants infected with crown rot caused by *F. graminearum* Group 1. The pathogen survives as mycelium in stubble (Wearing and Burgess, 1977) and stubble burning is associated with reduction in crown rot severity (Summerell, Burgess and Klein, 1989). Burning of stubble removes aerial inoculum in crown tissues although inoculum in tissues below ground still survives. Stored moisture is also lost to burning and the effectiveness of stubble burning is thus debatable (Simpfendorfer *et al.*, 2005). Management of Fusarium foot rot caused by *F. pseudograminearum* O'Donnell & T. Aoki and *F. culmorum* of wheat is heavily dependent on practices that delay planting dates for fall-sown winter wheat and nitrogen fertility balance coupled with the avoidance of water stress to the crop throughout the growing season (Cook, 1980).

Minimum tillage was shown to increase sorghum seedling diseases under low soil pH and temperature conditions (McLaren, 1987), however, no significant tillage treatment effect on grain yield and root and stalk rot of sorghum were observed (Flett, 1996b). Fusarium root rot can be reduced by using growing media that stimulate healthy root growth and discourages pathogen development (Couteaudier and Alabouvette, 1981) and rouging of diseased seedlings can prevent secondary spread of the pathogen. Cultural practices play an important role in the control of Fusarium root and stalk rot of sorghum (Doupnik, 1984). Disease incidence is greatly reduced (11-88 %) under minimum tillage when compared with conventional tillage practices. This is due to minimum tillage reducing soil erosion and conserving moisture both of which reduces plant stress (March, Leonardon and Principi, 1981).

Reduced tillage in cereal monoculture increases the potential for soilborne disease to carry over from crop to crop. In more balanced cropping sequences and rotation that includes non-hosts, the effect might be reduced (Bailey and Duczek, 1996). The use of tillage to bury crop residue is also effective however, no-till and reduced tillage practices may contribute to the increased incidence of Fusarium crown and root rot disease (Draper, 2000).

Integrated control of weed host species with glyphosate prior planting followed by minimum tillage will result in a reduction in Fusarium crown rot inoculum density and low levels of bare patch of cereals (Riesselman 1989; 1990). The most effective disease management tool to reduce Fusarium crown rot of wheat is crop rotation (Simpfendorfer *et al.*, 2005).

5.3 Crop rotation.

Crop rotation is the most important tool the farmer has to reduce the damage of root and crown rots of spring wheat and barley caused by *Fusarium* spp. Rotation with a crop other than wheat and barley preferably legumes, maize or hay may lessen the level of *Fusarium* root rot inoculum in soil in the succeeding wheat or barley and hay crops. Rotation should include the strategic use of crops such as chickpea, faba bean, field pea, canola, mustard, mungbean, sunflower, sorghum or cotton. Break crops sown in narrow row spacing produce a dense canopy and this increases the breakdown of infected cereal residue (Simpfendorfer *et al.*, 2005).

A two-three year rotation with wheat and summer fallow was found beneficial in reducing *Fusarium* root rot of wheat (Stack and McMullen, 1999). No single management strategy can eliminate *Fusarium* crown and root rot but crop rotation has proved to be the most effective control method. Rotation with non-grass crops such as broad leaf crops such as pulses is critically important. Crop rotation can be used to reduce the incidence of root and stalk rots of sorghum (Flett, 1996a)

Fusarium root rot can be a problem where continuous oats are grown, but this disease is seldom seen when oats are grown in rotation. Crop rotation can be used to reduce *Fusarium* crown rot of wheat but it is not widely used by farmers in Australia because a minimum of two years is required to reduce *F. graminearum* inoculum to satisfactory levels (Summerell and Burgess, 1988). Row spacing at which break crops are grown in crop rotation influences their effectiveness in controlling *Fusarium* crown rot of wheat. Row spacing of 30 or 38 cm rather than 50 or 100 cm provides more ground cover and proved more effective in reducing crown rot inoculum (Simpfendorfer *et al.*, 2005). An effective crown and root rot management strategy would include the use of crop rotation with a broad leaf break crop, selection of resistant wheat varieties and use of seed treatment fungicides (Draper, 2000).

Fusarium crown rot of wheat caused by *F. pseudograminearum* is associated with the amount of infected crop residues and yield loss is related to post flowering of wheat. Crop rotation thus offers an opportunity to break the crown rot cycle by denying *F. pseudograminearum* a host plant and allowing the natural decline of crop residues

that harbour the pathogen. Fallow management affects pathogen survival and determines infection as well as yield loss (Verrell, Moore and Simpfendorfer, 2003).

Crown rot is a stubble-borne pathogen and the mycelium survives inside cereal and grass weed residue, which provides initial inoculum to the following crop. Rotation with break crops (chickpea, faba bean, field peas and oil seeds) or summer crops such as sorghum in rotation with non hosts such as cotton, sunflower and mungbeans is the most crucial component of an integrated disease management system (Simpfendorfer *et al.*, 2005).

5.4 Chemical control measures.

The control of *Fusarium* diseases is complicated by the limited availability of registered fungicides. Integrated disease management, which combines fungicide application with other options such as host resistance and bio-control, would therefore supplement the lack of registered fungicides to control root rot disease in field. When disease pressure is high and the history of disease development is well understood chemicals might provide an efficient and economical control strategy. Biological control approaches have been proposed to control soil borne pathogens (Benhamou *et al.*, 1987; Chet, 1987; Harman, 1992; Kloepper, 1991) but none of them has reached the performance of chemical control. Several chemical fungicides are effective against soilborne pathogens and possess a competitive advantage compared to biocontrol agents. Conventional fungicides are cheaper and easier to use and their efficacy is mostly constant (Pharand, Carise and Benhamou, 2002).

Fungicides can protect germinating seeds and seedlings from *Fusarium* crown and root rot. Seed treatment will kill spores adhering to the seed coat and protect seeds against attack from soil borne or seed borne fungi (Stack and McMullen, 1999). Seed treatments, both fungicide and biological, are employed in crop production as a protectant against soilborne pathogens that may cause diseases for the first few weeks after planting. Therefore if seed treatment is effective, there should be little to no disease development when post infection stresses occurs (Dorrance *et al.*, 2003). Because of these limitations, integrated disease management systems (combining the use of fungicides and biocontrol agents) may provide a good measure of success.

Mycelial growth of *F. avenaceum*, *F. culmorum*, *F. graminearum* and *F. moniliforme* were greatly reduced when Diallate was added to growth media (Schneider and Pendery, 1983). Diallate reduced infection *Fusarium* spp. by direct or indirect inhibition or production of chemical changes in the host that retard pathogen growth (Altman and Schonbeck, 1978). Antibiotic treatment of carnation plants inoculated with *F. oxysporum* f. sp *dianthi* both delayed symptom expression and reduced disease severity comparable to that achieved with benlate (Mutitu and Muthomi, 1994).

Various fungicides have been registered for the suppression of common root rot in wheat caused by *Fusarium* spp. These include triadimefon and difenoconazole. These fungicides have been shown to significantly reduce common root rot on plants grown from treated seeds compared to untreated seeds (Stack and McMullen, 1999). Fenarimol, triadimefon, fenfuram and caboxin caused significant inhibition of linear growth of *F. graminearum* (*Gibberella zae*) on wheat in *in vitro* studies (Klein and Burgess, 1987). Furmecyclox and triadimefon used as seed dressings were found effective against pre- and post emergence damping-off and seedling blight of sorghum (McLaren and Rijkenberg, 1989). Control of seed- and soil-borne pathogens in wheat was successfully achieved through seed treatments with difenoconazole - metalaxyl (Dividend[®] 030 FS) and tebuconazole (Raxil[®] 015 ES) prior to planting (Ashley *et al.*, 2003). Barley, sorghum and wheat seeds were treated with carboxin and thiram (Vitavax 300) to protect the seedlings from pathogenicity of *A. alternata* (Fr : Fr) Keissl., *A. flavus* Link and *F. oxysporum* Schlecht. Emend. Snyder & Hansen (Hassan, 2000).

Benzimidazole was effective for reducing Fusarium wilt of basil but was seldom applied due to residues and inadequate levels of control (Minuto, Garibaldi and Gullino, 1994). Several studies have shown the potential for soil contamination and re-infestation through continued use of chemical compounds (Kvartskhava, 1957; Martini and Gullino, 1991; Elmer, Wick and Haviland., 1994; Keinath, 1994; Gamliel *et al.*, 1996). Moreover, air borne propagules have been found to make soil disinfestation only partially effective (Gamliel *et al.*, 1996). Chemical control has therefore not been encouraged. The low efficacy of chemical control measures and unsatisfactory level of control offered by the commercially available formulations of bio-control agents boost the urgency for other means of control for Fusarium crown and root rot disease (Reuveni *et al.*, 1997; Keinath, 1994; Minuto *et al.*, 1997).

Most published research on the efficacy of seed treatments for maize diseases consist of field tests performed under a variety of environmental conditions (Munkvold and O'Mara, 2002). Field tests can provide crucial information about the field performance of seed treatment with fungicides but information on the efficacy of fungicides against specific fungi is lacking (McGee, 1995). Control of Fusarium root rot is affected by fungicidal soil drenches but these treatments function primarily to limit the spread, rather than cure the disease (Landis, 1989). Soil pesticides offer relatively effective, although often only short-term, solutions for suppression of plant disease causing organisms. However, the future of soil pesticides appears questionable because of environmental and economic considerations (Bellus, 1991). Soilborne diseases are effectively controlled by repeated applications of fungicide, however, repeated applications of broad spectrum or persistent fungicides may result in soil contamination, fungicide resistance or harmful effects on non-target organisms (Someya *et al.*, 2000).

Chemicals pose problems to the environment, eliminate beneficial organisms, induce development of pathogen resistance and are not economically feasible. Biological control agents cause less disruption on the ecosystem and are highly specific and cheaper than chemicals. However, biocontrol is less effective and more variable than chemical control (Harman and Lumsden, 1990). Sole use of chemicals can result in nontarget effects on soil micro-organism populations (Pharand *et al.*, 2002). Biocontrol agents are however, composed of living organisms therefore their efficacy varies over a wide range of environmental, cultural and biotic conditions.

5.5 Biological control.

Baker and Cook (1974) and Paulitz (1996) suggested that biological control is a strategy to enhance plant density and promote growth using naturally occurring micro-organisms. Basic understanding of the soil and rhizosphere microbiology can simplify the identification of specific micro-organisms. These micro-organisms can be used directly for disease management, enhancement of plant growth or altered crop management practices can be used to enhance their populations (Mazzola and Gu, 2002; Mazzola *et al.*, 2002). Production of high value crops and lack of naturally occurring micro-organisms with potential to out compete introduced biological control agents for

suitable substrates makes the implementation of biological control agents potentially more feasible (Postma *et al.*, 2000; Paulitz and Belanger, 2001). Natural occurring bio-control agents may be sought on plant parts and have been successfully isolated from the rhizosphere of host plants (Wells, Bell and Jaworski, 1972; Backman and Rodriguez-Kabana, 1975). Biological control of soilborne pathogens has shown great promise and attracted much attention in recent years, but there are only few studies on the biocontrol of *Fusarium* crown rot in wheat (Weller, 1988; Hornby, 1990; Wong, Mead and Holley, 1996).

Severity of *F. graminearum* infection on wheat seedlings increased with the inoculation dose of the pathogen. Application of *Burkholderia (Pseudomonas) cepacia* (Burkholder) (A3R) as seed treatment or soil drench significantly reduced disease severity (Huang and Wong, 1998). Mao *et al.* (1997) and Mao *et al.* (1998) successfully evaluated biological control agents as seed treatments and observed a reduction in damping-off of corn, in potting medium infested with a combination of *Pythium* species and *F. graminearum*. Control of *Fusarium* diseases has been achieved using rhizobacteria on maize (Burkhead, Schisler and Slininger, 1994; Lambert *et al.*, 1987). A rifampicin-resistant isolate of *B. cepacia* (A3R) was shown to inhibit the growth of *F. graminearum* Group 1 *in vitro* and reduced crown and root rot severity of wheat in glasshouse and fields experiments (Huang and Wong, 1998). An antibiotic-negative mutant of *B. cepacia* protected maize from *Fusarium* wilt suggesting that other mechanisms of action may be responsible for the biocontrol of this isolate (McLoughlin *et al.*, 1992).

Sorghum is reported to be a good host of mycorrhizal fungi and the potential for exploiting these as biological buffers against a variety of biotic and abiotic stresses must be examined (Doupnik, 1984). In most plant production systems the population of beneficial rhizobacteria is too small to have a significant impact on root health. Introduction of selected plant health promoting rhizobacteria (PHPR) strains during spring or planting can enrich the rhizosphere and create a biological barrier for plant pathogens and pests (Cook and Baker, 1983).

Plant health promoting rhizobacteria (PHPR) have been identified as a biological control alternative to pesticides by achieving disease suppression without negative effects on the environment (Linderman, 1986; Weller, 1988). Superior rhizosphere colonizing isolates of non-pathogenic *F. oxysporum* in naturally infested

soil have a competitive advantage over soil micro-flora and might also offer protection from soilborne pathogens (Edel *et al.*, 1997).

Genetic diversity within species of both biocontrol agents, host plant and among members of a microbial species that suppresses disease may provide an answer to variability of biocontrol efficiency across space and time. The genetics of the host plant should be exploited for suppressiveness and hospitality to the biocontrol agents to enhance breeding or genetic modification of the host plant (Handelsman and Stabb, 1996). The integrated use of genetics, molecular and ecological approaches will form the basis for significant future advances in biological control. Understanding the ecology of microbial communities and the mechanisms of pathogen resistance to biocontrol agents is critical to sustain long term disease suppression (Handelsman and Stabb, 1996).

5.6 Soil amendments and additives.

Beneficial effects of organic composts in controlling major soilborne pathogens has been studied extensively (Hoitink and Fahy, 1986; Hadar and Grodecki, 1991; Hardy and Sivasithamparam, 1991). Incorporation of compost to soil has been shown to restrict pathogen growth and development (Pharand *et al.*, 2002) and proves that compost-amended potting mixes may play a key role in the control of root diseases.

The use of soil amendments to control diseases caused by soilborne plant pathogens is common (Huang and Huang, 1993). The addition of compost to soil has often been reported to reduce diseases caused by soilborne fungi (Zhang, Dick and Hoitink (1996) as well as increasing the water holding capacity and ion exchange capacity of the soil (Maynard, 1994). Incorporation of metabolizable organic matter into flooded soil greatly accelerated the decline of *F. oxysporum* f. sp. *conglutinans* compared with non-amended flooded soil (Mitchell and Alexander, 1962).

Amendment of field soil with composts suppressed root rot of sugar cane and increased plant growth. Soil amendment with organic materials therefore represents a potential management practice for crown and root rot disease (Dissanayake and Hoy, 1999). Different nitrogenous amendments (including urea) have been reported to increase beneficial microbial populations of *Nitrosomonas* spp. but not of *Nitrobacter* spp. which could lead to nitrate accumulation (Prior and Beramis, 1990).

Microbial densities in potato rhizosphere in compost-amended soils increased and affected pathogen inoculum potential and resulted in general or specific disease suppression (Hoitink, Stone and Han, 1997). The prospects for control of persistent soilborne pathogens by combining amendments with the application of air-tight plastic covers may provide an alternative for chemical disinfestation for high-value crops under conditions where other alternatives, such as soil solarisation or flooding, are not effective or feasible (Blok *et al.*, 2000).

Organic amendments may also be useful for management of soilborne diseases (Mazzola *et al.*, 2002). Incorporation of margosa and groundnut cakes into the soil reduced root rot caused by *Fusarium* spp. (Kaura and Singh, 1982). Use of naturally suppressive composts as part of integrated diseases control strategy offers exciting opportunities. These include the stability and persistence of the induced host response, efficiency of such substrates under commercial conditions and their suitability in an integrated crop protection system (Pharand *et al.*, 2002).

6.0 INTEGRATED CROP MANAGEMENT SYSTEMS.

Integrated crop management (ICM) embraces all activities in a crop production system and is composed of several management activities focusing on particular constraints such as IPM and integrated nutrient management (FAO, 1984; Schulten and van der Graaf, 1988). These management systems are dependent upon natural, technological and socio-economic resources and their interrelationships (FAO, 1991 b). Integrated crop management systems must be applied in an integrated way to ensure that they are mutually supportive in achieving the identified objectives for sustainable development (Heitefuss, 1989; Vereijken, 1989). Where possible, all the principles of management, eradication, exclusion, host resistance, and protection should be practiced (Kiewnick *et al.*, 2001).

Fusarium crown and root rot is difficult to control because the pathogen colonizes the soil and persists for long periods. The use of disease free transplants and avoiding over watering are sometimes very effective as is soil fumigation prior to planting and avoidance of physical damage of young plants which make them susceptible to *Fusarium* crown and root rot.

Ploughing in of crop residues, crop rotation and intercropping and breeding for disease resistance and the use of biological control measures is very important (Roberts, McGovern and Datnoff, 2001). Strategies for the management of *Fusarium* crown rot include the control of grass hosts prior to cropping, rotation of susceptible cereals with non host break crops, burning of infected stubble and growing of resistant or tolerant wheat varieties (Kirkegaard *et al.*, 2000).

Research involving root and stalk rots of any crop is difficult and complex. Numerous interactions probably occur, and biotic and abiotic factors e.g., other fungi, bacteria, insects, cultural practices, and in general any soil inhabitant could be involved. It is impossible to identify all potential interactions, but observations based on a single parameter may be misleading and the integration of these factors may thus provide the best control option.

The integration of management strategies that combines the use of resistant varieties, long balanced rotation, organic amendments, soil fertility, chemical, biological control agents and use of natural occurring toxic compounds in plant extracts could be effective (van Bruggen and Termorshuizen, 2003). The use of these combined practices usually produces the most reliable and stable plant disease management system.

7.0 SUMMARY.

Fusarium spp. are most important fungi associated with grain sorghum and occur in both temperate and tropical areas. In terms of prevalence *F. moniliforme* (*sensu lato*) is the major parasite on graminaceous crops including maize and sorghum. This species has been found to occur on at least 31 plant families however the host range of *F. moniliforme* was purported to more than 11000 species of plants (Bacon *et al.*, 1996). Grain sorghum hosts several *Fusarium* spp. including *F. equiseti*, *F. Verticillioides*, *F. napiforme*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. thapsinum* and *F. trincictum*. *Fusarium* spp. can result in economic losses of up to 100% in localized areas. Knowledge of causal organisms, host preferences, symptom expression, economic losses and predisposing factors may play an important role in the formulation of an integrated management system of crown and root rot caused by *Fusarium* spp. It is thus important to generate quantitative information on the relationship between agro-

ecological conditions such as soil type, moisture, pH and nutrient status on the management of crown and root rot pathogens. The incorporation of soil amendments can reduce diseases caused by soilborne fungi in addition to altering the effect of soil characteristics by increasing the water holding and ion exchange capacity of soil and thereby altering the organic matter content of soil.

Host plant resistance is the most effective method for controlling crown and root rot severity. Morphological and physiological sources of resistance are important tools for breeding cereals for crown and root rot resistance. Crown and root rot is caused by a complex of *Fusarium* spp., therefore development of resistant genotypes is difficult and complicated by environmental stress. No commercial cereal genotype is immune to the disease. However, genotypes with partial resistance can be identified within the germplasm collection.

Cultural practices can also provide effective control for crown and root rot of sorghum. Tillage systems, stubble management, intercropping and crop rotation are some examples. In the long term, it may be necessary to use natural or commercial biocontrol agents as seed and soil treatments since these could provide environmentally and economically safe control measures. Fungicides can also be considered for control of crown and root rot disease. Fungicides benomyl, benlate, triadimefon, difenoconazole, tebuconazole, carboxin and thiram and benzimidazoles used as seed treatments provided good control. Research on the development of integrated non-chemical approaches for pest management should therefore be continued because only with a combination of different approaches can an economically viable crop management system be developed. There is very little published information available on the effect of crop rotation, soil amendments, seed treatment on *Fusarium* crown and root rot of sorghum thus most of the examples cited were made in reference were made to other graminaceous crops mostly wheat.

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CHAPTER II

***FUSARIUM* SPP. ASSOCIATED WITH CROWN AND ROOT ROT DISEASE OF GRAIN SORGHUM**

ABSTRACT

The effects of genotype resistance on population densities of *Fusarium* spp. on sorghum roots and in soil were studied in the glasshouse using soil collected from three commercial fields as well as experimental plots in Bethlehem, Cedara and Potchefstroom. Six sorghum genotypes with unknown levels of resistance were evaluated. The more severe the root rot the greater was the number of colony forming units (CFU) of *Fusarium* spp. recovered. However, in the more resistant genotypes (PAN 8706W and PAN 8564) the numbers of colony forming units (CFU/g) recovered from roots were not associated with high crown and root rot severity. In the glasshouse, crown and root rot severity increased significantly ($P < 0.05$) with increasing initial inoculum levels for susceptible, but not for resistant genotypes. Crown and root rot severity was high for the susceptible (NK 283) and less for the resistant genotypes (PAN 8564 and PAN 8706W) in both field and glasshouse experiments. Roots were collected three times from plants in the glasshouse and twice in the field. Roots were dried, ground to powder suspended in sterile water and plated on to a selective medium to determine population densities. The population densities of the pathogen on plant roots were linearly related to crown and root rot disease; the more severe the disease, the larger the number of CFU of *Fusarium* recovered. A similar relationship was observed in soil, sampled around roots. Higher population densities were observed in soil than on roots. The most common *Fusarium* spp. associated with crown and root rot recovered from sorghum roots and soils in sorghum fields were *Fusarium oxysporum*, *F. solani*, *F. Verticillioides* and *F. thapsinum*. Other species recovered at significantly lower frequencies were, *F. equiseti*, *F. nygamai*, *F. pseudonygamai* and *F. proliferatum*.

INTRODUCTION

Fusarium crown and root rot of grain sorghum (*Sorghum bicolor* (L.) Moench) typically involves the cortical tissues first and subsequently the vascular tissues of the roots. Newly formed roots may exhibit distinct lesions of various sizes and shapes. Root rot is progressive, so older roots are often destroyed, leaving little plant anchorage. When sorghum crown and root rot is extensive, the plants are easily uprooted (Zummo, 1983).

Fusarium spp. have been reported to cause crown and root rot diseases on several crops (Elmer and Lacy 1987; LaMondia *et al.*, 1999; Sangalang, Summerell and Burgess, 1995). *Fusarium moniliforme* (*sensu lato*) one of the most cosmopolitan plant pathogens, is found in soils wherever sorghum is grown. The fungus persists on plant residues but also survives in the soil. *F. moniliforme* may also be a serious pathogen on maize, millet, rice and sugarcane (Sheldon, 1904; Voorhies, 1933; Ullstrup, 1936; Dickson, 1956; Bourne, 1961).

Fusarium spp. associated with root and stalk rot include: *F. equiseti* (Cda.) Sacc., *F. graminearum* Schwabe, *F. napiforme* (Marasas, Nelson & Rabie), *F. nygamai* Burgess & Trimboli (1986), *F. oxysporum* (Schlecht. Emend. Snyder & Hansen), *F. proliferatum* (Matsushima) Nirenberg, *F. semitectum* (Berk. & Rav.), *F. solani* (Mart.) Appel & Wollenw. Emend. Snyder & Hansen, *F. subglutinans* (Wollenw & Reinking), *F. thapsinum* Klittich, Leslie, Nelson & Marasas, and *F. trincictum* (Corda) Sacc. *F. verticillioides* (Sacc.) Nirenberg, but pathogenicity of these species on sorghum was not been established (Leslie, 2000).

However, sorghum seedlings inoculated with *F. thapsinum* resulted in reduced seedling vigour (Little and Margill, 2003). This was confirmed on sorghum seedling pathogenicity tests when *F. thapsinum* was found most virulent followed by *F. andiyazi*, then *F. verticillioides*, and finally *F. nygamai* and *F. pseudonygamai* with similar reaction (Leslie *et al.*, 2005). *Fusarium* spp. are always present in the soil profile in which sorghum roots are distributed and may infect the plants at any time during the growth period (Clafin, 2000). *Fusarium* spp. can also live in sorghum tissue with no obvious disease symptoms, and the absence of symptoms should therefore, not be equated with the absence of these fungi (Leslie, 2000).

Initial inoculum is an important factor affecting *Fusarium* crown and root rot. In general, increasing amounts of initial inoculum of soil borne root pathogens cause higher disease severities (Grogan, *et al.*, 1979; Marois and Mitchell, 1981; Pullman and Devay, 1982; Rush and Kraft, 1986; Elmer and Lacy 1987; Nicot and Rouse 1987; Harris and Ferris, 1991; Paplomatas, *et al.*, 1992; Ben-Yephet, Reuven and Genizi, 1994). For example, *Fusarium* crown and root rot disease of carnation increased significantly ($P \leq 0.05$) with increasing initial inoculum levels on susceptible, but not resistant cultivars (Ben-Yephet *et al.*, 1996).

The objectives of the present study were, (i) to study the relationship between initial inoculum and sorghum genotype resistance to crown and root rot; (ii) to determine the population densities of *Fusarium* spp. in field soils and on sorghum roots; and (iii) to characterize and identify the *Fusarium* spp. isolated from sorghum roots and field soils.

MATERIALS AND METHODS

Effect of initial inoculum and genotype resistance on *Fusarium* population densities in soil in the glasshouse.

The effect of initial inoculum and genotype resistance on increasing *Fusarium* population densities in the soil after six weeks of plant growth were studied in glasshouse trials. Experiments were conducted in 500 ml plastic pots using naturally infested soil collected from Bethlehem (28° 15' S and 28° 20' E), Cedara (29° 36' S and 30° 26' E) and Potchefstroom (26° 43' S and 23° 6' E), South Africa. Ten containers were prepared for each soil type.

Sorghum seeds from each of the commercial genotypes NK 283, PAN 8446 and PAN 8706W with unknown levels of resistance were surface disinfested by dipping seeds in 70% ethanol for 30 seconds (Salas *et al.*, 1999). This was followed by surface sterilization in 0.05% NaOCl for 2 minutes, three rinses in sterile distilled water and then drying on a paper towel. Three containers were seeded with each of the sterilized sorghum seeds at a rate of five seeds per pot, planted to a depth of 3 cm. One container remained unplanted and served as a control treatment being used to determine changes in the *Fusarium* population in soil over time. Two weeks after emergence, seedlings were fertilized with limestone ammonium nitrate (LAN-28% a.i) at a rate of 1 g per kg

soil. Fertilization was thereafter applied every two weeks and plants were watered once daily.

Pots were arranged in a randomized complete block design and maintained in the glasshouse for a period of six weeks with daily minimum and maximum temperatures of 21 °C and 30 °C, respectively. After six weeks, three plants were removed from each pot per replicate/treatment combination and washed in running tap water to remove soil debris. Roots were cut at the crown and used for the isolation of *Fusarium* spp., determining the *Fusarium* population densities and assessment of crown and root rot severity. Crown and root rot severity was quantified using a 0-5 scale (Correll, Puhalla and Schneider, 1986), where; 0 = no root discolouration; 1 = slight vascular discolouration in root tissue, 2 = extensive vascular discolouration in root tissue, 3 = vascular discolouration in crown; 4 = extensive vascular discolouration in crown, 5 = crown completely necrotic.

Fusarium population densities were determined in the unplanted soil and in the rhizosphere soils at the end of the trial. Soil was air dried, passed through a 200 µm sieve and a sub-sample of 5g of air dried soil was suspended in 30 ml of sterile distilled water (Tsao and Ocana, 1969; Martin, 1992; Edel *et al.*, 1997). The suspension was serially diluted (Hansen and Smith 1932; Nash and Snyder 1962) with sterile distilled water to a final dilution of 1:10,000 before 1 ml was pipetted onto Petri dishes containing rose bengal-glycerol-urea (RbGU) selective medium (Van Wyk, Scholtz and Los, 1986). The media was amended with 300 mg of streptomycin sulfate (Novo Nordisk Pty Ltd) to reduce bacterial contamination. Five replicate plates were prepared for each soil x cultivar combination. Petri dishes were incubated for 7 days at 25°C. *Fusarium*-like colonies growing on the RbGU medium were counted and averaged for the five replicates.

Colonies that developed from unplanted soil samples were counted and the difference compared with those from rhizosphere soil of each genotype in order to measure the increase in the population of soil fungi subsequent to the planting of sorghum (Ben-Yephet *et al.*, 1996). Colonies counted were expressed as colony forming units (CFU) per gram of soil (average CFU for five Petri dishes) (number CFU * final dilution factor of 1:10,000 CFUs/ml) / g dry soil = CFU g⁻¹ soil (Rice, 2004). The experiment was conducted three times in the glasshouse during the 2003/2004 season.

Effect of initial inoculum and genotype resistance on *Fusarium* population densities in field soil.

The effect of initial inoculum and genotype resistance on *Fusarium* population densities were studied in field experiments during 2003 / 2004 season at Bethlehem, Cedara and Potchefstroom. Five sorghum genotypes (NK 283, PAN 8446, PAN 8564, SNK 3939 and Buster) were treated with Concep II (herbicide seed safener) and planted in plots of 10 rows, 8 m in length with 10 cm intra-row spacing. An inter-row spacing of 0.85 m was used at Cedara and 1.2 m at both Bethlehem and Potchefstroom. A path of 1 meter was kept between plots and replications with the first and last row of each plot serving as a border row. Treatments were arranged in a randomized complete block design and replicated four times. Pre-plant fertilization with NPK 2:3:2 (21% a.i) at 300 kg per hectare prior to planting was applied throughout the duration of the trial. Weeds were controlled with Metalochlor/ Terbutylazine (a.i 125 / 375 g litre) at a rate of 4 litres per hectare.

After nine weeks, five plants were removed from plots for each replicate/ treatment combination and washed in running tap water to remove soil debris. Roots were cut at the crown and used for the isolation of *Fusarium* spp., determination of the *Fusarium* population densities and assessing crown and root rot severity. Crown and root rot severity and population densities were determined as described above. Inoculum concentration was determined in the unplanted soil and in the rhizosphere soil from planted plots at the end of the trial as described above (Ben-Yephet *et al.*, 1996). CFU per gram soil for determining inoculum concentration and population densities were determined as described (Rice, 2004) above.

Quantification of *Fusarium* spp. from field soils and sorghum roots.

The incidence of *Fusarium* spp. on six sorghum genotypes grown in the North West, Free State and Kwa-Zulu Natal provinces of South Africa was determined during the 2003 and 2004 season. Fungi were isolated from soil and sorghum roots collected from experimental plots at three locations (Bethlehem, Cedara and Potchefstroom) and rhizosphere soil that was collected from farmers fields (Koppies, Heilbron, Heildelberg and Standerton). Fungi were also isolated from plants in the glasshouse grown in soil collected from Bethlehem, Cedara and Potchefstroom (described above).

Quantification of *Fusarium* isolates and population densities from soil.

Soil samples were air dried in an air-conditioned glasshouse at day/night temperatures of 21°C and 30°C respectively for seven days. Soils were passed through a 200 µm sieve. A sub-sample of 5g was serially diluted (Hansen and Smith 1932; Nash and Snyder 1962) and suspended in water as described by Tsao and Ocana (1969), Martin, (1992) and Edel *et al.*, (1997). Five *Fusarium* colonies growing on RbGU medium (Van Wyk *et al.*, 1986) were randomly selected from each of the five replicate Petri dishes and subcultured on potato dextrose agar (PDA) and carnation leaf agar (CLA) in preparation for identification as described by Summerell, Salleh and Leslie (2003).

To determine the number of colony forming units per gram soil (CFU/g soil) a sub-sample of 5 g of air-dried soil from each replicate was added to 45 ml of sterile dilute water agar (0.1 %, wt/vol). The samples were shaken on a reciprocal shaker and serially diluted to final dilution of 1:10,000 CFUs/ml). Soil suspensions of 0.2 ml were spread on five, 90-mm-diameter Petri dishes (Dhingra and Sinclair 1986; Ben-Yephet *et al.*, 1996) containing RbGU selective medium (Van Wyk *et al.*, 1986). Petri dishes were incubated for 7 days at 25°C. Colonies were counted and results were expressed as CFU/g soil as described above (Rice, 2004).

Sub-culturing, purification and identification.

Cultures on CLA were sub-cultured and purified before identification (Summerell *et al.*, 2003). Purification was performed by means of single spore isolates, prepared on CLA using serial dilution (Hansen and Smith, 1932). Ten ml of sterile distilled water were added to the surface of a 7-day-old culture on CLA to prepare a conidial suspension. The spore suspension (1 ml) was spread on (90-mm-diameter) Petri dishes containing water agar (WA) and incubated overnight at 25 °C. Individual germlings were excised from the surface of the water agar using a small sterile spatula and transferred to PDA and CLA (90-mm-diameter half plates) with one germling in each compartment of the plate. Purified cultures on CLA and PDA were incubated at 25 °C for 7-14 days prior to final identification by means of the keys described by (Nelson, Toussoun and Marasas, 1983 and Summerell *et al.*, 2003).

Pigmentation and gross colony morphology were assessed following the colour codes of Nelson *et al.*, (1983) and Summerell *et al.*, (2003) and CLA cultures were examined for the presence of macro- and micro-conidia and conidiogenous cells.

Microscope slides were prepared from each of the cultures on PDA and CLA half plates and examined under a light microscope for the presence of heads and chains of macro- and micro-conidia, phialides and chlamydospores in sterile water and lactophenol mounts. Half-plate sub-cultures were subsequently transferred to 65-mm-diameter plates containing PDA and stored on PDA slants at 4 °C.

Quantification of *Fusarium* isolates and population densities from roots.

Fungi were isolated from the roots of sorghum genotypes cultivated in the glasshouse at Bloemfontein and in the field at Bethlehem, Cedara and Potchefstroom. Isolations were also performed from sorghum plants collected from farmer's fields at Heidelberg, Heilbron, Standerton and Koppies in the Free State province, South Africa.

Three and five plants were sampled from glasshouse and field experiments respectively. Soil was dislodged from roots by thoroughly washing with running tap water. Roots were surface sterilized with 0.5 % NaOCl for 2 minutes followed by washing three times in sterile distilled water (Stover and Waite 1953; 1954; Jones 1987; Gamliel *et al.*, 1996; Luo *et al.*, 1999). Roots were placed on sterile filter paper to remove excess water and cut into pieces of 0.3-0.5 mm. Five root segments were plated on each of five replicate Petri dishes (90-mm-diameter) containing RbGU selective medium (Van Wyk *et al.*, 1986). Colonies growing from root cultures were sub-cultured and purified for identification.

To determine population densities, roots removed from soil were left to dry for two weeks in the glasshouse at a day/night temperature of 30/21°C. Plants were then washed to remove soil particles, inserted into brown paper bags and left to dry for a further two weeks. After drying, the roots were ground in a Waring blender and passed through a 250 µm sieve. Ground root sub-samples (5 g) for each replicate were added to 45 ml of sterile dilute water agar (0.1 %, wt / vol).

The samples were shaken on a reciprocal shaker and then serially diluted with sterile dilute water agar to (1:10000). Root suspensions (0.2 ml) were spread on five, 90-mm-diameter Petri dishes for each treatment combination containing RbGU selective medium (Van Wyk *et al.*, 1986). The medium was amended with streptomycin sulphate (300 mg/l) (Novo Nordisk Pty Ltd) to reduce bacterial contaminants. Petri dishes were incubated for 7 days at 25°C under cool white fluorescent light. Colonies from ground root samples were counted to determine

population densities in the roots. Results were expressed as CFU/g root (CFUr) (average CFU for five Petri dishes) (number of CFU * final dilution factor) / g dry roots = CFU g⁻¹ root (Rice, 2004).

Data analysis.

Data were analyzed using the analysis of variance (ANOVA) and general linear model of SAS (SAS 9.1 SAS Institute Cary NC). Treatment means were compared using the Fisher's least significant difference (LSD) with ($P \leq 0.05$). The ANOVA and GLM means from individual treatments were compared if interactions were significant ($P \leq 0.05$). When interactions were not significant ($P \leq 0.05$) individual treatments were combined to evaluate effects of treatments in the two main effects.

RESULTS

Effect of initial inoculum and genotype resistance on *Fusarium* population densities in soil in the glasshouse.

Crown and root rot severity was significantly ($P \leq 0.01$) influenced by genotype but not location or location x genotype interaction. Highest crown and root rot severity was observed on plants grown in soil collected from Cedara compared with the least for Potchefstroom (Table 2.1). Crown and root rot severity ratings remained low in the more resistant genotype, PAN 8706W and the intermediate genotype, PAN8446 in all soils from the three locations (Table 2.1). In the susceptible NK 283, crown and root rot severity increased significantly ($P \leq 0.05$) with increasing amounts of initial inoculum associated with the test soils (Table 2. 1).

Genotype resistance and inoculum concentration significantly affected population densities of *Fusarium* spp. on plant roots and in soil subsequent to the growth of sorghum (Table 2.1). Increasing the amount of initial inoculum of soil had a greater effect on crown and root rot severity in the susceptible genotype (NK 283) than on the resistant genotype (PAN 8706W) (Table 2.1).

Effect of initial inoculum and genotype resistance on *Fusarium* population densities in the field soil.

Disease severity in the field was generally higher than in the glasshouse (Table 2. 2). Crown and root rot severity was significantly ($P \leq 0.01$) influenced by genotype and locality but not locality x genotype interaction. Crown and root rot severity in the five sorghum genotypes developed in accordance with their level of resistance to crown and root rot pathogen (Table 2.2). The resistant genotype PAN 8564 and the intermediate SNK 3939 and Buster had low crown and root rot severities compared to the most susceptible NK 283 and PAN 8446 in which high crown and root rot severities developed (Table 2.2). Crown and root rot severity increased significantly ($P \leq 0.05$) with increasing amounts of initial inoculum for the highly susceptible NK 283 and less susceptible PAN 8446. Both genotypes expressed high root rot severity even at low initial inoculum when compared to the resistant PAN 8564 (Table 2.2).

Quantification of *Fusarium* spp. in field soils and sorghum roots.

Genotype resistance and the amount of initial inoculum significantly ($P \leq 0.05$) affected the population densities of *Fusarium* spp. in plant tissues and in soil (Tables 2.1 and 2. 2). High population densities in soil used in glasshouse experiments were observed in those collected at Cedara and least at Potchefstroom. The population densities of the pathogens in plant tissues were related to genotype resistance. Larger numbers of CFU/g root were recovered from the susceptible genotypes (Table 2.1).

Population densities in roots were highest in those grown in soil collected at Cedara and least for soils from Potchefstroom (Table 2.1). Locality, ($P \leq 0.01$) and genotype significantly ($P \leq 0.01$) affected *Fusarium* population densities in roots for the glasshouse experiments. No significant location x genotype interaction was recorded. Soil population densities in the glasshouse were lower for resistant PAN 8706W followed by the moderately resistant PAN 8446 and highest for susceptible NK 283 across the three localities (Table 2. 1). The relationship between crown and root rot severity and the number of CFU/g root in the glasshouse was linear (Fig. 2.2) although the pathogens were also recovered from symptomless plants. A similar tendency was observed between disease severity and population density of *Fusarium* spp. in soil although the relationship was not significant.

General linear model (GLM) analysis indicated that *Fusarium* population densities in roots from field trials were significantly ($P \leq 0.01$) affected by location and genotype, and locality x genotype interaction ($P \leq 0.05$). Populations in soils were significantly ($P \leq 0.01$) affected by locality but not genotype or genotype x locality interactions. Field evaluations displayed highest soil population densities for Cedara and least for Potchefstroom (Table 2.2). In field experiments, changes in population densities followed similar trends to those in the glasshouse with the highest density recorded for the susceptible genotypes NK 283 and PAN 8446 followed by the intermediate genotypes SNK 3939 and Buster and least for the resistant genotype PAN 8564 (Table 2.2). The number of CFU recovered was related to the degree of genotype resistance. Soil population densities were higher after cultivation of the highly susceptible genotype NK 283 compared with the resistant genotype PAN 8564 (Table 2.2).

The number of *Fusarium* CFU/ g root recovered from plant roots was lower on the resistant genotypes (PAN 8564) compared to the susceptible genotype (NK 283) (Table 2.2). The relationship between crown and root rot severity and the number of CFU in roots was linear (Fig. 2.4) despite the pathogen also being recovered from symptomless plants. A similar relationship was observed between crown and root rot severity and population density of *Fusarium* spp. in soil (Fig. 2.5). In general, the results obtained in the glasshouse were corroborated in the field. Differences in CFU in the soil were higher than on the roots in all the genotypes at all localities despite the variation in soil inoculum concentrations for the glasshouse and field trials (Tables 2.1 and 2.2).

The frequencies of *Fusarium* spp. isolated from sorghum roots and field soils are indicated in Table 2.3. In experimental sorghum plots at Bethlehem, Cedara, and Potchefstroom, *F. oxysporum* was the most frequently recovered species followed by *F. solani* and *F. verticillioides* (Table 2.3). *F. thapsinum*, *F. nygamai*, and *F. equiseti* each constituted more than 2% of all the *Fusarium* spp. recovered.

F. subglutinans, *F. proliferatum* were the other principle *Fusarium* spp. recovered but they occurred at frequencies less than 2%. The remaining *Fusarium* spp. each constituted less than 1% of all *Fusarium* spp. recovered. In commercial sorghum fields as in experimental plots *F. oxysporum* and *F. solani* were the most frequently

recovered species. *F. verticillioides*, *F. thapsinum*, *F. proliferatum* and *F. equiseti* were recovered at relatively low frequencies (Table 2. 3).

DISCUSSION

The present study demonstrated that the severity of crown and root rot disease and population densities of *Fusarium* spp. on sorghum roots and in the rhizosphere are affected by host resistance and initial soil inoculum. The degree of increase and the final number of CFU were related to genotype resistance. The highest population densities developed on the highly susceptible NK 283 and the lowest on the resistant genotypes PAN 8564 and PAN 8706W. A similar relationship between initial inoculum and the severity of the resulting epidemic in susceptible crops by wilt pathogens has been demonstrated for several *F. oxysporum* pathosystems (Marois and Mitchel 1981; Rush and Kraft 1986; Elmer and Lacy 1987; Harris and Ferris 1991; Ben-Yephet *et al.*, 1994). Ben-Yephet *et al.* (1996) found that increases in the population densities of *F. oxysporum* f. sp. *dianthi* in soil, in relation to the amount of initial inoculum occurred for highly susceptible (Harmon), susceptible (Lior), and moderately resistant (Gailt) carnation cultivars, but not for the highly resistant cultivars (Eveline and Scarlette). These results are consistent with results by Elgersma, McHardy and Beckman (1972) and Alon, Katan, and Kedar (1974) who compared the production of *F. oxysporum* f. sp. *lycopersici* in stems of susceptible and completely resistant tomato cultivars following inoculation. They also found that the number of CFU/g of the pathogen on stems increased more in susceptible than in resistant cultivars.

In the present study, pathogen population densities were higher in soil than on roots. The high population densities in soil may have been due to most *Fusarium* spp. being free-living in soil, many of which are non-pathogenic and either saprophytic or antagonistic to pathogenic *Fusarium* spp. The method used for isolation did not differentiate between pathogenic or non-pathogenic species. Wang and Jeffers (2000) suggested that despite the frequent isolation of *F. solani* and *F. oxysporum* from hostas plants, these appear to be weak pathogens. Nelson, Toussoun and Cook (1981) reported that *F. oxysporum* is an important component of soil micro-flora throughout the world and its pathogenicity has often been questioned. Burgess (1981) suggested that certain strains of *F. oxysporum* are pathogenic and are responsible for wilts on various plant

species. Although soils harbour large populations of non-pathogenic *F. oxysporum* both pathogenic and non-pathogenic strains are able to persist through saprophytic growth on organic matter in soil.

F. oxysporum and *F. solani* were the two species most frequently recovered from plants with crown and root rot symptoms and sorghum field soils. Other species recovered were *F. verticillioides*, *F. thapsinum*, *F. pseudonygamai*, *F. nygamai*, *F. equiseti* and *F. proliferatum*. Although it was previously stated that “isolates from maize for example are usually *F. verticillioides* while those from grain sorghum are more commonly *F. thapsinum* (Leslie and Marasas, 2002)” in the current study more *F. verticillioides* was recovered from sorghum than *F. thapsinum* or *F. andiyazi*. These species are also likely to play a significant role in Fusarium crown and root rot of sorghum. This is in contrast to previous reports. Giorda, Martinez and Chulze (1995) found that the *Fusarium* spp. associated with root and stalk rot diseases in sorghum were *F. moniliforme* (*sensu lato*) *F. proliferatum*, *F. semitectum*, *F. scirpi*, *F. nygamai*. EL-Assiuty (1982) also primarily recovered *F. moniliforme* from diseased stalks of sorghum while Leslie (2000) reported that *F. proliferatum*, *F. graminearum* and *F. verticillioides* are widely distributed in sorghum growing areas of the world.

Sorghum genotypes differed in resistance to Fusarium crown and root rot disease. Low crown and root rot severity was recorded for the resistant genotypes PAN 8706W and PAN 8564 compared with the susceptible genotype NK 283 and PAN 8446 in the glasshouse and field experiments, respectively. No sorghum genotype was completely resistant to crown and root rot of sorghum.

The present study demonstrated that Fusarium crown and root rot of sorghum and population density on sorghum roots and in rhizosphere soil was affected by the degree of genotype resistance and the amount of initial inoculum in soil before planting. Genotype resistance significantly affected change to the population density of *Fusarium* spp. on plants and in soil. Differences in the genotype reactions for field and glasshouse experiments were observed and this may be attributable to the influence of environmental conditions and field histories. The consistent recovery of *Fusarium* spp. in sorghum roots and field soil strongly suggests that crown and root rot of sorghum is caused primarily by *Fusarium* spp. The present study thus has implications for the development of strategies for effective management of Fusarium crown and root rot of sorghum. Since disease severity of the six sorghum genotypes developed in accordance

with their level of susceptibility to the pathogen resistant genotypes may be used to reduce the accumulation of *Fusarium* spp. inoculum in soil which would reduce disease incidence.

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Table 2.1: The relationship between genotype resistance, population densities of *Fusarium* spp. on sorghum roots and in soil and crown and root rot severity in the glasshouse.

Genotype	Increase in inoculum concentration CFU/g soil	Population in soil (CFU/g)	Population in roots (CFU/g)	Root rot (0-5)
Bethlehem				
NK 283	380.19a ¹	7413.10a	5370.32a	3.75a
PAN 8446	372.51b	7244.36b	5011.87b	3.00b
PAN8706W	371.54b	6456.54c	4466.84c	2.50c
Mean	374.74	7038.00	4949.68	3.08
Cedara				
NK 283	354.81a	7943.28a	5572.34a	4.00a
PAN 8446	338.53b	7762.47b	4897.79b	3.25b
PAN8706W	316.23c	7234.23c	4677.35c	2.25c
Mean	336.52	7646.66	5049.16	3.25
Potchefstroom				
NK 283	302.00a	5659.54a	5323.61a	3.25a
PAN 8446	288.40b	5495.40a	4466.84b	2.50b
PAN8706W	234.42c	5373.21b	4265.80c	1.75c
Mean	274.94	5509.38	4685.42	2.50

¹Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05)

Table 2.2: The relationship between genotype resistance, population densities of *Fusarium* spp. on sorghum roots and in soil and crown and root rot severity in the field

Genotype	Increase in inoculum concentration (CFU/g soil)	Population in soil (CFU/g)	Population in roots (CFU/g)	Root rot (0-5)
Bethlehem				
NK 283	302.00a ¹	39810.72a	9332.53a	4.33a
SNK 3939	213.80b	10715.15b	6456.51bc	3.67b
PAN 8446	223.87ab	16218.10ab	8511.38ab	4.00a
Buster	222.01ab	9332.54c	6545.65bc	3.00bc
PAN 8564	199.53c	8128.31d	6456.54b	3.25bc
Mean	232.24	15180.96	7460.52	3.65
Cedara				
NK 283	354.81a	39761.32a	10000a	4.67a
SNK 3939	281.84b	15135.62bc	7413.10b	3.67b
PAN 8446	316.23ab	30902.95b	7943.28ab	4.33ab
Buster	225.49bc	10232.93b	8317.64ab	3.66b
PAN 8564	204.01bc	8912.51c	7244.36bc	3.33bc
Mean	276.48	20989.07	8183.68	3.93
Potchefstroom				
NK 283	239.09ab	19952.63a	8912.51a	4.33a
SNK 3939	229.88b	15488.17b	7942.28ab	3.99ab
PAN 8446	239.86ab	16218.10ab	7079.45b	4.00a
Buster	280.14a	8651.63bc	3801.89d	3.30b
PAN 8564	210.02bc	7413.10bc	6309.57c	3.33b
Mean	239.80	13544.73	6809.14	3.80

¹Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05)

Table 2.3: Frequencies of *Fusarium* spp. isolated from sorghum roots and field soils from Bethlehem, Cedara, Potchefstroom and farmers fields in 2003\04.

Species Name	Bethlehem	Cedara	Potch.	Farmers field	Total
<i>Fusarium oxysporum</i>	29	34	34	16	113
<i>Fusarium verticillioides</i>	17	3	7	4	31
<i>Fusarium solani</i>	16	20	28	14	78
<i>Fusarium thapsinum</i>	6		1	2	9
<i>Fusarium pseudonygamai</i>	4	2	1		7
<i>Fusarium nygamai</i>	3	5	1		9
<i>Fusarium subglutinans</i>	3	1	2		6
<i>Fusarium proliferatum</i>	3	1	1	2	7
<i>Fusarium polyphialidicum</i>	2	5			7
<i>Fusarium pseudocicinatum</i>	2				2
<i>Fusarium beomiforme</i>	2				2
<i>Fusarium equiseti</i>	2	4		2	8
<i>Fusarium lateritium</i>	1				1
<i>Fusarium andiyazi</i>	1				1
<i>Fusarium semitectum</i>	1				1
<i>Fusarium scirpi</i>	1				1
<i>Fusarium poae</i>	1				1
<i>Fusarium napiforme</i>	1				1
<i>Fusarium culmorum</i>		4	2		6
<i>Fusarium dlamini</i>		2			2
<i>Fusarium crookwellense</i>			3		3
<i>Fusarium chlamydosporum</i>			1		1
<i>Fusarium circinatum</i>			1		1
Total	95	81	84	40	378

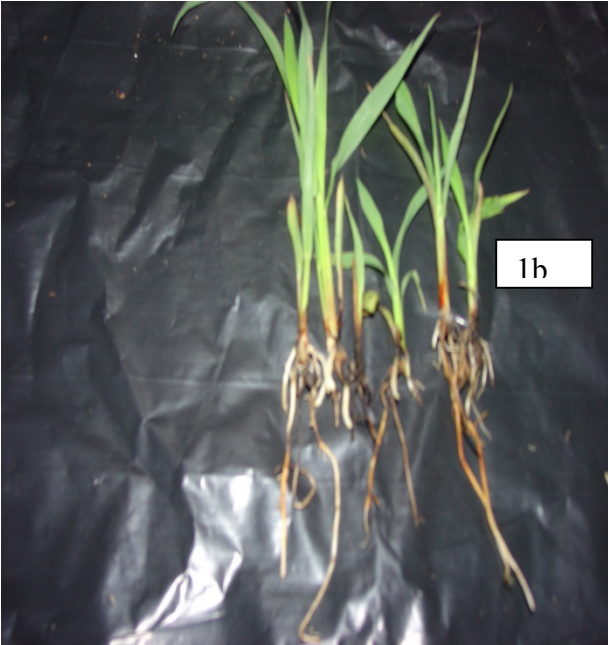


Figure 2. 1: Discolouration of the root system of (1a) PAN 8706W compared with dark coloured roots of (1b) NK 283.

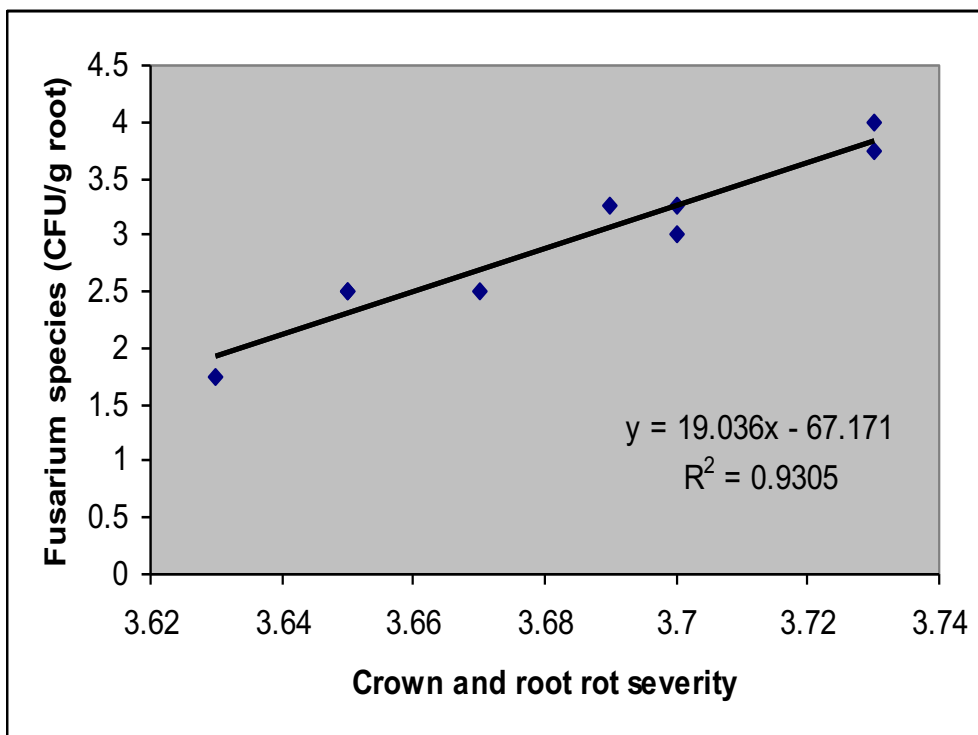


Figure 2.2: The relationship between crown and root rot severity and population density of *Fusarium* on sorghum roots in glasshouse experiments.

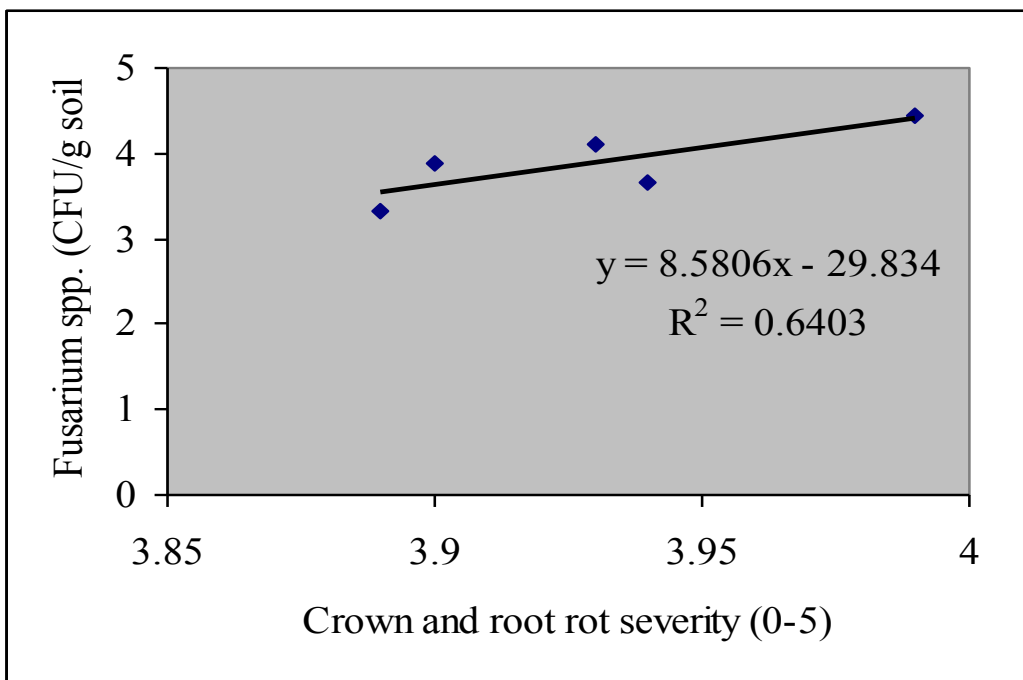


Figure 2.3: The relationship between crown and root rot severity and population density of *Fusarium* in field soil.

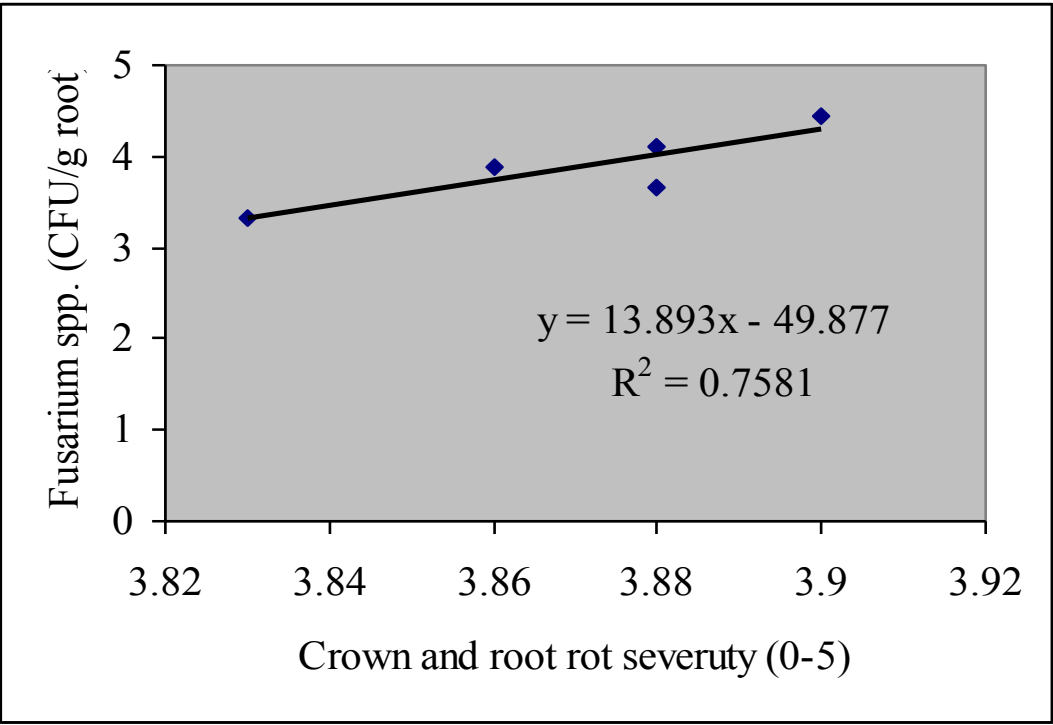


Figure 2.4: The relationship between crown and root rot severity and population density of *Fusarium* in field soil.

CHAPTER III

EFFECT OF SOILBORNE PATHOGENS AND *FUSARIUM* SPP. ON SORGHUM CROWN AND ROOT ROT

ABSTRACT

The effect of *Fusarium* spp. on host plant growth and development was studied in three glasshouse and two field experiments on six sorghum genotypes. There was a reduction in shoot and root mass with an increase in crown and root rot severity. Three hundred fungal isolates, recovered from field soil and sorghum roots, were characterized and identified according to species. Twenty isolates from the most frequently isolated species were selected to evaluate their effect on pre- and post-emergent sorghum. Genotype IA 57 was used as the host in the glasshouse. *Fusarium equiseti*, *F. thapsinum*, and *F. solani*, were consistently highly pathogenic while *F. nygamai*, *F. proliferatum*, *F. subglutinans*, and *F. verticillioides* were moderately pathogenic and *F. oxysporum*, *F. polyphialidicum*, and *F. pseudonygamai* were significantly less pathogenic in all experiments. *Fusarium equiseti* consistently caused the highest degree of root rot while the lowest degree was caused by *F. oxysporum* and *F. pseudonygamai* in both the pre- and post- emergent experiments. All *Fusarium* spp. tested were pathogenic to sorghum to varying extents. The present study demonstrated that the *Fusarium* spp. tested could cause crown and root rot of sorghum and that pathogenicity varied within species. The most pathogenic *Fusarium* spp. were *F. equiseti*, *F. nygamai*, *F. proliferatum*, and *F. thapsinum*. These species caused the highest crown and root rot severity ratings. The area under disease progress curve was highest for *F. nygamai* and *F. thapsinum*. Fresh plant mass did not always correlate with crown and root rot severity. A genotype response was observed with PAN 8706W displaying the highest shoot mass and PAN 8446 which displayed the lowest. NK 283 displayed largest AUDPC for crown and root rot severity while the smallest AUDPC was for PAN 8706W.

INTRODUCTION

Soil is a medium for plant growth and development as well as a habitat for soilborne pathogens. Understanding the population dynamics, ecology, and epidemiology of soilborne cereal pathogens may provide new management strategies that allow growers to adopt direct seeding systems without losses due to major soilborne diseases (Cook, 2001).

Soilborne pathogens of cereals are associated with the invasion of the crown and root tissues and interference with the capacity for efficient water and nutrient uptake. In wheat, soilborne pathogens are widely distributed and result in economic yield losses. Soilborne pathogens negatively influence wheat root growth and development from planting to harvest (Singleton, 2006). In sorghum (*Sorghum bicolor* (L.) Moench), soilborne pathogens most commonly associated with root rot diseases are *Macrophomina phaseolina* (Tassi), *F. moniliforme* (*sensu lato*) (Sheldon), *Periconia circinata* (L. Mangin) Sacc., *Pythium* spp, and *Colletotrichum graminicola* (Ces.), Wils. (Sny. *C. sublineolum* Henn. Kab. & Bubak), (Pande and Karunakar, 1992). *F. moniliforme* (*sensu lato*) is an important soilborne pathogen associated with root and stalk rot of maize and sorghum (Leslie *et al.*, 1990). Other soilborne pathogens associated with root and stalk rot are *F. thapsinum* Klittich Leslie Marasas and *F. proliferatum* (Matsushima) (Leslie, 2000).

Fusarium spp. are associated with sorghum throughout its life cycle, from seed germination to senescence. These fungi are associated with seedling blight, root and stalk rot, grain mould, storage diseases and mycotoxicoses (Leslie, 2000). Although *Fusarium* spp. can survive in sorghum with no obvious disease symptoms, the absence of symptoms should not be equated with the absence of the fungi (Leslie, 2000). *Fusarium* spp. associated with root and stalk rot include *Fusarium Verticillioides* (Sacc.) Nirenberg, *F. equiseti* (Cda.) Sacc., *F. graminearum* (Schw.), *F. napiforme* Marasas *et al.* (1987) *F. nygamai* Burgess & Trimboli, *F. oxysporum* Schlecht. emend. Snyder & Hansen, *F. proliferatum* (Matsushima) Nirenberg, *F. semitectum* Berk. & Rav., *F. solani* (Mart.) Appel & Wollenw. emend. Snyder & Hansen, *F. subglutinans* (Wollenw. & Reinking), *F. thapsinum* Klittich *et al.* (1997), and *F. trincictum* (Corda) Sacc. (Leslie, 2000).

Most of the listed *Fusarium* spp. are reported to colonize sorghum root and stalk tissues, but the pathogenic association with sorghum has not been proven. *Fusarium thapsinum* has however, been shown to reduce sorghum seedling vigour (Little and Magill, 2003). Pathogenicity tests of *Fusarium* spp. on cereal heads, or roots and crowns have been conducted and the most pathogenic species were *F. culmorum* (W. G. Smith) Sacc. and *F. graminearum* (Schw.) (Colhoun, Taylor and Tomlison, 1968; Uoti, 1976; Stack and McMullen, 1985; Kane and Smiley, 1987; Specht and Rush, 1988; Wilcoxson *et al.*, 1988; Mihuta-Grimm and Forster, 1989; Arseniuk, Goral, Czembor, 1993; Jenkinson and Parry, 1994; Wong *et al.*, 1995; Walker *et al.*, 2001; Golinski *et al.*, 2002).

Soilborne pathogens limit cereal production under many different environments (Saari, 1985; Weise, 1987) and their impact can be observed in terms of seedling and mature plant effects (Zillinsky, 1983; Weise, 1987). Seedling disease complexes are characterized by poor stands and pre- and post-emergence damping-off which may require replanting (Zillinsky, 1983; McLaren, 1987; Weise, 1987). The post-emergence presence of root rot pathogens result in plant failure to respond to nitrogen application leading to true nitrogen deficiencies (Huber and Graham, 1992).

A detailed understanding of variation in pathogenicity and virulence in the pathogen populations is necessary to enable effective selection for disease resistance in breeding programs. High variation in pathogenicity (aggressiveness) within and among species has been detected (Miedaner, Gang and Geiger, 1996; Miedaner and Schilling, 1996; Carter *et al.*, 2002). Leslie and Mansuetus (1995) suggested that it is unlikely that all strains of *F. moniliforme* are equally virulent and identification of the significant avirulent strains will require a more detailed analysis of appropriate populations.

Variation in pathogenicity within *Fusarium* spp. has been observed among the isolates of *F. lateritium* Nees recovered from sweet potato seed of vines grown in the glasshouse. Many isolates clearly induced strong chlorosis symptoms, some produced transient or less definitive response, and some consistently colonized the plant surface without inducing chlorosis (Clark, Hoy and Nelson, 1995). Similar, variation in virulence has been observed among isolates of *F. solani* and *F. oxysporum* by Wang and Jeffers (2000) who found that the isolates varied in virulence when hostas plants were inoculated.

The objectives of the present study were, (i) to evaluate the effect of soilborne pathogens on plant growth and the development of crown root rot in the glasshouse and field; (ii) to determine the pathogenic effect of *Fusarium* spp. isolated from field soils and sorghum roots on pre- and post-emergent sorghum; and (iii) to determine variation in pathogenicity among *Fusarium* spp. pathogenic to sorghum.

MATERIALS AND METHODS

Effect of soilborne pathogens on host plant growth and development.

Glasshouse experiments. Plastic pots (100 cm in height x 60 cm in diameter) were filled with field soils collected from Bethlehem (28° 15' S and 28° 20' E) in the Free State Province, Cedara (29° 36' S and 30° 26' E) in the KwaZulu Natal Province and Potchefstroom (26° 43' S and 23° 6' E) in the North West Province in South Africa. The soils were sandy loam (Bethlehem), clay loam (Cedara), and heavy clay (Potchefstroom). Soils were collected a week before use. Nine pots were prepared for each soil type.

Sorghum seeds from each of the commercial genotypes NK 283, PAN 8446 and PAN 8706W with variable levels of resistance were surface disinfested by dipping seeds in 70 % ethanol for 30 seconds (Salas *et al.*, 1999). This was followed by surface sterilization in 0.05% NaOCl for 2 minutes, three rinses in sterile distilled water and then drying on a paper towel. Sterilized seeds were planted at a rate of 5 seeds per pot to a depth of 3 cm. Pots were laid out in a randomized complete block design and replicated three times. Pots were maintained in the glasshouse at 30 / 21°C day and night regime and watered daily. Two weeks after emergence, seedlings were fertilized with limestone ammonium nitrate (LAN-28% a.i) at a rate of 1 g per kg soil. Fertilization was repeated every two weeks.

After six weeks, three plants were removed from each pot and the roots were thoroughly washed in running tap water. Plants were severed at the crown and fresh shoot and root mass measured. Crown and root rot severity was assessed on a 0-5 scale (Correll, Puhalla and Schneider, 1986) where; 0 = no root discoloration; 1 = slight vascular discoloration in root tissue, 2 = extensive vascular discoloration in root

tissue, 3 = vascular discolouration in crown; 4 = extensive vascular discolouration in crown, 5 = crown completely necrotic. The experiment was repeated three times.

Field experiments. Field trials were conducted in Bethlehem, Cedara and Potchefstroom during the 2003 / 2004 cropping season. Plots consisted of 10 rows 8 m in length with a 10 cm intra-row spacing. An inter-row spacing of 0.85 was used at Cedara and 1.2 m at Bethlehem and Potchefstroom respectively. Treatments were evaluated in a randomized complete block design and replicated four times. A path of 1 m was left between plots and replications and the first and last rows of each plot were regarded as border rows. Plots were fertilized with NPK 3:2:3 (21 % a.i) at 300 kg pre-planting.

Sorghum genotypes NK 283, SNK 3939, Buster, PAN 8446, and PAN 8564 were treated with Concep II (herbicide seed safener) and planted at two dates in the field from early-November to mid-December in 2003. Weeds were controlled with Metalochlor/ Terbutylazine (a.i 125 / 375 g litre) at a rate of 4 litres per hectare.

After nine weeks, five plants were randomly selected from the central three rows of each plot. Crowns and roots were gently rinsed in water to remove soil and dried with paper towels. Plants were then air dried at room temperature (22 - 24°C) for 30 minutes before individual plants were cut at the crown and fresh shoot and root mass measured. Crown and root rot severity was assessed on a 0-5 scale (Correll *et al.*, 1986) described above. The experiment was repeated during 2004/2005 season.

Effect of *Fusarium* isolates on pre- and post-emergent sorghum.

The sorghum genotype IA 57 was used as the host in the glasshouse to evaluate the effect of twenty *Fusarium* isolates (two from each species) on pre- and post-emergent sorghum in two independent experiments.

Oat grain inoculum. Inoculum was prepared by soaking 100g oat grains in 250 ml bottles in tap water overnight. Water was drained and grains were autoclaved twice for 20 min at 121°C on each of two consecutive days. Each bottle was seeded with potato dextrose agar plugs of the respective *Fusarium* isolates and five bottles were prepared for each isolate. Test isolates were *F. equiseti*, *F. nygamai*, *F. oxysporum*, *F. polyphialidicum* Marasas, *F. proliferatum*, *F. pseudonygamai* O'Donnell & Nirenberg, *F. solani*, *F. subglutinans*, *F. thapsinum*, and *F. verticilliioides*. The bottles were

maintained in a sporulation room at 25°C and shaken daily until seeds were completely colonized (approximately 21 days). The colonized seeds were then removed from the bottles, spread on aluminium foil and air dried at room temperature (25°C) which took approximately 10 days (Wang and Jeffers, 2000; Clark *et al.*, 1995; Dorrance *et al.*, 2003). Dry oat seeds were ground in a laboratory blender to pass through 200 µm sieve for inoculations.

Pre-emergent sorghum. Plastic glasshouse pots (500 ml) were filled with a steam sterilized soil: peat (3:1) mixture. The soil mixture was inoculated with 5 g of ground colonized oat grain inoculum and thoroughly mixed with the upper 5 cm layer of the soil (Van Wyk *et al.* 1988). Four pots were prepared per test isolate.

Seeds of sorghum genotype IA 57 were disinfested and sterilized as described above (Salas *et al.*, 1999). Five sterilized sorghum seeds were planted into the inoculated soil mixture to a depth of 3 cm. The pots were laid out in a randomized complete block design and replicated four times. Plants were watered daily and maintained in the glasshouse for six weeks at 30/21 °C day and night regime.

Two weeks after emergence seedlings were fertilized with limestone ammonium nitrate (LAN-28 % a.i) at a rate of 1 g per kg soil. The fertilizers were subsequently applied every two weeks. After six weeks, three plants were removed from each pot. Crowns and roots were gently rinsed in water to remove soil and dried with paper towels. Plants were then air dried at room temperature (22 – 24 °C) for 30 minutes before individual plants were cut at the crown and fresh shoot and root mass measured. Crown and root rot severity was determined on a 0-5 scale (Correll *et al.*, 1986) as described above. The experiment was conducted twice.

Post-emergent sorghum. Sorghum seeds of genotype IA 57 were disinfested and surface sterilized as described (Salas *et al.*, 1999) above. The sterilized seeds were pre-germinated in polystyrene trays lined with sterile germinating paper. The containers were covered with black polythene bags which served as a humidity chamber and placed in a glasshouse cubicle with a mean temperature of 25 °C (± 3) for 11 days.

Seedlings were removed from polystyrene trays and transplanted into 500 ml pots containing a steam sterilized soil: peat (3:1) mixture prepared and inoculated with ground colonized oat seeds inoculum prepared as described above (Wang and Jeffers, 2000; Clark *et al.*, 1995; Dorrance *et al.*, 2003). Five seedlings were transplanted into each of the four pots per test fungus. Pots were laid out in a randomized complete block design and replicated four times. Plants were watered daily and maintained in the glasshouse for six weeks at 30/21 °C day night regime. Two weeks after emergence/transplanting seedlings were fertilized with limestone ammonium nitrate (LAN-28% a.i) at a rate of 1 g per kg soil. The fertilizers were subsequently applied every two weeks. After six weeks, three plants were removed from each pot, treated and evaluated as described above. The experiment was conducted twice.

Variation in pathogenicity of *Fusarium* spp.

Inoculum was prepared as described above (Wang and Jeffers, 2000; Clark *et al.*, 1995; Dorrance *et al.*, 2003). Inoculum was prepared for each of the test isolates i.e *F. equiseti*, *F. nygamai*, *F. oxysporum*, *F. polyphialidicum*, *F. proliferatum*, *F. solani*, *F. subglutinans* and *F. thapsinum*. Pots (500 ml) containing steam sterilized soil: peat (3:1) mixture were prepared and inoculated with 5 grams of ground oat grain inoculum thoroughly mixed in the upper 5 cm of the soil (Van Wyk *et al.*, 1988).

Seeds of three sorghum genotypes (NK 283, PAN 8446 and PAN 8706W) were immersed in 70% ethanol for 30 seconds, surface sterilized in 0.05 % NaOCl for 2 minutes and rinsed three times in sterile distilled water (Salas *et al.*, 1999). Seeds were dried on a paper towel. Five seeds per genotype were planted in each of 36 pots to a depth of 3 cm. The treatments were arranged in a randomized complete block design and replicated four times. Plants were watered daily and maintained in the glasshouse for six weeks at 30/21°C day and night regime. Two weeks after emergence, seedlings were fertilized with limestone ammonium nitrate (LAN-28% a.i) at a rate of 1 g per kg soil. Fertilization at the same rate was repeated every two weeks.

Sampling was done three times at 14, 28, and 42 days after planting. Three plants were removed from each pot. Crowns and roots were gently rinsed in water to remove soil and dried with paper towels. Plants were then air dried at room temperature (22 - 24°C) for 30 minutes before individual plants were cut at the crown and fresh shoot and root mass measured. Crown and root rot severity was assessed on a 0-5 scale

(Correll *et al.* 1986) as described above. Plots of mean disease severity values on days after planting were used to produce an area under disease progress curve (AUDPC) using the formula of Shaner and Finney (1977). Where; $AUDPC = \sum_{i=1}^{n-1} \frac{X_i + X_{i+1}}{2} (t_{i+1} - t_i)$, in which X_i = root rot severity at the i th observation, t = time (days) at the i th observation, and n = total number of observations. The experiment was repeated.

Data analysis.

Data were analyzed using the general linear model (GLM) and ANOVA in SAS 9.1 (SAS Institute Cary NC). The data from repeated experiments were combined for analysis based on tests for homogeneity of variance. Treatment means were compared using the Fisher's least significant difference (LSD) with ($P \leq 0.05$). The ANOVA and GLM means from individual treatments were compared if interactions were significant ($P \leq 0.05$). Where interactions were not significant ($P \leq 0.05$) individual treatments were combined to evaluate effects of treatments in the two main effects. The pathogenicity or isolate aggressiveness was described as follows: N = non-pathogenic, no disease = 0; L=low (1 to 20 % disease) M= moderate (21 to 60 % disease) H= high (61-100 % disease) (Abad, Shew and Lucas, 1994).

RESULTS

Effect of soilborne pathogens on host plant growth and development

Glasshouse experiments. Data from three glasshouse experiments were homogenous and thus combined for analysis. No significant locality x genotype interaction was recorded. Locality significantly ($P \leq 0.0001$) affected shoot mass and root mass. Highest shoot and root mass were recorded on plants grown on soil collected at Cedara (Table 3.1). Genotype significantly ($P \leq 0.01$) affected shoot and root mass (Table 3.1). Greatest shoot and root mass was observed for NK 283 compared to least for PAN 8446 grown on the soil collected from Potchefstroom and root mass was least for PAN 8446 grown on soil collected from Bethlehem (Table 3.1).

Genotype significantly ($P \leq 0.05$) affected crown and root rot severity. Greatest mean crown and root rot severity ratings were recorded for plants grown in soil collected from Bethlehem (Table 3.1). Greatest crown and root rot severity was

recorded for NK 283 grown on soil collected from Bethlehem and least was observed for PAN 8706W grown on soil collected from Potchefstroom (Table 3.1).

Field experiments. Field data from the two experiments were homogenous and therefore combined for analysis. Genotype significantly ($P \leq 0.01$) affected root mass. Plants grown at Bethlehem had significantly greater mean root mass than Cedara (Table 3.2). Highest root mass was shown for PAN 8446 grown at Bethlehem compared to the least for Buster grown at Cedara (Table 3.2).

Genotype significantly ($P \leq 0.05$) affected crown and root rot severity. Mean crown and root rot severity was greatest on plants grown at Bethlehem compared to those grown at Potchefstroom (Table 3.2). Genotype NK 283 grown at Bethlehem had the greatest crown and root rot severity ratings compared to the least for SNK 3939 grown at Potchefstroom (Table 3.2).

Effect of *Fusarium* isolates on pre- and post-emergent sorghum.

Data for fresh shoot and root mass, crown and root rot severity from two independent experiments were homogeneous and thus combined for analysis.

Pre-emergent sorghum. In the pre-emergent sorghum, all the tested *Fusarium* spp. were pathogenic on the host IA 57. *Fusarium* isolates significantly affected shoot and root mass ($P \leq 0.0001$), crown and root rot ($P \leq 0.002$) and percentage emergence ($P \leq 0.05$). *F. equiseti* caused the greatest reduction in seed emergence compared to *F. subglutinans* and the control (Table 3.3).

F. pseudonygamai did not cause a significant reduction in shoot mass compared with the control treatment. Seedlings inoculated with *F. equiseti* had the lowest fresh shoot and root mass (Table 3.3). Concomitant decreases in seedling emergence, root and shoot mass were recorded. Crown and root rot severity in pre-emergent sorghum was highest for plants inoculated with *F. equiseti* and lowest for *F. oxysporum* and *F. pseudonygamai* (Table 3.3).

Post-emergent sorghum. In the post-emergent sorghum, all the tested *Fusarium* spp. were pathogenic. Significant ($P \leq 0.05$) differences in fresh shoot and root mass of seedling resulted. *F. oxysporum* did not cause a significant reduction in shoot and root

mass which were lowest for *F. equiseti* (Table 3.4). *Fusarium* spp significantly ($P \leq 0.05$) affected crown and root rot, ($P \leq 0.002$) percentage mortality. Results recorded for crown and root rot severity and percentage mortality were greatest for *F. equiseti* and least for *F. oxysporum* (Table 3.4). There was a linear relationship between shoot mass and root rot severity and also between root mass and root rot severity for both the pre- and post-emergent sorghum (Figs. 3.1 and 3.2).

Variation in pathogenicity of *Fusarium* spp.

Data on fresh shoot and root mass, crown and root rot severity, and AUDPC from two independent experiments were homogeneous and thus combined for analysis. Genotype ($P \leq 0.0001$) and *Fusarium* spp. ($P \leq 0.04$) significantly affected percentage emergence. No significant interaction between cultivar and *Fusarium* spp. was evident. Seedling emergence varied among the *Fusarium* isolates and the genotypes from 30% to 100% with the greatest variable effect observed for *F. nygamai* (Table 3.5). The greatest genotype effect on seedling emergence was observed on sorghum genotype NK 283 compared to PAN 8446 (Table 3.5).

Genotype ($P \leq 0.01$) and *Fusarium* spp. ($P \leq 0.05$) significantly influenced shoot mass and root mass. Genotype x *Fusarium* spp. interactions did not significantly influence shoot and root mass. Mean fresh shoot and root mass were lowest for plants inoculated with *F. solani* compared to the control (Table 3.5). All eight *Fusarium* spp. varied significantly in terms of shoot mass and root mass of sorghum seedlings when compared to un-inoculated control plants (Table 3.5). Sorghum genotypes differed in response to *Fusarium* isolates. Mean fresh shoot and root mass were highest for PAN 8706W and lowest for PAN 8446 (Table 3.5). A significant genotype x *Fusarium* spp. interaction ($P \leq 0.001$) effect on crown and root rot severity and ($P \leq 0.01$) AUDPC were recorded. Crown and root rot severity ratings ranged from 2.00 to 5.00 and AUDPC ranged from 84.00 to 210.00. Crown and root rot severity varied greatly between the *Fusarium* isolates and sorghum genotypes (Table 3.5). Crown and root rot severity and AUDPC were consistently greatest for genotype NK 283 compared to the least for PAN 8706W while PAN 8446 was intermediate (Table 3.5).

DISCUSSION

The present study aimed at evaluating the effect of soilborne pathogens on plant growth and the pathogenicity of *Fusarium* spp. on sorghum. Verma, Morrall and Tinline (1976) suggested that a reduction in root growth would explain the association of lower yield and biomass production of individual plants with severe root rot ratings. Disease severity did however, not always correspond with a reduction in plant mass. Wang and Jeffers (2000) found that disease severity in container grown hostas did not always correlate well to plant weight and similar trends have been reported for other root rot diseases on crops such as beans, sugar beet and peas (Burke and Barker, 1966; Harveson and Rush, 1988; Rush and Craft, 1986). In the present study, NK 283 often displayed both the highest root mass and crown and root rot severity. The root system for this genotype was generally more discoloured when compared with PAN 8706W which displayed the lowest root mass with little or no discolouration. Kokko *et al.* (1994) found that regardless of the differences in crown and root rot ratings, wheat plants with larger root systems were usually most discoloured. This suggests that plants with larger root systems are more likely to be discoloured simply because of the increase in the root surface area that provides the pathogen with more potential infection sites. Plants with larger root systems may also compensate for root loss damage caused by soilborne pathogens (McLaren, 2002).

Twenty isolates of *Fusarium* spp. tested in the two pre- and post-emergent sorghum experiments were found to be pathogenic to varying degrees on inoculated sorghum plants. In the present study the greater pathogenicity on sorghum seedlings of the isolates of *F. equiseti*, *F. nygamai*, *F. solani*, *F. subglutinans* and *F. thapsinum* and *F. verticillioides*, than other *Fusarium* isolates is consistent with previous reports and with Wang and Jeffers (2000) who found that *F. oxysporum*, *F. solani* and *Fusarium* spp were pathogenic to hostas and caused crown and root rot severity.

F. equiseti consistently caused greatest reductions in shoot and root mass and exhibited the greatest crown and root rot severity ratings in both pre- and post emergent sorghum. This was also recorded in the virulence tests. Arsenuik *et al.*, (1993) and Fernandez and Chen (2005) reported that *F. equiseti* caused retardation on plant emergence, growth and development of wheat seedlings. Similarly, Akinsanmi *et al.*, (2004) found that *F. equiseti* caused crown rot and head blight of wheat. In contrast, Wong *et al.* (1995) found *F. equiseti* to be least pathogenic on winter wheat when

compared with the ability of the highly pathogenic *F. graminearum* Schwabe and *F. culmorum* (W. G. Smith) Saccardo to induce Fusarium head blight.

Variation in virulence of eight *Fusarium* spp. found to be pathogenic on sorghum and cause crown and root rot of sorghum were evaluated in the glasshouse. The symptoms displayed on the inoculated plants ranged from very faint lesions on the outer leaf sheath to intense discolouration on the leaf sheath and browning of the crown. The significant effect on inoculated seedlings was observed for *F. nygamai* which resulted in 60% inhibition of emergence on NK 283 and a 100% emergence for PAN 8446. *F. nygamai* also caused variation on plant growth with highest shoot and root mass recorded for PAN 8706W and lowest for NK 283. The greatest disease severity in terms of crown and root rot and AUDPC were observed on NK 283 and least for PAN 8706W. Overall, sorghum genotype PAN 8706W indicated a significant resistance reaction across all the measured parameters. It resulted in the highest, seed emergence, shoot and root mass with the lowest diseases severity ratings in terms of crown and root rot severity and AUDPC. PAN 8446 was intermediate and NK 283 was susceptible.

Variation in pathogenicity was demonstrated among the tested *Fusarium* isolates with *F. equiseti*, *F. nygamai*, *F. proliferatum* and *F. thapsinum* being most virulent species on sorghum as measured by AUDPC of crown and root rot severity, *F. polyphialidicum* and *F. subglutinans* were intermediate whereas *F. solani* and *F. oxysporum* were least virulent. The *Fusarium* isolate aggressiveness was genotype dependent. The current study results are consistent with Wang and Jeffers (2000) who found variation in virulence among isolates of *Fusarium* spp., *F. oxysporum* and *F. solani* on inoculated hostas plants. Another form of variation in pathogenicity was reported by Clark *et al.* (1995) who found that many isolates of *F. lateritium* induced strong chlorosis symptoms, some produced a transient or less definitive response and some consistently colonized the plant surface without inducing chlorosis. Walker *et al.* (2001) found that *F. graminearum* population in North Carolina was diverse and some isolates produced on average 80 to 100% disease severity on the three wheat cultivars tested, whereas others were much less virulent. An ability to detect differences in virulence may thus be a function of the number of isolates tested.

The present study found that crown and root rot of sorghum resulted from inoculum in the soil, which may have been produced during the previous year(s) of

cropping. It was also evident that these soilborne pathogens produce resistant spores or survive as mycelium in decaying roots and are capable of causing crown and root rot in subsequent crops. Differences in pathogenicity among *Fusarium* spp. appeared related to the source of the isolates and all the tested *Fusarium* isolates were proven pathogenic at varying extent to grain sorghum and capable of causing crown and root disease. The current study demonstrates the need to consider variability in pathogen aggressiveness in screening and selection of host plant resistance.

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Table 3.1. Crown and root rot severity associated with soils from three localities and its effect on plant growth

Genotype	Shoot mass (g)			Root mass (g)			Crown and root rot severity (0-5)		
	Bethlehem	Cedara	Potchefstroom	Bethlehem	Cedara	Potchefstroom	Bethlehem	Cedara	Potchefstroom
NK 283	8.88b ¹	10.23a	11.12a	5.12a	3.56b	5.90a	3.56a	2.89a	2.78b
PAN 8446	8.91ab	10.08ab	7.90b	3.80b	5.84a	4.30b	2.67b	2.89a	3.22a
PAN 8706W	10.07a	8.74b	9.04ab	4.07ab	4.39ab	4.61ab	2.22c	2.00b	1.99c
Mean	9.29	9.69	9.36	4.29	4.60	4.94	2.81	2.59	2.66

¹ Means within the same parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).

Table 3.2. Crown and root rot severity associated with soils from three localities and its effect on plant growth

Genotype	Root mass (g)			Crown and root rot severity (0-5)		
	Bethlehem	Cedara	Potchefstroom	Bethlehem	Cedara	Potchefstroom
Buster	31.07bc ¹	17.62c	36.95a	3.67ab	2.67c	3.56a
NK 283	33.45b	20.52b	31.55b	3.17b	3.50a	3.00b
PAN 8446	43.42a	18.35bc	27.85c	3.83a	3.17b	2.67bc
PAN 8564	29.13c	29.57a	32.78ab	3.00c	3.33ab	3.33ab
SNK 3939	36.40ab	23.97ab	28.65bc	3.17b	3.33ab	2.50c
Mean	34.69	22.00	31.56	3.37	3.20	3.00

¹ Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).

Table 3.3: Effect of *Fusarium* spp. on shoot mass, root mass, crown and root rot severity and percentage emergence on sorghum genotype IA 57 planted in the glasshouse for pre-emergent analysis

<i>Fusarium</i> isolates	Shoot mass	Root mass	Root rot severity	Emergence %
<i>F. equiseti</i>	0.37e	0.11e	4.38a	56.50b
<i>F. nygamai</i>	2.35de	0.62d	3.25b	43.94cd
<i>F. oxysporum</i>	5.96bc	2.64bc	2.38c	45.63c
<i>F. polyphialidicum</i>	2.70d	1.18cd	2.88bc	50.23bc
<i>F. proliferatum</i>	3.77cd	1.58c	2.89bc	47.00c
<i>F. pseudonygamai</i>	10.65ab	5.50ab	2.00c	56.75b
<i>F. solani</i>	6.18b	2.85bc	3.13b	55.00b
<i>F. subglutinans</i>	3.87cd	1.36cd	3.38b	62.75ab
<i>F. thapsinum</i>	4.34c	1.86c	3.37b	53.38bc
<i>F. verticillioides</i>	2.14de	0.85d	3.63ab	51.88bc
Control	13.66a	8.63a	0.00e	78.57a

¹Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05)

Table 3.4: The effect of *Fusarium* spp. on shoot mass, root mass, crown and root rot severity and percentage mortality on sorghum genotype IA 57 planted in the glasshouse for post-emergent analysis

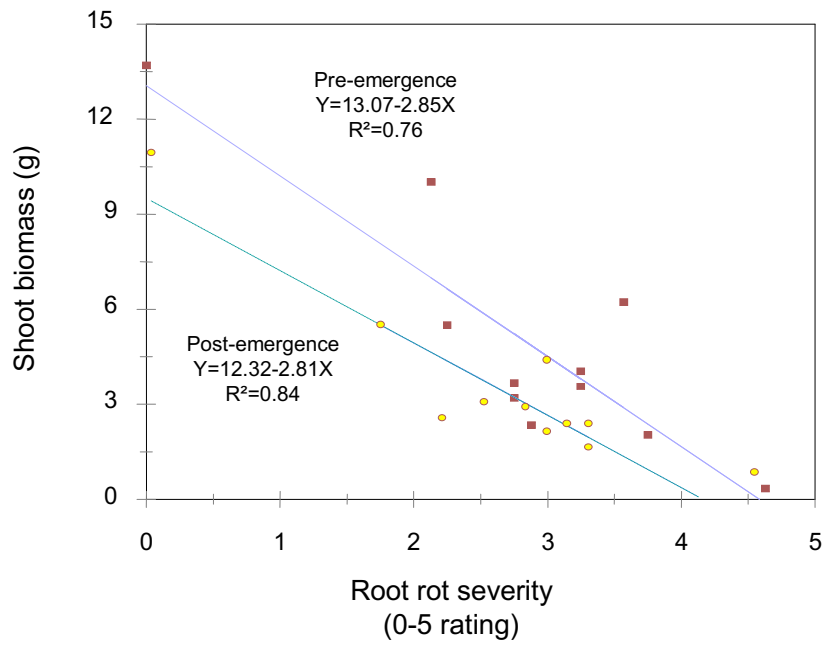
<i>Fusarium</i> isolates	Shoot mass	Root mass	Root rot severity	Mortality %
<i>F. equiseti</i>	0.81e	0.44d	4.63a	50.25b
<i>F. nygamai</i>	1.66d	0.49d	3.63ab	55.01ab
<i>F. oxysporum</i>	5.43ab	1.83b	2.38c	29.88d
<i>F. polyphialidicum</i>	2.53c	0.79cd	3.25b	53.50b
<i>F. proliferatum</i>	2.65c	0.77cd	3.38b	44.25c
<i>F. pseudonygamai</i>	4.95b	1.67b	3.00b	50.51b
<i>F. solani</i>	3.31bc	1.23c	3.38b	51.75b
<i>F. subglutinans</i>	2.50c	0.89cd	2.75bc	48.75bc
<i>F. thapsinum</i>	2.81c	1.01c	3.63ab	54.88b
<i>F. verticillioides</i>	2.01cd	0.58cd	3.50ab	61.13a
Control	11.02a	6.15a	1.00c	28.25d

¹ Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05)

Table 3.5: The effect of *Fusarium* spp on variation in pathogenicity on sorghum genotypes tested in the glasshouse

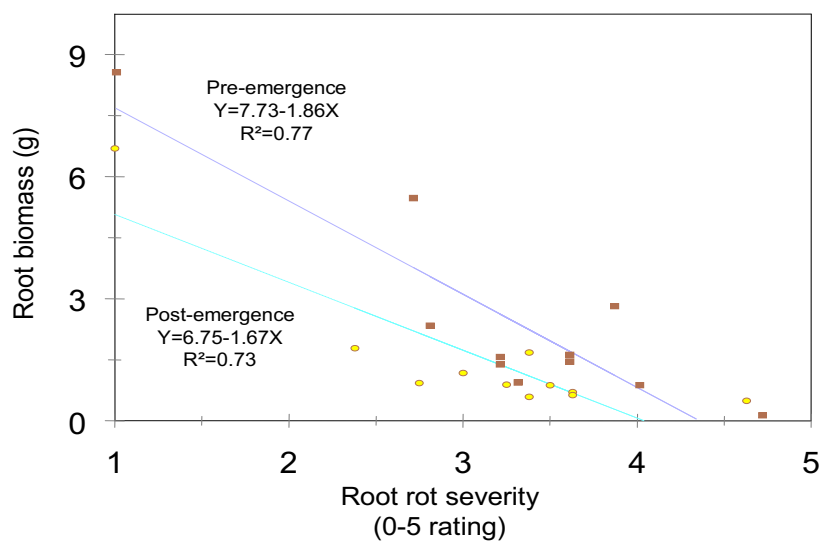
<i>Fusarium</i> isolates	Emergence %			Shoot mass (g)			Root mass (g)			Root rot (g)			AUDPC		
	NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W
<i>F. equiseti</i>	50.00bc	60.00b	100.00a	1.70d	2.05bc	7.10a	0.45e	1.75d	6.05a	5.00a	5.00a	2.00bc	210.00a	210.00a	84.00d
<i>F. nygamai</i>	40.00c	100.00a	90.00ab	3.00c	3.90ab	7.25a	1.55c	2.30c	5.30b	5.00a	4.50ab	3.00ab	210.00a	189.00ab	126.00b
<i>F. oxysporum</i>	60.00b	60.00b	100.00a	3.05c	2.85b	6.35ab	1.85c	3.30b	4.85bc	3.50bc	3.50bc	2.50b	147.00c	147.00c	105.00c
<i>F. polyphialidicum</i>	60.00b	80.00ab	100.00a	4.30b	1.80bc	5.55b	3.50b	0.85e	3.35c	3.00c	5.00a	2.50b	126.00d	210.00a	105.00c
<i>F. proliferatum</i>	60.00b	60.00b	90.00ab	4.10b	2.90b	6.05ab	1.20c	2.20c	5.40b	5.00a	4.00b	3.00ab	210.00a	168.00b	126.00b
<i>F. solani</i>	30.00d	40.00c	80.00b	1.45d	1.30c	2.60c	1.25c	0.55e	2.10d	4.50ab	3.00c	2.00bc	189.00ab	126.00d	84.00d
<i>F. subglutinans</i>	80.00a	60.00b	90.00ab	6.00ab	4.55b	5.85b	3.15b	3.25b	3.15c	4.50ab	3.50bc	2.00bc	189.00ab	147.00c	84.00d
<i>F. thapsinum</i>	80.00a	60.00b	80.00b	5.10b	1.30c	1.70d	4.05ab	0.80e	1.40e	4.00b	5.00a	3.50a	168.00b	210.00a	147.00a
Control	70.00ab ¹	100.00a	100.00a	7.30a	7.60a	6.80ab	6.15a	6.50a	5.20b	0.00d	0.00d	0.00c	0.00e	0.00e	0.00e
Mean	58.89	68.89	92.22	4.03	5.47	3.14	2.57	2.39	4.09	3.83	3.72	2.28	161.00	156.00	95.67

¹Means with a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).



The relationship between root rot severity and shoot biomass

Fig. 3.1: The relationship between shoot biomass and crown and root rot severity on sorghum genotype IA 57 evaluated for pre-emergent and post-emergent sorghum



The relationship between root biomass and root rot

Fig. 3.2: The relationship between root biomass and crown and root rot severity on sorghum genotype IA 57 evaluated for pre-emergent and post-emergent sorghum

CHAPTER IV

ASSESSING THE RESISTANCE OF SORGHUM GENOTYPES TO CROWN AND ROOT ROT OF *FUSARIUM* SPP. IN THE GLASSHOUSE

ABSTRACT

Reliable glasshouse assays are required to differentiate types of resistance to *Fusarium* crown and root rot of sorghum. Four inoculation techniques were used to inoculate sorghum plants with single spore isolates of *Fusarium equiseti*, *F. proliferatum*, *F. solani*, and *F. thapsinum*. Two sources of inoculum, colonized oat seeds and conidial suspensions, were used to inoculate wounded and non-wounded sorghum genotypes Buster, NK 283, PAN 8446, PAN 8564, PAN 8706W and SNK 3939. Disease symptoms occurred consistently on sorghum plants where wounded crowns and roots were inoculated with ground colonized oats grain or drenched with a conidial suspension of 1×10^6 conidia per ml. Sorghum genotypes SNK 3939 and Buster were most susceptible compared to least susceptible PAN 8706W. Fresh shoot and root mass were most reduced when plant crown and roots were wounded and inoculated with ground colonized oats grain. The effect of inoculum concentration on crown and root rot was evaluated on three sorghum genotypes, IA 57, TX 2853 and TX 2793 selected from root rot evaluation trials. Conidial suspensions prepared from isolates of *F. proliferatum* and *F. thapsinum* were applied to the crown and roots at concentrations of 1×10^4 , 1×10^5 , 1×10^6 and 1×10^7 conidia per ml. Disease severity increased and fresh shoot and root mass of inoculated plants decreased when the inoculum concentration was increased over the range of 1×10^4 to 1×10^7 conidia per ml. Genotype IA 57, was most susceptible, TX 2853 was intermediately susceptible and TX 2793 was least susceptible. The isolate of *F. thapsinum* was more aggressive and caused greater crown and root rot severity than the *F. proliferatum* isolate.

INTRODUCTION

Fusarium spp. cause crown and root rot of sorghum (*Sorghum bicolor* L. (Moench)). Symptoms of crown and root rot can include pre- and post-emergence seedling blight, but the most obvious symptoms appear near maturity with the crown or leaf sheath showing a honey brown discolouration at the base of the plant. Infection begins in rootlets where it remains latent in symptomless plants until maturity when root necrosis takes place inducing rotting and lodging (Giorda, Martinez and Chulze, 1995).

In order to develop a breeding program for resistance of sorghum to *Fusarium* spp. an efficient inoculation technique must be developed that can differentiate between genotypes. Inoculation under controlled conditions is the primary means to determine the pathogenicity of a species and virulence of an isolate as well as to evaluate susceptibility of the host plants. Natural inoculum in the field is often unevenly distributed and the levels may not be high resulting in low disease incidence and frequent disease escapes (Dodman and Wildermuth, 1987).

Compared with natural infection, inoculation of corn with *Fusarium* ear rot increased disease severity and decreased variability within and among treatments (Clements, *et al.*, 2003). Where investigations are aimed at assessing the genetics of resistance to crown and root rot, inoculation techniques and disease assessment must ensure that resistance is expressed and disease escapes are minimized. High levels of resistance identified from inoculation trials will thus enable breeders to develop genotypes that are useful to producers during disease-free growing seasons and during seasons when disease development is favoured. Field evaluations are conducted once per season and breeding programs frequently rely on glasshouse procedures to achieve rapid progress toward the development of resistant germplasm. Breeding programs usually attempt to develop methods that mimic natural infection in the glasshouse processes and specifically detect types of resistance most effective in the field (Engle, Madden and Lipps, 2003).

Methods to induce crown rot and determine cultivar resistance include seed inoculation with spore suspension and inoculation of soil in pots with colonized corn-meal-sand (Purss, 1966). Seed inoculation was found to be an effective inoculation technique for screening wheat cultivars for field resistance to crown rot caused by *Gibberella zeae* (Wildermuth and Purss, 1971). Inoculation methods such as injecting the crown of the plant with conidial suspensions, drenching *Fusarium* conidial

suspensions into soil surrounding the base of potted plants have been studied (Dodman and Wildermuth, 1987). Mixing the soil in pots with ground grain colonized with *Fusarium* spp. has been used to screen wheat plants for resistance to crown rot (Dodman and Wildermuth, 1987). Grain colonized with *F. graminearum* Group 1 (Fig.1) was used as inoculum to conduct field inoculations for determining the resistance of several wheat cultivars to crown rot (Dodman *et al.*, 1985).

Inoculum concentration can directly influence the development of disease (Dodman and Wildermuth, 1987; Reid, Hamilton and Mather, 1995; Borges, Resende and Von-Pinho, 2001). High spore concentrations have been reported to cause *Fusarium* diseases in some resistant cultivars of wheat and maize (Dodman and Wildermuth, 1987; Reid *et al.*, 1995). It is therefore, important to choose an inoculum concentration for pathogenicity tests so that the pathogenicity of isolates or the susceptibility of host plants will not be masked by extraordinarily low or high inoculum levels (Wang and Jeffers, 2000). *F. graminearum* inoculum concentration of 5×10^5 spores/ ml has been shown to give maximum response of maize genotypes, ranging from resistant to very susceptible (Reid *et al.*, 1995). Stalks of maize hybrids inoculated with inoculum concentrations of 1×10^4 , 5×10^5 , and 1×10^7 conidia per ml yielded significant differences in resistance to stalk rot caused by *F. moniliforme* (*sensu lato*) (Reid *et al.*, 1995).

The objectives of the present study were, (i) to identify inoculation techniques that could be used to consistently identify sorghum genotypes with resistance to *Fusarium* crown and root rot; and (ii) to evaluate the effect of inoculum concentration on crown and root rot infection.

MATERIALS AND METHODS

Inoculum production.

Conidial suspension. Inoculum was prepared from cultures of *Fusarium* spp. isolated from infected sorghum roots and associated rhizosphere soil. Individual *Fusarium* isolates were cultured in Petri dishes on potato dextrose agar (PDA) and maintained at 22 to 24°C in a sporulation room under ultra-violet fluorescent lighting (Nelson, Toussoun and Marasas, 1983). Petri dishes were positioned lid side up in a single layer and allowed to grow for 14 days. Sterile distilled water (10 ml) was then added to the

surface of the agar colony and scraped with a spatula handle to dislodge micro- and macro-conidia (Engle *et al.*, 2003; Rose Parker and Punja, 2003; Mitter *et al.*, 2006).

The concentration of macro- and micro-conidia suspensions was determined using conidial counts made with a haemocytometer (Engle *et al.*, 2003; Rose *et al.*, 2003; Mitter *et al.*, 2006). Conidial suspension was adjusted with sterile distilled water to the required concentrations (Clark, Hoy and Nelson, 1995; Wang and Jeffers, 2000). Suspensions were either prepared fresh daily or alternatively one-day-old suspensions that had been stored at -4°C were used (Engle *et al.*, 2003).

Oats grain inoculum. Twenty aliquots each containing 100g of oats seed were prepared by soaking seeds in tap water overnight, decanting water and autoclaving at 121°C for 20 min once on two consecutive days. Each bottle was seeded with PDA plugs from cultures of the *Fusarium* isolates to be tested.

Bottles were maintained in a sporulation room at 22 to 24°C (shaking them daily) until the seeds were completely colonized by mycelium after approximately 21 days. Colonized seeds were removed from bottles, spread on aluminium foil and air dried at room temperature (22 to 24°C) for 10 days (Clark *et al.*, 1995; Wang and Jeffers, 2000; Dorrance *et al.*, 2003). Half of the dried colonized oat seeds were ground in a laboratory blender while the remaining portion was used as whole grain inoculum.

Glasshouse inoculations.

Inoculum was prepared using each one of the following *Fusarium* isolates: *F. equiseti* (Cda.) Sacc., *F. proliferatum* (Matsushima) Nirenberg, *F. solani* (Mart.) Appel & Wollenw. emend. Snyder & Hansen and *F. thapsinum* Klittich, Leslie, Marasas as described above.

Seeds of sorghum genotypes NK 283, SNK 3939, PAN 8446, PAN 8564, PAN 8706W and Buster were surface disinfested by dipping seeds in 70 % ethanol for 30 seconds. This was followed by surface sterilization in 0.05 % NaOCl for two minutes before seeds were rinsed three times in sterile distilled water (Salas *et al.*, 1999). Sterilized seeds were planted in vermiculite in polystyrene seed trays which were placed in a glasshouse cubicle with temperatures of 25°C to 27°C and 12 hours of white light supplemented by fluorescent light. After emergence, seedlings were allowed to grow for 10 days. Seedlings were transplanted into 250 ml pots containing a soil: peat

(3:1) mixture inoculated with each of the isolates. Whole grain and ground grain inoculations (Figs. 4.1) were made by mixing 5 grams of respective colonized grains into the soil prior transplanting. Drenches were applied subsequent to transplanting by applying 50 ml of 1×10^6 conidia per ml suspension (Dodman and Wildermuth, 1987). Conidial suspension treatment included dipping of seedlings into 1×10^6 conidia per ml conidial suspension for 10 minutes (Fig. 4.2). The effect of wounding was determined by cutting the roots of seedlings 5 cm below the crown using a scalpel prior to transplanting as well as wounding the crown by means of 2-mm-deep incisions at right angles to each other (Wang and Jeffers, 2000).

A factorial design was used with six sorghum genotypes x four inoculation techniques x four *Fusarium* spp. isolates replicated four times. The experiment was conducted twice. Two weeks after transplanting seedlings were fertilized with limestone ammonium nitrate (LAN -28 % a.i) at a rate of 1 g per kg soil. Fertilization at the same rate was repeated every two weeks. Plants were watered daily and maintained in the glasshouse at 30 / 21°C day and night regime.

After six weeks, three plants were removed from each pot. Crowns and roots were gently rinsed in water to remove soil and then blotted dry using paper towels. Plants were air dried at room temperature (22 - 24°C) for 30 minutes before individual plants were severed at the crown prior to measuring fresh shoot and root mass. Crown and root rot severity was assessed on a 0-5 scale (Correll, Puhalla and Schneider, 1986) where; 0 = no root discoloration; 1 = slight vascular discoloration in root tissue, 2 = extensive vascular discoloration in root tissue, 3 = vascular discoloration in crown; 4 = extensive vascular discoloration in crown, 5 = crown completely necrotic.

Inoculum concentration.

Conidial suspensions were prepared from isolates of *F. thapsinum* and *F. proliferatum* as previously described (Engle *et al.*, 2003 and Rose *et al.*, 2003; Mitter *et al.*, 2006). Four concentrations of 1×10^4 , 1×10^5 , 1×10^6 and 1×10^7 conidia per ml were adjusted using a haemocytometer (Dodman and Wildermuth, 1987; Reid *et al.*, 1995a; Wang and Jeffers, 2000; Borges *et al.*, 2001).

Seeds of sorghum genotypes, IA 57, TX 2853 and TX 2793 were surface disinfected and sterilized as described above (Salas *et al.*, 1999). Sterilized seeds were

planted in polystyrene trays in vermiculite and placed in a glasshouse cubicle as described above. After emergence, seeds were allowed to grow for 10 days before seedlings were removed from germination trays and roots were washed under running tap water to remove the growing medium. Roots were cut 5 cm below the crown and the crowns were wounded with a scalpel by making four 2-mm-deep holes at right angles to one another (Wang and Jeffers 2000). Wounded plants were dipped for 10 min into the four different conidial concentrations of each *Fusarium* isolate. Five seedlings of inoculum treatment were transplanted into 250 ml pots containing an uninfested steam sterilized soil: peat (3:1) mixture. Control treatment was similarly treated by being dipped in sterile distilled water.

A factorial experimental design was used including three sorghum genotypes, four inoculum concentrations and a control and two *Fusarium* spp., replicated four times. The experiment was conducted twice. Two weeks after transplanting, seedlings were fertilized with limestone ammonium nitrate (LAN -28 % a.i) at a rate of 1 g per kg soil. Fertilization at the same rate was repeated every two weeks. Plants were watered daily and maintained in the glasshouse at 30 / 21°C day night regime for a six-week growth period.

After six weeks, three plants were removed from each pot. Crowns and roots were gently rinsed in water to remove soil and blotted dry with paper towels. Plants were then air dried at room temperature (22 - 24°C) for 30 minutes before individual plants were severed at the crown and fresh shoot and root mass was measured. Crown and root rot severity was assessed on a 0-5 scale (Correll *et al.*, 1986) as described above.

Data analysis.

Data were analysed using the general linear model (GLM) and ANOVA in SAS version 9.1 (SAS Institute Cary NC). Data were used to generate the means of fresh shoot and root mass, percentage mortality and crown and root rot severity of each genotype and associated *Fusarium* isolates. Data from repeated experiments were combined for analysis based on results of tests for homogeneity of variances. Treatment means were compared using Fisher's protected least significant difference (LSD) with ($P \leq 0.05$). Means from individual treatments were compared if interactions

were significant ($P \leq 0.05$). Where interactions were not significant ($P \leq 0.05$) individuals treatments were combined to evaluate the main effects.

RESULTS

Glasshouse inoculations.

Sorghum plants that were wounded and dipped in sterile distilled water and or planted in steam sterilized soil inoculated with un-colonized oat seeds displayed no symptoms. Analysis of variance indicated a significant ($P \leq 0.05$) genotype effect on shoot and ($P \leq 0.01$) on root mass. There was a significant ($P \leq 0.05$) genotype x inoculation technique x *Fusarium* spp. interaction on fresh shoot and root mass, indicating that genotypes responded differently to the inoculation techniques.

Fresh shoot and root mass was more greatly influenced by the ground colonized oat seeds technique than the soil drench technique (Table 4.1). NK 283 inoculated with *F. solani* conidial suspension displayed the highest fresh shoot and root mass compared to least for PAN 8446 inoculated with ground colonized grain inoculum of *F. equiseti* (Table 4.1). *F. equiseti* caused a greater reduction in shoot and root mass of sorghum seedlings than the other isolates tested (Table 4.1).

There was a significant ($P \leq 0.01$) genotype x inoculation technique x *Fusarium* spp. interaction on crown and root rot severity. SNK 3939 and Buster inoculated with *F. equiseti* colonized ground grain inoculum were more susceptible to crown and root rot pathogens compared to the more resistant PAN 8706W (Table 4.1). *F. equiseti* was the most aggressive isolate and caused the highest degree of crown and root rot severity while *F. solani* was the least aggressive (Table 4.1). The highest degree of crown and root rot severity occurred on wounded plants inoculated with ground colonized oats grain (Table 4.1).

Inoculum concentration.

Variances associated with disease severity in the two experiments were homogeneous, thus data were combined for analysis. There was a significant ($P \leq 0.01$) genotype x inoculum concentration x *Fusarium* spp. interaction on percent mortality

which indicated that genotypes differed in susceptibility to the isolates of *Fusarium* spp. tested. Genotype IA 57 had the highest percentage mortality compared to TX 2793 which had the lowest (Table 4.2). *F. thapsinum* was the most aggressive isolate and resulted in the highest mortality compared to *F. proliferatum* with the least (Table 4.2). Mortality was highest at the highest inoculum concentration of 1×10^7 conidia per ml (Table 4.2).

Genotype x *Fusarium* spp. interaction significantly ($P \leq 0.05$) affected fresh shoot and root mass as did genotype x inoculum concentration x *Fusarium* spp. interaction. Genotype TX 2793 showed the most shoot and root growth compared to IA 57 which had the least (Table 4.2). *F. thapsinum* caused a greater reduction in shoot and root mass than *F. proliferatum* (Table 4.2). Fresh shoot mass ranged from 0.71 to 3.79 g and root mass from 0.31 g to 2.34 g for the concentrations of 1×10^4 to 1×10^7 conidia per ml respectively (Table 4.2). Fresh shoot and root mass were significantly lower for the 1×10^7 conidia per ml inoculum concentration (Table 4.2).

Genotype x inoculum concentration interaction significantly ($P \leq 0.01$) affected crown and root rot severity. Genotype IA 57 was most susceptible and a higher crown and root rot severity was recorded than in TX 2793 (Table 4.2). *F. thapsinum* caused more crown and root rot severity than *F. proliferatum* (Table 4.2). Crown and root rot severity increased incrementally for concentrations between 1×10^4 and 1×10^7 conidia per ml and was greatest at the highest concentrations of 1×10^6 and 1×10^7 conidia per ml (Table 4.2). Overall fresh shoot and root mass were significantly affected by inoculum concentration. Crown and root rot severity and percent age mortality increased with an increase in inoculum concentration from 1×10^4 to 1×10^7 (Table 4.2).

DISCUSSION

The present study was conducted to determine whether glasshouse inoculation techniques could be used to identify sorghum genotypes with resistance to *Fusarium* crown and root rot. The study evaluated the effect of inoculum concentration on crown and root rot in relation to genotype resistance. A technique involving wounded crowns and roots of sorghum seedlings planted in steam sterilized soil: peat (3:1) mixture inoculated with ground colonized oat grain was tested. The technique was reliable and

precise as highly reproducible crown and root rot severity data were obtained using relatively few replications in repeated experiments. The colonized grain inoculation technique has been used successfully to screen crown rot severity in wheat cultivars in pots in the glasshouse and under field conditions (Dodman and Wildermuth, 1987).

When plants are wounded, initial infection and pathogen penetration takes place through wounds and there is little resistance to penetration (Doman and Wildermuth, 1987). Many disease pathogens enter plant tissues through wounds caused either mechanically or by insects. Direct exposure of fresh wounds and the entire root system to inoculum was responsible, at least in part for the success of this inoculation technique. Wang and Jeffers (2000) consistently reproduced disease symptoms caused by *Fusarium* spp., only when hostas plants were wounded and wounds were exposed to inoculum. The wounding of plant crowns and roots is believed to mimic natural infection. Chi, Childers and Hanson (1964) and Stephens *et al.* (1993) found that the wounding of roots disrupts physical defence barriers in plants. This also accelerates colonization and penetration of the roots, and increases the incidence and severity of root diseases caused by *Fusarium* spp. The results also suggest that the resistant genotypes had a greater ability to restrict the development of crown rot. This is indicative that initial penetration of spores produced less crown rot severity in resistant than in susceptible genotypes (Dodman and Wildermuth, 1987).

The second inoculation technique involved drenching soil with a conidial suspension applied on the base of the stem above the soil surface. This technique was found to be effective when studying resistance to crown rot caused by *F. pseudograminearum* (Dodman and Wildermuth, 1987 and Mitter *et al.*, 2006). The success of this inoculation technique depends on the placement of the inoculum for initial infection. The crown rot pathogen survives on infected crop residues but does not survive when these are buried in soil (Burgess *et al.*, 2001). The placement of inoculum on the crown tissues of wounded or non-wounded plants makes intuitive sense for a crown-infecting pathogen (Mitter *et al.*, 2006). However, the exact mechanisms of infection and host resistance mechanism are not known, but the inoculation technique has the potential to bypass any root-mediated host-resistance mechanisms. This may account for the observed genotype x inoculation technique interactions. Other inoculation techniques were developed to screen wheat for crown rot resistance but some of these have failed to reproduce the relative levels of field

resistance. Some bioassays did not yield good correlation between field tolerance and seedling blight (Purss, 1966). Other inoculation techniques have however, shown good correlations between seedling (Klein *et al.*, 1985; Wildermuth and McNamara, 1994) or adult resistance (Wallwork *et al.*, 2004) and the performance of cultivars in the field.

Inoculation techniques aimed to improve reproducibility and precision of the inoculation technique when inoculations were repeated to reproduce crown and root rot symptoms. Inoculum was placed on the crown and within the root zone area. Purss (1966) and Burgess *et al.* (1993) suggested that leaf sheaths and stem bases are the primary infection sites for crown rot causal agent (*F. pseudograminearum*). The techniques facilitate the ranking of sorghum genotypes according to their resistance to crown and root rot resistance and *Fusarium* isolates according to their aggressiveness.

Inoculation techniques where whole oats grain was colonized and wounded plants were dipped into a conidial suspension were least effective in terms of inducing crown and root rot severity. Variable reactions were observed for shoot and root growth and disease development on plants inoculated with these techniques. The inoculation technique suffered inherent variability and deficiencies associated with the delivery of the pathogen inoculum through the soil and on the plant. These findings are consistent with Wallwork *et al.* (2004) who did not produce high level of susceptibility to *Fusarium* crown rot on durum wheat using the terrace system which was as variable as the field assay.

Wounding of plants prior to planting in steam sterilized soil infested with ground colonized oats grain significantly reduced shoot and root mass. The soil drench technique did not however inhibit shoot and root mass growth or development. Disease severity ratings did not always correspond to plant biomass. A similar phenomenon was reported for other root rot diseases (Burke and Barker, 1966; Harveson and Rush, 1998; Wang and Jeffers, 2000).

The present study compared six sorghum genotypes for resistance to crown and root rot using four inoculation techniques. Glasshouse inoculation tests indicated that sorghum genotypes reacted differently to *Fusarium* crown and root rot according to the procedures used to inoculate the plants. The inoculation technique using ground colonized grain consistently ranked sorghum genotypes according to their field resistance. The technique resulted in high crown and root rot and caused reductions in

shoot and root mass. Genotype responses were observed with SNK3939 and Buster being highly susceptible, NK 283, PAN 8446 and PAN 8564 showed intermediate reactions while PAN 8706 was resistant. Dodman *et al.* (1985) also found that resistance to Fusarium crown rot in wheat varieties was expressed in glasshouse and field inoculations when ground colonized grain inoculum was used. They further suggested that resistance was best expressed where inoculum was applied onto or in soil, rather than directly onto plants. In another study, Dodman and Wildermuth (1987) using ground colonized grain, observed high levels of Fusarium crown rot on the cultivars Oxley and Puseas but not in Gala.

In the present study, four *Fusarium* spp. isolates differing in their aggressiveness were used. The results indicated that *F. equiseti*, inoculated as ground colonized grain, was significantly more aggressive and consistently caused the greatest degree of crown and root rot severity compared to other isolates. Shoot and root mass were highest for plants inoculated with conidial suspension of *F. solani* compared to least plants inoculated with ground colonized oats grain of *F. equiseti*.

In the present study, severity of crown and root rot and mortality of sorghum seedlings was significantly influenced by increasing conidial concentration over the range of 1×10^4 to 1×10^7 conidia per ml. This is consistent with Dodman and Wildermuth, (1987) who found that Fusarium crown rot of wheat increased with increasing conidial concentration over the range of 1×10^3 to 1×10^6 conidia per ml. Plant mortality increased with increase in inoculum concentration. The results were also consistent with the findings of (Wang and Jeffers, 2000) who found that as the inoculum dosage increased so did plant mortality. High spore concentrations (1×10^6 conidia per ml) produced significantly more disease than all other concentrations and a significant difference among cultivars in their level of resistance to crown rot was observed (Dodman and Wildermuth, 1987; Reid *et al.*, 1995a; Borges *et al.*, 2001). Dodman and Wildermuth, (1987) also reported a reduction in seedling emergence at the highest (1×10^6 conidia per ml) spore concentration. This is consistent with the previous observations that the number of infective propagules on root rot surfaces is proportion to the levels of inoculum used (Rodriguez-Gavez and Mendgen, 1995; Wang and Jeffers, 2000; Biggs, 2004). Genotype reaction was significantly influenced by inoculum concentration and three genotypes were categorized based on their differential response to inoculations and inoculum concentrations. Genotype IA 57 was

highly susceptible, TX 2853 showed an intermediate reaction while TX 2793 was resistant.

Screening of sorghum germplasm for high levels of resistance to *Fusarium* crown and root rot can be effected using inoculation techniques where the pathogen is incorporated into the soil. Repeated screening of promising germplasm using this technique yielded 14 wheat cultivars with high level of resistance to crown rot (Liu *et al.*, 2004). Crown and root rot severity of sorghum caused by various *Fusarium* spp. is greatly influenced by the inoculation technique used under controlled conditions. Wounding of crowns and roots of plants followed by transplanting them into soil infested with ground oats seeds colonized by *Fusarium* spp. was shown to be more effective than drenching soil with a conidial suspension. An inoculum concentration of 1×10^6 conidia per ml is recommended for future use in similar inoculations as it consistently resulted in disease development. The inoculation techniques described here show great potential for glasshouse assays to differentiate resistance to crown and root rot of sorghum.

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Table 4.1 Effect of inoculation techniques on plant biomass and development of crown and root rot of sorghum genotypes with variable level of resistance six weeks after inoculation

Sorghum genotype	Inoculation technique	<i>F. equiseti</i>			<i>F. proliferatum</i>			<i>F. solani</i>			<i>F. thapsinum</i>		
		Shoot mass (g)	Root mass (g)	Root rot (0-5)	Shoot mass (g)	Root mass (g)	Root rot (0-5)	Shoot mass (g)	Root mass (g)	Root rot (0-5)	Shoot mass (g)	Root mass (g)	Root rot (0-5)
Buster	Control	2.40bc ¹	1.91c	0.00d	2.63b	2.50b	0.00d	3.63ab	3.50a	0.00d	3.40bc	2.72ab	0.00d
	Drench ^a	3.60a	2.52b	3.00ab	4.00a	3.01ab	2.05b	1.81cd	0.80de	1.00c	4.11a	2.20b	2.00b
	Intact ^b	2.61b	2.44bc	2.00bc	2.11bc	1.89c	2.00b	2.20c	2.01bc	1.00c	1.92d	1.22c	2.00b
	Powder ^c	0.72d	0.65d	3.50a	0.81d	0.46d	3.00a	0.70d	0.31de	2.00b	1.52d	1.01c	2.50ab
	Suspension ^d	2.52b	2.30bc	1.00cd	2.83b	2.11bc	1.00c	2.66bc	2.00bc	1.00c	3.44bc	2.00b	1.00c
	Mean	2.37	1.96	1.90	2.48	1.99	1.61	2.20	1.72	1.00	2.88	1.83	1.50
NK 283	Control	3.65a	3.55a	0.00d	3.84a	3.30a	0.00d	4.90a	3.71a	0.00d	4.12a	3.21a	0.00d
	Drench	3.91a	3.05ab	1.50c	3.81a	3.15ab	2.00b	3.33b	3.25ab	2.50ab	3.85ab	2.40b	2.55ab
	Intact	2.45bc	1.45cd	2.50b	3.25ab	2.75b	2.00b	1.53cd	1.05cd	1.50bc	2.11cd	1.95bc	1.50bc
	Powder	0.42d	0.25d	3.00ab	0.85d	0.70d	3.00a	0.72d	0.50de	3.00a	0.80e	0.35d	3.00a
	Suspension	3.31ab	2.85b	2.00bc	3.62a	3.10ab	1.55bc	4.33a	3.05ab	2.00b	3.14bc	2.88ab	1.00c
	Mean	2.75	2.23	1.80	3.07	2.60	1.71	2.96	2.31	1.80	2.80	2.16	1.61
PAN 8446	Control	3.55a	3.20ab	0.00d	3.42ab	2.41bc	0.00d	3.44b	2.65b	0.00d	2.41cd	1.65bc	0.00d
	Drench	1.86c	1.51c	2.00bc	2.13bc	2.00bc	3.00a	2.15c	1.42cd	3.00a	1.65d	1.55bc	3.00a
	Intact	3.00ab	2.53b	1.00cd	2.80b	1.80c	1.00c	2.10c	1.90c	1.00c	2.00cd	1.81bc	1.00c
	Powder	0.25d	0.10d	3.00ab	0.80d	0.75d	3.00a	0.52d	0.30de	3.00a	0.55e	0.35d	3.00a
	Suspension	2.21bc	2.00bc	2.00bc	3.13ab	3.07ab	1.00c	4.01a	3.72a	1.00c	3.01bc	3.12a	1.00c
	Mean	2.17	1.87	1.60	2.31	2.01	1.60	2.44	2.00	1.60	1.92	1.70	1.60
PAN 8564	Control	3.01ab	2.25bc	0.00d	1.82c	1.50cd	0.00d	2.95bc	2.22bc	0.00d	3.16bc	1.90bc	0.00d
	Drench	2.72b	2.20bc	2.00bc	3.45ab	3.21a	1.00c	3.44b	2.80b	2.00b	2.40cd	2.00b	2.00b
	Intact	1.56c	1.22cd	1.00cd	2.13bc	1.83c	1.00c	1.57cd	1.02d	1.00c	2.12cd	1.02c	2.00b
	Powder	0.39d	0.25d	3.00ab	0.83d	0.70d	1.00c	0.65d	0.20de	2.00b	1.13de	0.71d	2.00b
	Suspension	3.87a	1.89c	1.00cd	2.31bc	2.10bc	1.00c	2.89bc	2.11bc	1.00c	2.28cd	2.20b	1.00c
	Mean	2.31	1.56	1.40	2.11	1.87	0.80	2.30	2.03	1.20	2.22	1.57	1.40
PAN 8706W	Control	2.40bc	2.00bc	0.00d	3.37ab	2.01bc	0.00d	3.73ab	3.10ab	0.00d	2.77c	2.50ab	0.00d
	Drench	3.12ab	1.50c	1.25cd	1.89c	1.10c	1.00c	2.31c	2.01bc	1.00c	2.15cd	1.93bc	1.00c

	Intact	1.91c	1.52c	1.00cd	2.50b	1.50c	1.00c	1.70cd	1.15d	1.00c	2.00cd	2.74ab	1.00c
	Powder	0.42d	0.30d	1.00cd	0.62d	0.31d	1.00c	0.40d	0.30de	1.00c	0.51d	0.40d	1.00c
	Suspension	3.30ab	3.01ab	1.00cd	2.60b	2.11bc	1.00c	1.65cd	1.25d	1.00c	2.13cd	2.00b	1.00c
	Mean	2.23	1.67	0.85	2.20	1.41	0.80	1.96	1.56	0.80	1.91	1.91	0.80
SNK 3939	Control	2.95b	2.35bc	0.00d	3.50a	2.25bc	0.00d	1.85cd	1.90c	0.00d	2.65c	2.07b	0.00d
	Drench	3.06ab	2.70b	2.50b	3.75a	3.00ab	2.55ab	4.00a	3.21ab	2.00b	3.95ab	3.75a	2.50ab
	Intact	1.35cd	1.20cd	2.50b	1.80c	1.58c	2.00b	1.75cd	1.21d	1.50bc	2.80c	2.41b	2.00b
	Powder	0.45d	0.25d	3.50a	0.60d	0.55d	3.15a	0.70d	0.61de	3.00a	0.72d	0.55d	3.00a
	Suspension	2.60b	2.08bc	1.50c	2.01bc	1.60c	2.00b	3.05b	2.60b	2.00b	2.35cd	2.15b	2.00b
	Mean	2.08	1.72	2.00	2.45	1.80	1.94	2.27	1.91	1.70	2.49	2.19	1.90

^aDrench (soil drenching with conidial suspension), ^bintact (whole colonized oat grain inoculum), ^cpowder (ground colonized oats grain), ^dsuspension (wounded roots and crowns dipped in conidial suspension).

¹ Means within a parameter followed by the same letter are not significantly different ($P>0.05$) according to Fisher's protected least significant difference (LSD) tests.

Table 4.2 Effect of four inoculum concentrations 1×10^4 ; 1×10^5 ; 1×10^6 ; 1×10^7 conidia per ml of *F. thapsinum* and *F. proliferatum* and an untreated control on plant biomass and crown and root rot development on sorghum genotypes with variable reactions

Genotype	Inoculum concentration	<i>F. thapsinum</i>				<i>F. proliferatum</i>			
		Mortality (%)	Shoot mass (g)	Root mass (g)	Root rot (0-5)	Mortality (%)	Shoot mass (g)	Root mass (g)	Root rot (0-5)
IA 57	1×10^4	47.50c ¹	1.94ab	0.94ab	3.50bc	29.25bc	3.01ab	1.41ab	2.58bc
	1×10^5	56.30b	0.99b	0.53b	3.63b	36.77b	1.43b	0.70b	3.17b
	1×10^6	67.50ab	0.90b	0.50b	4.63ab	46.73ab	1.48b	0.73b	4.17ab
	1×10^7	72.51a	0.71bc	0.31c	4.75a	51.74a	0.88c	0.35c	4.41a
	Control	0.00d	5.80a	2.65a	0.00c	0.00c	7.13a	2.71a	0.33c
	Mean	44.80	2.07	0.98	3.15	32.89	2.55	1.18	2.93
TX 2853	1×10^4	31.70c	2.74ab	1.34ab	3.25b	28.81bc	2.90ab	1.36ab	2.75b
	1×10^5	39.10b	2.35b	0.95b	3.75ab	37.53b	1.49bc	0.59c	3.75ab
	1×10^6	49.22ab	1.87bc	0.75bc	3.67ab	50.09ab	2.21b	0.90b	3.63ab
	1×10^7	52.57a	1.35c	0.57c	4.17a	57.56a	1.26c	0.49c	4.50a
	Control	0.00d	7.13a	3.70a	0.00c	0.00d	7.01a	2.93a	0.50c
	Mean	34.52	3.08	1.47	2.97	34.80	2.98	1.25	3.03
TX 2793	1×10^4	15.01c	3.79ab	1.83ab	2.50b	17.79bc	2.29bc	1.13b	2.45b
	1×10^5	20.98b	3.21b	1.36b	2.88ab	18.40b	4.53a	2.34a	2.85ab
	1×10^6	26.03ab	1.90bc	0.82bc	3.13a	34.71ab	2.30bc	1.09bc	2.90ab
	1×10^7	26.37a	1.38c	0.59c	3.13a	47.59a	3.05ab	1.33ab	3.05a
	Control	0.00d	6.78a	4.06a	0.00c	0.00c	2.90b	1.18b	0.00c
	Mean	17.68	3.41	1.73	2.33	23.70	3.01	1.41	2.25

¹ Means within a parameter followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's protected least significant difference (LSD) tests.

A



B



C



Fig. 4.1 Colonized oat seeds (A) used as ground (B) or whole grain (C) as inoculum for the evaluation of sorghum seedlings for resistance to crown and root rot and seedling blight.

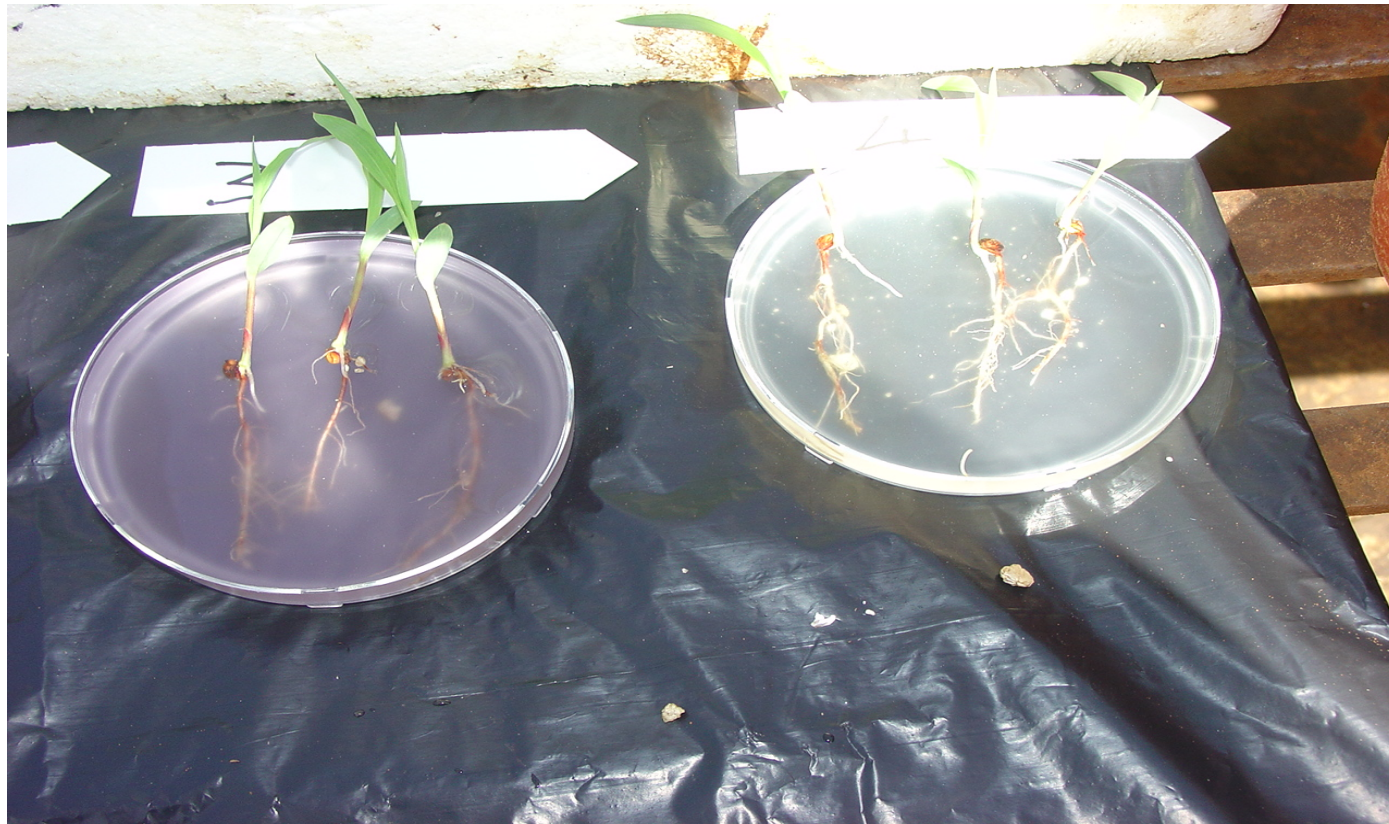


Figure 4.2 Sorghum seedlings with wounded crown and roots dipped in conidial suspension (1×10^6 conidia/ml) and planted in uninfested soil mix

CHAPTER V
RESISTANCE TO FUSARIUM CROWN AND ROOT ROT IN SELECTED
SORGHUM GENOTYPES

ABSTRACT

Resistance to pre- and post-emergence damping-off caused by *Fusarium* spp. was investigated among sorghum genotypes in the glasshouse. Results provided a reliable indication of the performance of resistant (TX 2793), tolerant (TX 2853) and susceptible (IA 57) genotypes. *Fusarium thapsinum* was more virulent than *F. proliferatum* in both the pre- and post-emergence damping-off tests. Twenty one sorghum genotypes were evaluated for resistance to Fusarium crown and root rot severity caused by *F. equiseti*, *F. proliferatum*, *F. solani* and *F. thapsinum*. The availability of the new sources of resistance could provide a tool for managing Fusarium crown and root rot of sorghum. Nine sorghum genotypes resulted in crown and root rot severity ratings ranging from 0.63 to 2.75 and these were selected as having partial resistance to crown and root rot. Immunity to Fusarium crown and root rot was not found. However, TX 2793 showed a significantly high level of resistance. Sorghum genotypes PAN 8706W, PAN 8446, PAN 8564, SNK 3939, NK 283 and Buster were evaluated for age related resistance to crown and root rot severity caused by *F. equiseti*, *F. proliferatum*, *F. solani* and *F. thapsinum*. Resistance of sorghum genotypes to crown and root rot caused by *Fusarium* spp. as assessed by shoot and root mass, root rot severity and AUDPC increased with plant age. Resistant genotypes PAN 8706W and PAN 8564 developed less crown and root rot severity and had lower AUDPCs than the susceptible NK 283 at all plant ages. PAN 8564 showed the highest fresh shoot and root mass for the six-week-old compared to least for PAN 8706W for the 2-week-old plants. Plants inoculated with *F. equiseti* indicated significantly higher crown and root rot and AUDPC compared to least for plants inoculated with *F. proliferatum*. In split root experiments the antagonist (*Trichoderma harzianum*) and the pathogen were physically separated from each other. Root colonization by *Trichoderma harzianum* (Tricho-Plus) reduced crown and root rot severity, verifying the mechanism of action as induced systemic resistance. *T. harzianum* (Tricho-Plus) inoculated into autoclaved soil significantly reduced the crown and root rot indicating the ability of the antagonistic to colonize roots extensively and reduce colonization by the pathogen.

INTRODUCTION

Root and stalk rots are among the most prevalent diseases of sorghum (Reed, Partridge and Norquist, 1983) noted that breeding for resistance to these diseases remains a research priority in sorghum improvement programs. Root and stalk rots are caused by a complex of diverse fungal pathogens and infection is enhanced under conditions of environmental stress (Thompson, 1963; Edmunds, 1964; Esechie, Maranville and Ross, 1977; Zummo, 1980; Jordan, Clark and Seetharama, 1984; Bramel-Cox *et al.*, 1988). The use of cultivars that are resistant to both biotic and abiotic stresses is generally considered to be the most effective and environmentally sound approach for addressing stalk and root rot diseases (Tuinstra *et al.*, 2002).

Currently, 13 *Fusarium* spp. (*F. equiseti*, *F. graminearum*, *F. moniliforme*, *F. napiforme*, *F. oxysporum*, *F. nygamai*, *F. proliferatum*, *F. semitectum*, *F. solani*, *F. subglutinans*, *F. thapsinum* and *F. trincinctum*) are known to be associated with sorghum and it is likely that sources of partial resistance to *Fusarium* spp. exist in sorghum (Claflin, 2000). However, more species were found associated with sorghum roots and rhizosphere soils in South Africa. These include, *F. andiyazi*, *F. beomiforme*, *F. chlamydosporum*, *F. circinatum*, *F. culmorum*, *F. crookwellense*, *F. equiseti*, *F. dlamini*, *F. lateritium*, *F. napiforme*, *F. nygamai*, *F. oxysporum*, *F. polyphialidicum*, *F. pseudociccinatum*, *F. pseudonygamai*, *F. proliferatum*, *F. poae*, *F. scirpi*, *F. semitectum*, *F. solani*, *F. subglutinans*, *F. thapsinum* and *F. Verticillioides* which have been isolated from sorghum roots and rhizosphere soil (Marasas *et al.*, 2001)

Germplasm has to be screened for resistance to several soil-borne plant pathogens including *Fusarium* spp. The development of resistant cultivars involves germplasm characterization and identification of sources of resistance. Tuinstra *et al.* (2002) emphasized the importance of developing populations to derive segregating progenies, and the identification of superior genotypes to bring together the required traits into a single plant. The type of resistance desired (monogenic, polygenic) as well as the genetic control of resistance for example dominant, recessive or polygenic, available in the germplasm resources will dictate speed and dependability of progress (Duncan and de Milliano, 1995).

Pre- and post-emergence damping-off has been used as an indication of host resistance to root rot pathogen however, reactions of plants are known to be affected by plant age and this has been used to distinguish between types of resistance (Koch and Mew, 1991). Both adult and seedling plants are of economic importance. Duncan and

de Milliano (1995) indicated that the levels of juvenile and adult plant resistance to most pathogens differ. Juvenile resistance may not be active during reproductive development and sequential analysis of disease development is therefore required. Kuhn, Ohm and Shaner (1978) and Parlevliet (1989) identified latent period as an important component of partial resistance.

Induced resistance exists when seemingly healthy plants occur in a field or glasshouse, despite exposure to a continuous supply of inoculum from diseased plants at close proximity (Duncan and de Milliano, 1995). Induced systemic resistance (ISR) is the process whereby treatment of plants with plant growth promoting rhizobacteria (PGPR) elicits host plant defense (van Loon and Glick, 2004). Induction of systemic-acquired resistance by sub-optimal doses of inoculum has been found for pearl millet against downy mildew by (Kumar *et al.*, 1993).

The fungus *Trichoderma harzianum* Rifai has been used to induce systemic resistance in bio-control experiments (Chet *et al.*, 1979; Elad, Chet and Henis, 1982; Papavizas and Lumsden, 1980) and has been shown to increase plant growth. In a split root bioassay, *T. hamatum* Rifai 382 (T₃₈₂) inoculated into a compost-amended potting mix significantly reduced the severity of Phytophthora root and crown rot of cucumber on paired roots in the peat mix (Khan *et al.*, 2004).

The objectives of the present study were (i) to investigate variation in pathogenicity of *Fusarium* spp. causing pre- and post-emergence damping-off; (ii) to determine the extent to which the expression of the quantitative resistance varies with plant age; (iii) to identify sources of partial resistance to *Fusarium* crown and root rot in sorghum genotypes; and (iv) to determine whether *T. harzianum* could induce systemic resistance against crown and root rot in split root sorghum plants.

MATERIALS AND METHODS

Oat seed inoculum.

Twenty aliquots each containing 100g of oat seeds were prepared by soaking in tap water overnight, decanting water and autoclaving at 121°C for 20 min on two consecutive days. Each bottle was seeded with potato dextrose agar plugs (PDA) (Wang and Jeffers, 2000; Clark, Hoy and Nelson, 1995; Dorrance *et al.*, 2003) one from each of the cultures of *Fusarium equiseti* (Cda.) Sacc. *F. proliferatum* (Matsushima)

Nirenberg, *F. solani* (Mart.) Appel. & Wollenw. emend. Snyder & Hansen, and *F. thapsinum* Klittich *et al.*, (1997),

Bottles were maintained in the sporulation room at 22 – 24°C (shaking them daily) until the seeds were completely colonized by mycelium (after approximately 21 days). Colonized seeds were removed from bottles, spread on aluminium foil and air dried at room temperature (22 to 24°C) for 10 days (Wang and Jeffers, 2000; Clark *et al.*, 1995; Dorrance *et al.*, 2003). Dried colonized oat seeds were ground in a laboratory blender.

Conidial suspensions.

Conidial suspension inoculum was prepared from cultures of *Fusarium* spp. isolated from infected sorghum roots and associated rhizosphere soil. Isolates of *F. equiseti*, *F. proliferatum*, *F. solani*, and *F. thapsinum* were cultured on PDA in 90 mm Petri dishes and maintained at 22 to 24°C in a sporulation room under ultra-violet fluorescent lighting (Nelson, Toussoun and Marasas, 1983). Petri dishes were positioned flat side up in a single layer and allowed to grow for 14 days. Subsequently, 10 ml of sterile distilled water was added to the surface of the culture which was scraped with a spatula handle to dislodge the micro- and macro-conidia (Engle, Madden and Lipps, 2003; Rose Parker and Punja, 2003).

The mycelial mat was removed from the Petri dishes and passed through four layers of cheese cloth. The concentration of macro- and micro-conidia suspensions for each isolate was determined using conidial counts made with a haemocytometer (Engle *et al.*, 2003 and Rose *et al.*, 2003). Conidial suspensions were adjusted with sterile distilled water to 1×10^6 conidia per ml (Wang and Jeffers, 2000; Clark *et al.*, 1995). Suspensions were either prepared fresh daily or alternatively one-day-old suspensions that had been stored at -4°C were used (Engle *et al.*, 2003).

Pre- and post-emergence damping-off.

Pre-emergence damping-off. To test for pre-emergence damping-off, seeds of three sorghum genotypes TX 2793, TX 2853 and IA 57 (selected from the root rot evaluation lines) were surface disinfested by dipping seeds in 70 % ethanol for 30

seconds followed by surface sterilization in 0.05 % NaOCL for 5 minutes. Seeds were rinsed three times in sterile distilled water (Salas *et al.*, 1999).

Sterilized seeds were planted in 250 ml pots containing steam sterilized soil: peat (3:1) mixture inoculated with 2.5 g of ground colonized oats grain inoculum prepared as described by Wang and Jeffers, (2000); Clark *et al.*, (1995); Dorrance *et al.*, (2003) above from *Fusarium* isolates of *F. proliferatum* and / or *F. thapsinum*. The inoculum was thoroughly mixed with the upper 5 cm (Van Wyk *et al.*, 1988) of steam sterilized soil: peat (3:1) mixture. Seeds were planted in direct contact with the inoculum. Five seeds were planted per pot to a depth of 3 cm. Control treatments were similarly treated with un-colonized oats grain. Pots were laid out in a randomized complete block design and replicated four times. Two weeks after emergence seedlings were fertilized with limestone ammonium nitrate (LAN -28 % a.i) at a rate of 1 g per kg soil. The fertilizers were subsequently applied every two weeks. Pots were watered daily and maintained in the glasshouse for six weeks at 30 / 21 °C day and night regime.

After four weeks three plants were removed from each pot. Plants were cut into two at the crown. Fresh shoot and root mass were measured. Crown and root rot severity was assessed on a 0-5 scale (Correll, Puhalla and Schneider, 1986) where; 0 = no root discoloration; 1 = slight vascular discoloration in root tissue, 2 = extensive vascular discoloration in root tissue, 3 = vascular discoloration in crown; 4 = extensive vascular discoloration in crown, 5 = crown completely necrotic. The experiment was conducted twice.

Post-emergence damping-off. To test for post-emergence damping-off, sorghum seeds were surface disinfested and sterilized as described above (Salas *et al.*, 1999). Sterilized sorghum seeds of genotypes TX 2793, TX 2853 and IA 57 were pre-germinated in square plastic trays (100 seeds) lined with germinating paper. The germinating containers were covered with black polythene bags which served as a humidity chamber and placed in a glasshouse cubicle with a mean temperature of 25° C (\pm 3) for 11 days.

Seedlings were removed from plastic trays and transplanted into 250 ml pots containing soil: peat (3:1) mixture inoculated with 2.5 g ground colonized oat seeds prepared as described by Wang and Jeffers (2000); Clark *et al.*, (1995); Dorrance *et al.*, (2003) with isolates of *F. proliferatum* and or *F. thapsinum*. Five seedlings were

transplanted into each pot per test fungus. The controls were similarly treated and planted in steam sterilized soil: peat (3:1) mixture thoroughly mixed with ground uncolonized oat seeds. Treatments were laid out in a randomized complete block design replicated four times. Fertilization, watering and the temperatures in the glasshouse were as described above.

After six weeks three plants were removed from each pot. Crowns and roots were gently rinsed in water to remove soil and plants were then air dried at room temperature (22 - 24°C) for 30 minutes before individual plants were cut at the crown and fresh shoot and root mass were measured. Crown and root rot severity was assessed on a 0-5 scale (Correll *et al.* 1986) as described above. The experiment was conducted twice.

Sources of partial resistance.

Glasshouse experiments. Ground colonized oat seed inoculum was prepared as described above for each of the *Fusarium* isolates, *F. equiseti*, *F. solani*, *F. proliferatum* and *F. thapsinum*. The inoculum (2.5 g) was thoroughly mixed with the upper 5 cm (Van Wyk *et al.*, 1988) of the steam sterilized soil:peat (3:1) mixture in 250 ml plastic pots. Five pots replicated four times were prepared for each treatment combination.

Sorghum seeds were selected from a diverse collection of germplasm in the All Disease and Insect Nursery (ADIN) and Southern Africa Breeding Nursery (SABN) provided by Texas A & M as well as selected commercial varieties (Table 5.2a). Seeds of each of the test genotypes were surface disinfested and sterilized as described Salas *et al.* (1999) above. Five seeds were planted in each pot to a depth of 3 cm in direct contact with the inoculum. The treatments were arranged in a randomized complete block design replicated four times. Pots were maintained in the glasshouse for six weeks and watered daily. Fertilization and temperatures in the glasshouse were as described above.

Sampling was done three times at 14, 28, and 42 days after planting. Three plants were removed from each pot. Crowns and roots were gently rinsed in water to remove soil and dried with paper towels. Plants were then air dried at room temperature (22 - 24°C) for 30 minutes before individual plants were cut at the crown and fresh shoot and root mass were measured. Crown and root rot severity was assessed on a 0-

5 scale (Correll *et al.* 1986) as described above. Plots of mean disease severity values on days after planting were used to produce an area under disease progress curve (AUDPC) using the formula of Shaner and Finney (1977). Where; $AUDPC = \sum [(X_i + X_{i+1})/2 (t_{i+1} - t_i)]$ $i=1$, in which X_i = root rot severity at the i th observation, t = time (days) at the i th observation, and n = total number of observations. The experiment was repeated.

Field experiments. Thirteen sorghum genotypes (Table 5.2b) were evaluated under field conditions at Cedara (29° 36' S and 30° 26' E) in the KwaZulu-Natal Province, South Africa. Seeds were treated with (seed safener) Concep II. prior to planting. Plots consisted of single row plots 8 m in length with 10 cm intra-row spacing. An inter-row spacing of 0.85 was used. Plots were fertilized with 300 kg (3:2:3) pre-plant. Weeds were controlled with Metalochlor/Terbuthylazine (a.i 125 / 375 g/ l) at 4 litres per ha. Treatments were evaluated in a randomized complete block design and replicated four times.

After nine weeks, five plants were randomly selected from each plot. Crowns and roots were gently rinsed in water to remove soil and plants were air dried at room temperature (22 - 24°C) for 30 minutes before individual plants were cut at the crown and fresh shoot and root mass were measured. Crown and root rot severity was assessed on a 0-5 scale as described (Correll *et al.*, 1986) above.

Age related resistance.

Seeds of sorghum genotypes, Buster, PAN 8706W, PAN 8564, PAN 8446, NK 283 and SNK 3939 were disinfested and surface sterilized as described previously (Salas *et al.*, 1999), sown in un-infested steam sterilized soil: peat (3:1) in 250 ml pots and allowed to grow for two, four and six weeks prior to inoculation (Hartman and Wang, 1992; Koch and Mew, 1991). Five pots replicated four times were prepared for each treatment combinations. Inoculations were performed using conidial suspensions prepared as described (Engle *et al.*, 2003 and Rose *et al.*, 2003) above. Soil surrounding the plant base was drenched with 50 ml of conidial suspensions (1×10^6 conidia per ml) of each *Fusarium* spp. isolate, *F. equiseti*, *F. proliferatum*, *F. solani*, and *F. thapsinum* (Dodman and Wildermuth, 1987).

The experiment was a split – split plot design with sorghum cultivars as main plot, *Fusarium* spp. as sub-plot and age at inoculation time as sub-sub plot, replicated four times. Plants were maintained in the glasshouse for two, four and six weeks after inoculation. Fertilization, watering and the temperatures in the glasshouse were as described above. The experiment was conducted twice.

Disease assessment was done at 14, 28 and 42 days after inoculation. Three plants were removed from each pot and treated as described above. Crown and root rot severity was assessed on a 0-5 scale (Correll *et al.* 1986) as described above. Plots of mean disease severity values on days after planting were used to produce an area under disease progress curve (AUDPC) using the formula of Shaner and Finney (1977). Where; $AUDPC = \sum (X_i + X_{i+1}) / 2 (t_{i+1} - t_i)$ $i=1$, in which X_i = root rot severity at the i th observation, t =time (days) at the i th observation, and n = total number of observations.

Induction of systemic resistance.

A split root bioassay for detection of systemic effects in roots as described by Larkin, Hopkins and Martin (1996) Zhang, Dick and Hoitink (1996) and Khan *et al.* (2004) was used to evaluate induced systemic resistance in sorghum. Soil was autoclaved at 121°C for 30 min on each of two consecutive days. Two 500 ml pots were taped together and each contained autoclaved treated soil with a pathogen added. The growth media in the respective paired pots were separated to avoid cross contamination by inoculum during watering of plants. Ground colonized oat seeds inoculum (5 g) prepared as described above (Wang and Jeffers, 2000; Clark *et al.*, 1995; Dorrance *et al.*, 2003) from each of the three *Fusarium* spp. exc (*F. thapsinum*, *F. proliferatum*, *F. equiseti*) was placed on the bottom half of the soil in each pot, followed by soil without the pathogen in the top half of the pot.

Seeds of genotypes SNK 3939, NK 283, PAN 8564 and PAN 8706W were surface sterilized as described above (Salas *et al.*, 1999) and planted (100 seeds) on moist germinating paper in square plastic containers. The seeded containers were covered with black polythene bags to act as a humidity chamber then placed in a glasshouse cubicle with temperatures of 25°C to 27°C. After emergence the developing root tip was excised. The excised root was allowed to grow for further nine days in the germinating containers. This resulted in a proliferation of several rootlets near the point

of excision. The roots of 12-day-old seedlings were separated into (two batches) for transplanting into the dual pot system (Zhang *et al.*, 1996; Larkin *et al.*, 1996; Khan *et al.*, 2004). Two separate experiments were conducted one with and one without antagonist.

The split root was transplanted and covered with uninfested soil (Larkin *et al.*, 1996). This enabled roots to grow for 2–3 days through uninfested soil before being challenged by the pathogen in the bottom half of the pot. An antagonist was introduced into one pot of each pair. The antagonist was introduced through drenching the root and soil surrounding the plant base with 50 ml of *T. harzianum* (Tricho-Plus) suspension in sterile distilled water (0.25 g in 1 litre). The antagonist was also able to colonize the roots before the pathogen. The experiment consisted of 16 split root plants for each treatment replicated four times. Fertilization, watering and the temperatures in the glasshouse were as described above. The trial was arranged in a randomized complete block design.

The severity of crown and root rot in the split root seedlings was determined 42 days after infestation using a 0-6 scale (recommended for split root trials) described by Khan *et al.* (2004) where; 1 = symptomless; 2 = mild root rot (one to three small lesions per root system); 3 = moderate root rot (one to three large lesions per root system); 4 = severe root rot and crown rot; 5 = severe root and crown rot and large stem lesion 6 = dead plant. The experiment was conducted twice.

Data analysis.

Data were analyzed using the general linear model (GLM) and ANOVA in SAS 9.1 (SAS Institute Cary NC). The data from repeated experiments were combined for analysis based on tests for homogeneity of variance. Treatment means were compared using the Fisher's least significant difference (LSD) with ($P \leq 0.05$). The ANOVA and GLM means from individual treatments were compared if interactions were significant ($P \leq 0.05$). Where interactions were not significant individuals treatments were combined to evaluate effects of treatments in the two main effects.

RESULTS

Pre- and post-emergence damping-off.

Pre-emergence damping-off. Data from the two experiments were homogenous and data were combined for analysis. Pre-emergence damping-off of sorghum seedlings was evident 7 days after inoculation and seedlings continued to die during the subsequent six weeks. *Fusarium* spp. and genotype ($P \leq 0.0001$) and *Fusarium* spp. x genotype interaction significantly ($P \leq 0.05$) affected percentage damping-off. Percentage damping-off was highest for IA 57 inoculated with *F. thapsinum* and lowest for TX 2793 inoculated with *F. proliferatum* (Table 5.1a).

Shoot and root mass were significantly ($P \leq 0.0001$) affected by genotype and *Fusarium* spp.. Genotype x pathogen interaction was not significant. Genotype TX 2793 inoculated with *F. proliferatum* displayed the greatest shoot and root mass compared to lowest for IA 57 inoculated with *F. thapsinum* and *F. proliferatum* respectively (Table 5.1a). *F. proliferatum* was least aggressive in terms of shoot and root mass compared to *F. thapsinum* (Table 5.1a).

Genotype, *Fusarium* spp. and genotype x *Fusarium* spp. interactions significantly ($P \leq 0.0001$) affected crown and root rot severity. Genotype IA 57 was most susceptible to *F. thapsinum* and *F. proliferatum* and displayed greatest crown and root rot severity compared to the resistant TX 2793 (Table 5.1a). The reaction of TX 2853 was intermediate (Table 5.1a). The results indicated that the isolate of *F. thapsinum* was highly aggressive and resulted in the most damping-off and crown and root rot severity compared with the isolate of *F. proliferatum* (Table 5.1a).

Post-emergence damping-off. Data for the two experiments were homogenous and were thus combined for analysis. Genotype x *Fusarium* spp. interaction significantly ($P \leq 0.0001$) affected percentage damping-off. Genotype TX 2853 inoculated with *F. thapsinum* displayed the highest percent damping-off compared to lowest for TX 2793 (Table 5.1b).

Genotype and *Fusarium* spp. significantly ($P \leq 0.0001$) affected shoot and root mass. Genotype x *Fusarium* spp interaction was not significant. Highest shoot mass was recorded for TX 2793 inoculated with *F. proliferatum* and lowest was for TX 2853 inoculated with *F. thapsinum*. Root mass was highest for TX 2853 inoculated with *F. proliferatum* and lowest for IA 57 inoculated with *F. thapsinum*. Genotype and *Fusarium* spp. and genotype x *Fusarium* spp. interaction significantly ($P \leq 0.0001$) affected crown and root rot severity. Genotype IA 57 and TX 2853 inoculated with *F.*

proliferatum and *F. thapsinum* respectively displayed the greatest crown and root rot severity compared to least for TX 2793 inoculated with *F. proliferatum*. Genotype IA 57 was most susceptible to damping-off and crown and root rot severity while TX 2793 was resistant (Table 5.1b) and TX 2853 expressed an intermediate reaction Table 5.1b). *F. thapsinum* was highly aggressive and resulted in highest percent damping-off (Table 5. 1b) while *F. proliferatum* was less aggressive.

Sources of partial resistance.

Data for the two glasshouse trials were homogeneous and were therefore combined for analysis. Genotype significantly ($P \leq 0.0001$) affected shoot and root mass, crown and root rot severity and AUDPC. Shoot mass ranged from 0.35 to 9.20 g and the root mass ranged from 0.18 to 6.30 g for 88B885*GB102B inoculated with *F. equiseti* and TX 2793 inoculated with *F. solani* respectively (Table 5.2a). The effect of *Fusarium* spp. was variable and dependent on sorghum genotype resistance and the level of isolate aggressiveness (Table 5.2a).

Crown and root rot severity was highest for the susceptible lines Tx2895*(SC170*R4671) and PL2120*87EO366 inoculated with *F. thapsinum* and Macia*Dorado inoculated with *F. solani* and *F. proliferatum* compared to the lowest recorded for TX 2793 inoculated with *F. proliferatum* (Table 5.2a). Crown and root rot severity ratings ranged from 0.63 to 5.00 (Table 5.2a). Nine sorghum genotypes were identified having partial resistance to crown and root rot disease severity with the disease ratings ranging from 0.63 to 2.88 (Table 5.2a).

In field experiments, genotype significantly ($P=0.001$) affected shoot and root mass and crown and root rot severity. Shoot mass was highest for (86ED361*Macia)-HF39 and least for 86D361*Macia-HF41 (Table 5.2b). Root mass was highest for B*GB102B-13-1-2 and least for (86D361*Macia)-HF41 (Table 5.2b). Crown and root rot severity ratings in the field ranged from 2.67 to 5.00 (Table 5.2b). Good levels of sources of partial resistance to crown and root rot severity were observed on four genotypes (PAN 8564, PAN 8446, 86E0361*8B2668 and BTx635) which displayed crown and root rot severity ratings ranging from 2.67 to 3.33 (Table 5.2b), with the highest for NK 283 and least for PAN 8564 / PAN 8446 (Table 5.2b).

Age related resistance.

Results indicated significant ($P \leq 0.0001$) effects of genotype, time and pathogen, time x *Fusarium* spp. and genotype x time x pathogen interactions on shoot and root mass. Shoot and root mass were highest for PAN 8564 inoculated with *F. equiseti* at six weeks after planting (Table 5.3). GLM analysis indicated a significant ($P \leq 0.0001$) effect of genotype, time and *Fusarium* spp., genotype x time x pathogen and time x pathogen interactions on crown and root rot severity and AUDPC. Crown and root rot severity and AUDPC were greatest for SNK 3939 inoculated with *F. equiseti* and *F. thapsinum* at two weeks after planting compared to least for PAN 8706W inoculated with *F. proliferatum*, *F. solani* and *F. thapsinum* at six weeks respectively. Interactions indicated that genotypes responded differently to plant age at time of inoculation. GLM analysis indicated that plants inoculated at two and four week-old expressed the highest degree of crown and root rot severity (with the highest for four-week-old plants) compared with six-week-old plants (Table 5.3). AUDPC was significantly lower for the two- and six-week-old plants (42.00) than four-week-old plants (84.00) for PAN 8706W (Table 5.3).

The reaction of sorghum genotypes were variable depending on their level of resistance, virulence of the isolate used for inoculations and plant age at time of inoculation. However, genotype PAN 8706W consistently showed least AUDPC and crown and root rot severity at all plant ages (Table 5.3). Plants inoculated two weeks after planting were most susceptible to crown and root rot severity compared to least susceptible six-week-old plants. Four-week-old plants were intermediate (Table 5.3).

SNK 3939 and NK 283 were most susceptible to crown and root rot severity and AUDPC at all plant ages. The reactions of PAN 8446 and Buster were intermediate while PAN 8706W and PAN 8564 were least susceptible (Table 5.3). Disease severity differed significantly between *Fusarium* spp., plant age and resistance (Table 5.3).

Induction of systemic resistance.

In the antagonist-infested soil treatments, there was a significant ($P \leq 0.05$) genotype and genotype x *Fusarium* spp. interaction effect on shoot and root mass and crown and root rot severity. PAN 8706W inoculated with *F. proliferatum* displayed

the highest shoot and root mass compared to lowest for PAN 8564 inoculated with *F. equiseti* (Table 5.4a). *F. thapsinum* caused the greatest crown and root rot severity on the most susceptible genotype SNK 3939 compared with the least effect on the resistant genotype PAN 8706W (Table 5.4a). The treatment with the antagonist isolate (inducer treatment) of the commercial biocontrol *T. harzianum* (Tricho-plus) was highly effective in reducing crown and root rot severity (Tables 5.4a and b). The effect of the *Fusarium* spp. on the measured parameters of the treated side was variable based on the virulence of the *Fusarium* spp.

In the *Fusarium* inoculated soil treatment without antagonist genotype, genotype x *Fusarium* spp. interaction significantly ($P \leq 0.01$) affected shoot mass and ($P \leq 0.05$) root mass. Highest shoot and root mass were recorded for PAN 8706W inoculated with *F. proliferatum* compared to lowest for PAN 8564 inoculated with *F. equiseti* (Table 5.4b). There was a significant ($P \leq 0.01$) genotype, *Fusarium* spp. and genotype x *Fusarium* spp. interaction on crown and root rot severity. Crown and root rot severity was greatest for SNK 3939 inoculated with *F. equiseti* and lowest for PAN 8706W inoculated with *F. proliferatum* and *F. thapsinum* (Table 5.4b).

The significant interactions effect suggests that the tested genotypes differed in their response to the effect of *Fusarium* spp. The antagonist treated soil resulted in somewhat lower crown and root rot severity than the pathogen treatment soil where the antagonist was not added (Tables 5.4a and 5.4b). *T. harzianum* enhanced plant growth and development as observed by the higher shoot and root growth and reduced the crown and root rot severity compared with the highest recorded in the pathogen inoculated soil treatment (Table 5.4a).

DISCUSSION

In both pre- and post-emergence damping-off inoculation studies sorghum genotypes could be placed into three categories, namely the resistant group with crown and root rot severity ratings of 0-1.50 which included TX 2793, the intermediate group (2-2.50) which included TX 2853 and the susceptible group (>2.50) which included IA 57. The present study results are consistent with Van Wyk *et al.* (1988) who found three levels of resistance on wheat cultivars in terms of percent damping-off as tolerant,

intermediate and susceptible to *F. graminearum* Group 1 evaluated in the pre- and post-emergence percentage damping-off.

Fusarium spp. that cause root and stalk rot of sorghum have been reported to persist in the soil on the crop debris and weed hosts (Tuinstra *et al.*, 2002). This was shown by the high crown and root rot severity ratings observed in the field where the investigation was dependent on natural infection. In the present study glasshouse investigations were not dependent on natural infection and inoculations were used. Ground colonized oat grain inoculum mixed in the top 5 cm of the soil in pots was used to inoculate sorghum plants evaluated for sources of partial resistance in the glasshouse seeds were planted in direct contact with the inoculum. This inoculation technique used may have contributed to the consistency of the results. Similarly, Kraft (1975) used this method in screening pea lines for resistance to *Fusarium* root rot. However, Purss (1966) concluded that seedling blight reaction in the glasshouse gave no guide to the tolerance of wheat in the field to infection by *F. graminearum*.

In the present study, several genotypes of sorghum with good level of partial resistance to *Fusarium* crown and root rot of sorghum were identified in both field and glasshouse experiments. The best sources of partial resistance were observed among nine genotypes determined by crown and root rot severity and AUDPC. None of the evaluated genotypes was immune to crown and root rot severity pathogens. However, genotype TX 2793 displayed a disease severity of 0.00 to 0.72 which was indicative of resistance to *Fusarium* crown and root rot severity. The glasshouse results were corroborated in the field. Generally crown and root rot severities were higher in the field compared to the glasshouse.

Screening of sorghum seedlings for age related resistance under glasshouse conditions was effective and gave repeatable results. This is contrary to the view of Duncan and de Milliano (1995) who suggested that screening juvenile plants under artificial conditions is generally not effective. In the present study, younger plants were more susceptible than older plants. The overall results indicated that highest root rot severities were observed in plants inoculated at two and four weeks with the latter displaying the greatest crown and root rot severity. Plants inoculated at six weeks after planting were more resistant.

Kauffman *et al.* (1973) and Ezuka, Watanabe and Horino (1974) reported a similar trend for susceptible and intermediate rice cultivars as seedlings and adult

plants. The current study findings are consistent with Van Wyk *et al.* (1988) who found that the wheat cultivar SST 107 was more tolerant in the mature plant stage, but proved highly susceptible to *Fusarium* crown rot in the seedling stage. Mew, Vera-Cruz and Reyes (1981) found that lesions on susceptible rice cultivars inoculated 90 days after sowing were shorter than those of the same cultivar inoculated 30 days after sowing. Reports of age related resistance vary, but younger plants are generally more susceptible than older plants as was reported for other crops such as peppers and rice (Kim and Wang, 1989; Reifschneider *et al.*, 1986). Koch and Mew (1991) found that in all rice cultivars, susceptibility to *X. oryzae* decreased as the plant increased in age.

In the present study, crown and root rot severity was highest for plants of intermediate age (four weeks) in all genotypes compared to two and six-week old plants. Hartman and Wang (1992) found that older and younger plants had less disease than plants of intermediate age. The present study indicated differences in response of six sorghum genotypes to *Fusarium* crown and root rot at all plant ages evaluated. The genotypes could clearly be divided into those that were highly susceptible, (SNK 3939 and NK 283), intermediate (Buster and PAN 8446) and least susceptible (PAN 8706W and PAN 8564). Differences among genotypes of the intermediate groups were difficult to ascertain at all plant ages, and there was no evidence that differences were significantly easier to detect in older plants than in younger plants. Most importantly, it is desirable for highly resistant lines to be resistant at all plant ages.

The split-root technique employed in the present study was successful in separating the pathogen from the antagonist inoculated soil treatments. Individual control plants as well as the split-root plants showed no cross contamination on roots between the two pots. Treatment with an antagonistic isolate of *T. harzianum* drenched into soil surrounding plant base induced an increase in shoot mass, root mass and a decrease in crown and root rot severity. Larkin *et al.* (1996) demonstrated that selected isolates of *F. oxysporum* from suppressive soils induced systemic resistance to *Fusarium* wilt. Similarly, Davis (1967); Maraite (1982); Biles and Martyn (1989), working in split-root experiments on water melon, observed that induced resistance by non-pathogenic *F. oxysporum* resulted in a reduction or delay in disease development.

Martyn, Dillard and Biles (1990) found that the effect of induced systemic resistance could be overcome by an increase in pathogen inoculum density. Hillocks (1986) working with induced resistance to *Fusarium* wilt of cotton, concluded that the

mechanism was vascular occlusion induced by a non-pathogenic strain of *F. oxysporum* which limited the movement of the pathogen within vascular tissue. Larkin *et al.* (1996) mentioned that induced resistance probably involves reducing or restricting the extent of pathogen colonization or movement within vascular tissue rather than the prevention of root infection.

In the present study, induction of systemic resistance in sorghum was demonstrated when treatment with *T. harzianum* resulted in lower disease levels in autoclaved soil plus antagonist compared to autoclaved soil without antagonist. Khan *et al.*, (2004) reported evidence for the induction of systemic response in cucumber by *Trichoderma hamatum* 382 (T382) in split root experiments. The systemic effect induced by T 382 supports results reported for another *Trichoderma* isolate, *Trichoderma harzianum* T 203 by (Yedidia, Benhamou and Chat, 1999). In the present study, the significant interaction was observed because the inducer treatment had no effect if the pathogen was not present. Larkin *et al.* (1996) found that the mechanism of action is competition and thus they require inoculum levels substantially greater than the pathogen to successfully prevent infection.

Results of the present study have implications for the development of a sorghum cultivar program. Differences in resistance were recorded between the sorghum genotypes in terms of pre- and post-emergence damping off. Genotype TX 2793 showed a high degree of resistance in both the pre- and post-emergence damping-off TX 2853 was intermediate and IA 57 was susceptible. Quantitative resistance was evident at all plant ages as a reduction in crown and root rot with increase in plant age although immunity to *Fusarium* crown and root rot was not found. However, good sources of partial resistance to *Fusarium* crown and root rot were found to exist among the sorghum germplasm evaluated. The use of *T. harzianum* to elicit systemic resistance in sorghum could be a useful tool in disease management program. Therefore, resistance to crown and root rot can be improved by evaluating resistance in the sorghum germplasm collection and utilizing the most resistant genotypes while avoiding the susceptible ones.

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Table 5.1a: The effect *Fusarium* spp. on pre-emergence damping-off, shoot and root mass growth and crown and root rot severity of sorghum

Genotype	<i>Fusarium</i> spp.	Damping-off (%)	Shoot mass (g)	Root mass (g)	Root rot (0-5)
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IA 57	<i>F. proliferatum</i>	66.75ab ¹	7.83d	2.85e	3.50ab
	<i>F. thapsinum</i>	67.25a	7.20d	3.38d	3.75a
	Control	0.00d	12.05bc	8.73c	0.00d
TX 2853	<i>F. proliferatum</i>	42.25c	11.18c	7.00cd	2.00bc
	<i>F. thapsinum</i>	51.75b	11.76c	8.23c	2.50b
	Control	0.00d	12.58bc	7.20cd	0.00d
TX 2793	<i>F. proliferatum</i>	35.00cd	17.90ab	16.25a	1.25c
	<i>F. thapsinum</i>	44.00bc	15.65b	10.25b	1.00cd
	Control	0.00d	20.80a	13.23ab	0.00d

¹ Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).

Table 5.1b: The effect *Fusarium* spp. on post-emergence damping-off, shoot and root mass growth and crown and root rot severity of sorghum

Genotype	<i>Fusarium</i> spp.	Damping-off (%)	Shoot mass (g)	Root mass (g)	Root rot (0-5)
IA 57	<i>F. proliferatum</i>	57.50bc ¹	18.60c	11.48cd	3.50a
	<i>F. thapsinum</i>	62.50ab	19.70bc	10.68c	3.00a
	Control	0.00e	19.38bc	14.60bc	0.00b
TX 2853	<i>F. proliferatum</i>	58.00b	21.76b	16.25ab	2.50b
	<i>F. thapsinum</i>	73.50a	15.23d	10.58c	3.25a
	Control	0.00e	22.93ab	19.65a	0.00b
TX 2793	<i>F. proliferatum</i>	18.75c	22.65ab	15.40b	1.00c
	<i>F. thapsinum</i>	15.50d	18.83c	13.72c	1.25c
	Control	0.00e	25.20a	15.25b	0.00d

¹ Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).

Table 5.2a: Effect of *Fusarium* spp. on, shoot weight (g), root weight (g) disease severity (0-5) and area under disease progress curve (AUDPC) on 21 sorghum cultivars plus control for genotypes tested for sources of partial resistance

Sorghum genotype	<i>F. equiseti</i>				<i>F. solani</i>				<i>F. thapsinum</i>				<i>F. proliferatum</i>			
	Shoot mass (g)	Root mass (g)	Root rot (0-5)	AUDPC	Shoot mass (g)	Root mass (g)	Root rot (0-5)	AUDPC	Shoot mass (g)	Root mass (g)	Root rot (0-5)	AUDPC	Shoot mass (g)	Root mass (g)	Root rot (0-5)	AUDPC
Buster	3.16a ¹	2.98a	3.50b	147.00e	1.03cd	0.54c	3.73bc	156.45c	4.31ab	2.36b	4.00b	168.00b	4.21a	3.63a	3.25c	126.00d
PAN8564	2.36ab	1.59ab	2.88c	120.75f	0.98d	0.62c	3.50bc	147.00cd	1.11d	0.76cd	3.83bc	160.65bc	1.09c	0.89bc	3.73bc	156.45c
PAN8706W	1.78b	1.00b	2.38cd	99.75gh	2.95b	1.73b	1.75d	73.50ef	2.88c	1.83bc	3.00c	126.00de	1.50bc	1.26b	1.75de	73.50fg
PAN8446	1.01bc	0.68bc	4.00ab	168.00cd	1.80c	0.95c	4.00b	168.00bc	3.33bc	2.05b	3.00c	126.00de	2.45b	1.51b	4.00b	168.00bc
SNK3939	0.60c	0.30c	4.75a	199.50ab	1.94c	1.13bc	4.00b	168.00bc	3.65b	2.67ab	2.75cd	115.50e	1.43c	0.93bc	4.25b	168.00bc
NK283	1.98b	1.28b	4.00ab	168.00cd	2.35bc	1.53b	2.88cd	120.75de	4.25ab	2.33b	4.00b	168.00b	1.31c	0.59bc	3.00c	126.00d
Sureno	1.85b	1.31b	2.52c	105.00g	0.91d	0.54c	2.75c	115.50e	3.27bc	1.88bc	3.00c	126.00de	3.28ab	1.82b	2.00d	84.00ef
BTx635	1.01bc	0.56bc	2.88c	120.75f	1.35cd	0.88c	3.00c	126.75d	1.70d	1.15c	2.75cd	115.50e	0.81cd	0.61bc	2.75cd	115.50de
86E0361*8B2668	0.59c	0.28c	3.63b	152.25de	0.90d	0.55b	3.75bc	157.50c	0.90de	0.63cd	3.50bc	147.00cd	0.53cd	0.25c	3.50bc	147.00cd
Tx2895*(SC170*R4671)	1.44bc	1.18b	4.88a	204.75a	2.78b	1.73b	4.25b	178.50b	2.13cd	1.22c	5.00a	210.00a	0.61cd	0.23c	4.00b	168.00b
PL2120*87EO366	1.14bc	1.09b	4.88a	204.75a	1.40cd	0.75c	3.00c	126.75d	0.73de	0.50cd	5.00a	210.00a	0.60cd	0.38c	4.75ab	199.50ab
90EO328*CE151	0.90c	0.60bc	3.13bc	131.25ef	1.53c	1.33bc	2.75cd	115.50e	1.73d	1.03c	3.25c	136.50d	1.30c	0.78bc	3.00c	126.00d
(86ED361*Macia)-HF41	0.55c	0.30c	3.88b	162.75d	1.04cd	0.55c	3.73bc	156.45c	0.95de	0.63cd	4.00b	168.00b	0.73cd	0.48c	3.75bc	157.50c
ICSV1089BF*Macia	0.51c	0.33c	4.25ab	178.50c	0.68d	0.40cd	4.50ab	189.00ab	1.05d	0.63cd	4.00b	168.00b	0.58cd	0.33c	4.00b	168.00bc

B1*BTx635	0.66bc	0.35c	4.38ab	183.75bc	1.08cd	0.89c	3.73bc	156.45c	1.13d	0.73cd	4.00b	168.00b	0.90cd	0.61bc	4.75ab	199.50ab
(86ED361*Macia)- HF39	1.31bc	1.22b	4.50a	189.00b	0.98d	0.62c	3.50bc	147.00cd	0.58de	0.35d	4.75ab	199.50ab	0.43d	0.28c	4.25b	178.50b
SRN39*87ED366	1.06bc	0.66bc	3.88b	162.75d	1.23cd	0.78c	3.75bc	157.50c	0.88de	0.55cd	4.00b	168.00b	0.77cd	0.32c	3.75bc	157.50c
88B885*GB102B	0.35cd	0.18c	4.00ab	168.00cd	0.55d	0.24cd	3.75bc	157.50c	0.53de	0.33d	4.00b	168.00b	0.55cd	0.38c	4.00b	168.00bc
Macia*Dorado	0.59c	0.35c	4.38ab	183.75bc	0.95d	0.58c	5.00a	210.00a	1.11d	0.73cd	3.75bc	157.50c	0.80cd	0.45c	5.00a	210.00a
B1*B9501	0.46cd	0.24c	2.75c	115.50fg	0.70d	0.48cd	3.75bc	157.50c	0.70de	0.45d	3.00c	126.00de	0.73cd	0.48c	2.50cd	105.00e
TX2793	3.04a	1.75ab	0.71d	30.00h	9.20a	6.30a	0.00e	0.00f	4.69a	3.04a	0.72d	30.00f	3.45ab	1.90bc	0.63e	26.25g

¹ Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05) .

Table 5.2b: Effect of *Fusarium* spp. on, shoot weight (g), root weight (g) and disease severity (1-5) on 13 sorghum genotypes planted under natural field conditions at Cedara

Sorghum Genotype	Shoot mass (g)	Root mass (g)	Root rot (0-5)
(86ED361*Macia)-HF39	260.27a ¹	62.63bc	4.67ba
NK283	256.37ba	49.93bc	5.00a
Tx2895*(SC170*R4671	222.97bac	46.30bc	4.04bac
88B885*GB102B	215.60bac	57.33bc	4.68ba
Buster	206.53bdac	57.17bc	3.68bdc
B1*GB102B-13-1-2	197.17ebdac	101.17a	3.67bdc
PAN8446	182.73ebdac	77.23ba	2.66d
PAN8564	160.93ebdfc	63.80bc	2.67d
Macia*Dorado	125.77edfc	54.43bc	3.66bdc
PL2120*87EO366	116.97edf	34.40c	4.65ba
86E0361*8B2668	102.87ef	49.90bc	3.32dc
BTx635	73.33f	32.48c	3.33dc
(86ED361*Macia)-HF41	65.70f	32.40c	4.00bac

¹ Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).

Table 5.3 The effect of plant age at time of inoculation with *Fusarium* spp. on mean shoot mass (g), mean root mass (g) and root rot severity (0-5 scale) and area under the disease progress curve (AUDPC) of six sorghum genotypes

Sorghum genotype	<i>Fusarium</i> spp.	2 weeks				4 weeks				6 weeks			
		Shoot mass (g)	Root mass (g)	Root rot (0-5)	AUDPC	Shoot mass (g)	Root mass (g)	Root rot (0-5)	AUDPC	Shoot mass (g)	Root mass (g)	Root rot (0-5)	AUDPC
SNK 3939	<i>F. equiseti</i>	1.32c ¹	1.05b	4.75a	199.50a	3.18b	2.65b	4.25a	178.50a	3.33c	2.58bc	2.75a	115.50a
	<i>F. proliferatum</i>	2.07b	2.00a	3.25bc	136.50cd	3.08b	2.93b	3.25b	136.50c	1.65de	1.88cd	2.25ab	94.50b
	<i>F. solani</i>	1.72bc	1.13b	4.25ab	178.50b	2.88bc	2.68b	4.25a	178.50a	1.20e	1.00d	2.50a	105.00ab
	<i>F. thapsinum</i>	3.00a	2.10a	4.75a	199.50a	2.05c	1.53c	3.00b	126.00cd	2.28d	2.20c	1.50b	63.00cd
	Control	2.70ab	2.25a	0.00e	0.00g	2.53bc	1.73c	0.00d	0.00f	4.10d	3.73ab	0.00c	0.00e
NK 283	<i>F. equiseti</i>	2.00b	1.42b	4.50a	189.00ab	2.83bc	2.15bc	4.00a	168.00ab	1.58de	1.10d	2.25ab	94.50b
	<i>F. proliferatum</i>	1.92bc	1.53ab	2.25cd	94.50e	3.68ab	3.38ab	3.50ab	147.00bc	3.60bc	3.15b	1.75b	73.50c
	<i>F. solani</i>	3.05a	1.73ab	3.00bc	126.00d	2.84bc	2.01bc	3.25b	115.50d	1.73de	0.98de	1.50b	63.00cd
	<i>F. thapsinum</i>	1.30c	1.00b	3.25bc	136.50cd	2.78bc	2.01bc	3.50ab	147.00bc	1.98de	1.28d	2.25ab	94.50b
	Control	1.10c	1.18b	0.00e	0.00g	1.48d	1.13cd	0.00d	0.00f	2.90cd	1.63cd	0.00c	0.00c
Buster	<i>F. equiseti</i>	1.23c	0.88bc	2.25cd	94.50e	4.05a	3.13ab	4.25a	178.50a	4.13b	3.68ab	1.75b	73.50c
	<i>F. proliferatum</i>	1.90bc	1.65ab	2.75c	115.50de	2.97bc	2.60b	3.50ab	147.50bc	4.30b	3.58ab	1.25bc	52.50d
	<i>F. solani</i>	2.52ab	1.80ab	4.50a	189.00ab	3.05b	2.95b	3.50ab	147.50bc	2.58cd	2.10c	1.75b	73.50c
	<i>F. thapsinum</i>	2.46b	2.11a	2.50c	105.00de	3.55ab	2.85b	4.25a	178.50a	2.48d	3.35b	1.25bc	52.50d
	Control	1.31c	1.23b	0.00e	0.00g	2.60bc	2.51b	0.00d	0.00f	3.21c	3.15b	0.00c	0.00e
PAN 8446	<i>F. equiseti</i>	1.50bc	1.20b	4.25ab	178.50b	3.42b	2.88b	3.25b	136.50c	1.25e	1.21d	2.50a	105.00ab
	<i>F. proliferatum</i>	2.50ab	1.98ab	2.75c	115.50de	1.03d	0.73d	3.25b	136.50c	1.75de	1.50cd	2.00ab	84.00bc

	<i>F. solani</i>	1.71bc	1.23b	3.00bc	126.00d	2.85bc	2.20bc	3.25b	136.50c	2.00d	1.48d	2.00ab	84.00bc
	<i>F. thapsinum</i>	2.20b	1.80ab	4.00ab	168.00bc	0.73de	0.43de	3.75ab	157.50b	4.55ab	2.85bc	2.25ab	94.50b
	Control	1.60bc	1.35b	0.00e	0.00g	4.45a	4.15a	0.00d	0.00f	1.78de	1.68cd	0.00c	0.00e
PAN 8564	<i>F. equiseti</i>	1.60bc	1.23b	3.75b	157.50c	1.95cd	1.75c	3.25b	136.50c	6.23a	5.13a	1.75b	73.50c
	<i>F. proliferatum</i>	1.70bc	1.00b	2.75c	115.50c	2.10c	1.85c	2.75bc	115.50d	4.63ab	3.30b	1.50b	63.00cd
	<i>F. solani</i>	1.05c	0.85bc	3.00bc	126.50d	2.83bc	2.35bc	2.75bc	115.50d	2.18d	1.83cd	1.75b	73.50c
	<i>F. thapsinum</i>	1.72bc	1.27b	2.00cd	84.00ef	3.28b	2.65b	3.25b	136.50c	1.68de	1.73cd	2.25ab	94.50b
	Control	3.10a	2.28a	0.00e	0.00g	3.12b	2.38bc	0.00d	0.00f	1.88de	1.65cd	0.00c	0.00e
PAN 8706W	<i>F. equiseti</i>	0.82d	1.00b	2.00cd	84.00ef	3.48b	3.03ab	1.50cd	63.00e	1.03e	0.83de	1.25bc	52.50d
	<i>F. proliferatum</i>	2.10b	1.45b	1.00d	42.00f	1.33d	1.13cd	1.50cd	63.00e	3.18c	2.40c	1.00bc	42.00de
	<i>F. solani</i>	3.11a	2.38a	1.01d	42.00f	2.53bc	2.05bc	1.75cd	73.50de	4.45b	2.55bc	1.00bc	42.00de
	<i>F. thapsinum</i>	1.22c	1.20b	1.00d	42.00f	2.52bc	1.93c	2.00c	84.00d	2.35d	2.28c	1.00bc	42.00de
	Control	2.50ab	1.23b	0.00e	0.00g	3.35b	2.63b	0.00d	0.00f	0.90ef	0.65de	0.00c	0.00e

¹ Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).

Table 5.4a: Effect of *Fusarium* spp. and soil treatment plus antagonist on mean shoot mass, root mass and crown and root rot of sorghum genotypes evaluated for induced systemic resistance in split root plants

Genotype	<i>Fusarium</i> isolate	Shoot mass (g)	Root mass (g)	Root rot (0-5)
NK 283	<i>F. equiseti</i>	21.70b ¹	4.83b	3.05a
	<i>F. proliferatum</i>	18.15c	5.27ab	3.00a
	<i>F. thapsinum</i>	25.57a	5.63a	2.00b
	Control	22.81	6.24	0.00
PAN 8564	<i>F. equiseti</i>	5.63d	3.40c	3.20ab
	<i>F. proliferatum</i>	20.93a	4.23b	2.33b
	<i>F. thapsinum</i>	20.80a	5.28a	3.33a
	Control	20.15	4.23	0.00
PAN 8706W	<i>F. equiseti</i>	31.17ab	5.70b	1.33ab
	<i>F. proliferatum</i>	38.53a	12.23a	2.00a
	<i>F. thapsinum</i>	23.03b	3.73c	1.00b
	Control	20.94	6.72	0.00
SNK 3939	<i>F. equiseti</i>	24.97ab	6.33a	3.67ab
	<i>F. proliferatum</i>	18.20b	3.80c	3.30b
	<i>F. thapsinum</i>	29.70a	5.63b	4.00a
	Control	17.28	4.24	0.00

¹ Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).

Table 5.4b: Effect of *Fusarium* spp. soil treatment without antagonist on mean shoot mass, root mass and crown and root rot of sorghum genotypes evaluated for induced systemic resistance in split root plants.

Genotype	<i>Fusarium</i> isolate	Shoot mass (g)	Root mass (g)	Root rot (0-5)
NK 283	<i>F. equiseti</i>	19.70b ¹	4.60a	3.67a
	<i>F. proliferatum</i>	20.60ab	4.43a	3.65a
	<i>F. thapsinum</i>	21.90a	3.30b	3.66a
	Control	18.74	4.35	0.00
PAN 8564	<i>F. equiseti</i>	5.30c	1.97c	3.77a
	<i>F. proliferatum</i>	18.27b	4.23ab	3.64a
	<i>F. thapsinum</i>	20.47a	5.83a	3.68a
	Control	16.04	4.28	0.00
PAN 8706W	<i>F. equiseti</i>	21.67b	5.47b	3.00a
	<i>F. proliferatum</i>	33.26a	11.20a	2.33b
	<i>F. thapsinum</i>	18.03c	3.03c	2.33b
	Control	16.50	7.65	0.00
SNK 3939	<i>F. equiseti</i>	24.17b	7.63a	4.33a
	<i>F. proliferatum</i>	15.33c	4.53c	4.05b
	<i>F. thapsinum</i>	30.70a	5.67b	4.00b
	Control	14.22	4.90	0.00

¹Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).

CHAPTER VI

THE ROLE OF EDAPHIC FACTORS ON FUSARIUM CROWN AND ROOT ROT DISEASE OF SORGHUM

ABSTRACT

The effects of soil type, soil moisture, soil pH and fertility and soil amendments on crown and root rot of sorghum caused by *Fusarium* spp. were studied in the glasshouse in 2004 and 2005. Four soil types collected from Potchefstroom, Bethlehem, Cedara and Bloemfontein were evaluated with four *Fusarium* spp. (*F. equiseti*, *F. proliferatum*, *F. solani*, and *F. thapsinum*) on three sorghum genotypes NK 283, PAN 8446 and PAN 8706W. Bloemfontein and Potchefstroom soils were suppressive while Bethlehem and Cedara soils were conducive to crown and root rot severity. The study demonstrated that *Fusarium* spp. can survive in a wide range of soil types. Sorghum genotypes, NK 283, PAN 8446 and PAN 8706W and *Fusarium* spp., *F. equiseti*, *F. proliferatum*, *F. solani* and *F. thapsinum*, and an untreated control were evaluated at four root zone moisture levels 25, 50, 75 and 100 % (MHC). Shoot and root mass, percent emergence and crown and root rot severity were greatest at 50 and 75 % compared to 25 and 100 %. NK 283 exhibited the highest crown and root rot severity. *F. equiseti* caused a reduction in percent emergence in NK 283 and PAN 8446 at 50 and 75% moisture levels and shoot mass and root mass. *F. thapsinum* consistently resulted in greatest crown and root rot severity ratings. The effect of soil pH and nitrogen on crown and root rot severity were studied on three sorghum genotypes (NK 283, PAN 8446, PAN 8706W) with four *Fusarium* spp., *F. thapsinum*, *F. proliferatum*, *F. solani* and *F. equiseti*. The treatments consisted of (normal-N) single nitrogen application at the recommended rate, nitrogen application at a 1.5 times the recommended rate (high-N) and the recommended rate applied in three splits (split-N). The results indicated that normal-N and high-N significantly increased crown and root rot ratings. NK 283 displayed highest crown and root rot severity at normal and high nitrogen application rates. PAN 8446 resulted in highest shoot and root mass. PAN 8706W consistently resulted in least crown and root rot severity. The effect of soil amendments on crown and root rot pathogen infection was studied. Three soil amendments namely compost, kraal and chicken manure together with three sorghum genotypes (NK 283, PAN 8446, PAN 8706W) and four *Fusarium* spp. (*F. equiseti*, *F. proliferatum*, *F. solani* and *F. thapsinum*) were evaluated. Kraal manure was detrimental to plant growth while chicken manure was most effective in decreasing the crown and root rot severity. Crown and root rot was significantly higher for NK 283, least for PAN 8706W and PAN 8446 was intermediate.

INTRODUCTION

Soils that are naturally suppressive to *Fusarium* wilt diseases of numerous crops occur in many regions of the world (Toussoun, 1975; Scher and Baker, 1980; Schneider, 1984; Alabouvette, 1986; Nelson, Burgess and Summerell, 1990). In suppressive soils disease does not readily develop even though the pathogen and susceptible hosts are present (Larkin, Hopkins and Martin, 1996; Mazzola, 2002). Physical characteristics of soils are involved in suppressiveness. Suppressive soils tend to have high clay and organic matter content, which supports diverse populations of antagonistic bacteria and actinomycetes (Stotzky, 1966; Cook and Baker, 1983; Alabouvette, 1986; Amir and Alabouvette, 1993).

Suppressiveness is primarily a result of inhibition of chlamydospore germination and a reduction in saprophytic growth of pathogens. In contrast, conduciveness enhances the development of soil-borne pathogens (Smith, 1977; Scher and Baker, 1980; Scher and Baker, 1982; Sneh *et al.*, 1984; Elad and Baker, 1985; Alabouvette, 1986; Lemanceau, 1989; Louvet, 1989; Couteaudier and Alabouvette, 1992; Alabouvette, Lemanceau and Stinberg, 1993).

Crop production is seriously affected by soilborne diseases that cause heavy economic losses (Jarvis, 1989). Manipulating environmental conditions to control *Fusarium* diseases such as adjustment of moisture through soil solarisation has successfully been applied in many countries for the control of soilborne pathogens (Katan, 1981). Soil flooding has been found an effective alternative for controlling soil borne plant pathogens in specific areas or under specific conditions (Strandberg, 1987). The efficiency of soil solarization in killing *F. oxysporum* generally decreases with increasing soil moisture content (Al-Karraghoul and Al-Kayssi, 2001). The effect of moisture on *Fusarium solani* infested soil at moisture levels of 100, 70 and 25 % and resultant seed rot, germination, plant growth and root rot severity on beans was evaluated in the glasshouse (Tu and Tan, 2004). Watering schedule significantly affected both disease incidence and severity with the least disease incidence and severity observed on plants growing in wet and dry conditions (Wang and Jeffers, 2002). Disease development has been reported to be favoured by rainfall followed by cool weather in a “subnormal” rainfall season (Tachibana, 1968) and high temperature and soil moisture under greenhouse conditions (Lewis and Papavizas, 1977). Kendig, Rupe and Scott (2000) suggested that irrigation management is a key factor to obtaining profitable yield in the presence of certain soil borne pathogens.

Disease control has been achieved by the manipulation of soil pH and fertility. The concentration of major and minor nutrient elements and their interaction with pH on inoculum potential and disease development needs to be determined. It has been advised that soil pH be maintained above 5.8 in all fields where grain sorghum is planted (Mitchell and Mask, 2004).

Composts are increasingly gaining popularity not only from an ecological point of view but also as a means to recycle pollutants. Their potential in soilborne disease suppression clearly meets the needs of sustainable agriculture with low environmental effects (Pharand, Carisse and Benhamou, 2001). Composted organic materials have been used with varying success for suppression of soil-borne diseases of several crops (Daft, Poole, and Hoitink, 1979; Stephens and Stebbins, 1985; Lumsden, Millner and Lewis, 1986; Chen, Hoitink, and Schimithener, 1987; Nelson, Craft and Feldman, 1993; Theodore and Toribio, 1995; Craft and Nelson, 1996). Organic amendment of agricultural soil typically improves soil quality and suppresses soilborne diseases (Stone, Scheuerell and Darby, 2004).

The objectives of the present study were to determine the effects of (i) soil type; (ii) soil moisture levels; (iii) nitrogen application rate and timing; and (iv) soil amendments on the development of crown and root rot of sorghum.

MATERIALS AND METHODS

Oat seeds inoculum.

Twenty aliquots each containing 100g of oat seed were prepared by soaking seeds in tap water overnight, decanting the water and autoclaving at 121°C at 20 min on each of two consecutive days. Each bottle was seeded with a potato dextrose agar plugs from cultures of *Fusarium* spp. *F. equiseti* (Cda.) Sacc., *F. proliferatum* (Matsushima) Nirenberg, *F. solani* (Mart.) Appel & Wollenw. emend. Snyder & Hansen and *F. thapsinum* Klittich *et al.*, (1997) one isolate of each *Fusarium* spp. was used. Five bottles were prepared for each fungus.

Bottles were maintained in a culture room at 22 – 24°C (shaking them daily) until the seeds were completely colonized by mycelium after approximately 21 days. Colonized seeds were removed from bottles, spread onto aluminium foil and air dried at room temperature (22 to 24°C) for 10 days (Clark *et.al.*, 1995; Wang and Jeffers,

2000; Dorrance *et al.*, 2003). Dried colonized oat seeds were ground in a laboratory blender.

Soil type.

Four soil types were collected from Bethlehem (28° 15' S and 28° 20' E), Bloemfontein (29° 12' S and 28° 10' E) in the Free State Province, Cedara (29° 36' S and 30° 26' E) in the KwaZulu-Natal Province and Potchefstroom (26° 43' S and 23° 6' E) in the North West Province, South Africa. These soils had varying properties, were sandy loam grey (Bethlehem), loamy sand red (Cedara), loam sandy brown Bloemfontein and heavy clay dark brown soil (Potchefstroom).

The soils were autoclaved at 121° C for 30 minutes on each of two consecutive days. Pots (500 ml) were filled with autoclaved soil and inoculated with 5 g of ground oat seeds inoculum colonized with the respective test organism (*F. equiseti*, *F. proliferatum*, *F. solani* and *F. thapsinum*) and thoroughly mixed with the upper 5 cm of the soil (Van Wyk *et al.*, 1988). Seeds of three sorghum genotypes, NK 283, PAN 8446 and PAN 8706W were surface disinfested by dipping in 70 % ethanol for 30 seconds, sterilized in 0.05 % NaOCL for 2 minutes and rinsed three times in sterile distilled water (Salas *et al.*, 1999). Seeds were sown in direct contact with the inoculum at the rate of five seeds per pot planted to a depth of 3 cm. Non-inoculated pots served as control treatment.

The experiment was a factorial design with four soil types x four *Fusarium* spp. and a control x three sorghum genotypes replicated four times. Plants were maintained in the glasshouse at 21/30°C day and night regime. Plants were watered daily and allowed to grow for six weeks after inoculation. Two weeks after planting seedlings were fertilized with limestone ammonium nitrate (LAN-28% a.i) at a rate of 1 g per kg soil. Fertilization was repeated every two weeks.

After six weeks three plants were removed from each pot. Crowns and roots were rinsed in water to remove soil. Plants were air dried at room temperature (22 - 24°C) for 30 minutes before individual plants were cut at the crown and fresh shoot and root mass were measured. Crown and root rot severity was assessed on a 0-5 scale (Correll *et al.*, 1986) where; 0 = no root discolouration; 1 = slight vascular discolouration in root tissue, 2 = extensive vascular discolouration in root tissue, 3 =

vascular discolouration in crown; 4 = extensive vascular discolouration in crown, 5 = crown completely necrotic. The experiment was repeated.

Soil moisture.

A preliminary study was conducted to determine the moisture holding capacity of soils. Identically prepared (250 ml) pots were filled with dry soil, weighed, saturated with distilled water, allowed to drain overnight and re-weighed (Dorrance *et al.*, 2003). Each pot held approximately 88 g of water; calculated as the average difference between the pre-saturation and final weight of five pots. Root zone moisture levels were set at 25, 50, 75 and 100 % moisture holding capacity (Dorrance *et al.*, 2003; Tu and Tan 2004) by adding 22, 44, 66, and 88 ml of water to pots which were maintained according to the methods described by (Kleinhenz and Palta, 2002).

Soil was air dried in the glasshouse in rectangular metal containers for two months prior to planting. Pots, 250 ml in diameter were filled up with air dry soil and inoculated with 2.5 g of ground colonized oat seeds inoculum prepared from isolates of *F. thapsinum*, *F. proliferatum*, *F. solani* and *F. equiseti* as described above (Clark *et al.*, 1995; Wang and Jeffers, 2000; Dorrance *et al.*, 2003). Inoculum was thoroughly mixed in the upper 5 cm of the soil (Van Wyk *et al.*, 1988) in each pot. An uninoculated control treatment was included.

Seeds of three sorghum genotypes (NK 283, PAN 8446 and PAN 8706W) were surface disinfested by dipping seeds into 70 % ethanol for 30 seconds (Salas *et al.*, 1999), sterilized in 0.05% NaOCl for 2 min followed by rinsing three times in sterile distilled water. Five sterilized seeds were planted in each pot to a depth of 3 cm in direct contact with the inoculum. Pots were maintained and watered at four root zone moisture levels in the glasshouse. Fresh water was delivered with a measuring cylinder daily. Fertilization and glasshouse temperatures used were as described above. The experiment was a factorial design including four root zone moisture levels x 3 sorghum genotypes x 4 *Fusarium* spp. and un-inoculated control. After six weeks plants were removed from each pot and treated as described above. Crown and root rot severity was assessed on a 0-5 scale (Correll, Puhalla and Schneider, 1986) as described above. The experiment was repeated.

Soil pH and fertility.

Determination of soil pH. Soil used for the experiments was air dried in the glasshouse and a sub-sample of 50 g was poured into 100 ml distilled water (1:2 ratio) stirred for 1 min, allowed to settle and stirred again 30 minutes later. Soil was allowed to settle at the bottom of the beaker. The supernatant was strained through two layers of cheese cloth (Trough, 1948). The pH of the solution was determined. The soil pH was established at 5.8.

Determination of nitrogen fertilizer. The amount of nitrogen applied for each treatment was calculated using the formula of Millar, Turk and Foth (1965). Bulk density (BD) was used to estimate hectare furrow slice (100 m x 100 x 0.15 m) (plough depth) as described by Millar *et al.* (1965) with some modifications.

Hectare = 100m x 100m

a) Bulk density = Mass of oven dry soil / volume

b) Therefore mass = BD x volume

Assuming BD of soil = 1.3 g /cm³

Then mass of soil in hectare = BD*depth*hectare

$$= 1.3 \text{ g/cm}^3 \times 15 \text{ cm} \times 10^8 \text{cm}^2$$

$$= 19.6 \times 10^8 \text{g}$$

$$= 19.6 \times 10^9 \text{g}$$

Rate of application = 150 kg/ha

$$= \frac{150 \text{ kg}}{1.96 \times 10^9 \text{g}} = \frac{150000 \text{g}}{1.96 \times 10^9 \text{g}}$$

Soil in pot = 250 g

Therefore the amount added = $\frac{150000 \text{g}}{1.96 \times 10^9 \text{g}} = \underline{x}$

$$\frac{150000 \text{g}}{1.96 \times 10^9 \text{g}} = \frac{x}{250 \text{g}}$$

$$= \frac{150000 \text{g} \times 250 \text{g}}{1.96 \times 10^9 \text{g}} = 0.019133 \text{ g (Full rate)}$$

$$= 0.019133 \text{ g}$$

The sorghum genotypes received nitrogen at recommended rates of 150 kg / ha / year. Treatments consisted of three nitrogen applications;

- Normal-N=Nitrogen fertilizer applied at the recommended rate as a single application.
- High-N=the recommended rate of nitrogen fertilizer x 1.5 applied as a single pre-planting application.

- Split-N=the recommended rate of nitrogen fertilizer split into three equal portions and applied at planting then at two- and four-weeks after planting.

The amount of nitrogen was applied in granular form mixed with soil in each of the 250 ml pots was as described above. Nitrogen was uniformly mixed with 250 g of steam sterilized soil in a plastic bag to ensure uniform distribution of the nitrogen within the soil. The soil in pots was inoculated with 2.5 g of ground colonized oat seed inoculum of *F. equiseti*, *F. proliferatum*, *F. solani* and *F. thapsinum* prepared as described above (Clark *et al.*, 1995; Wang and Jeffers, 2000; Dorrance *et al.*, 2003). Inoculum was thoroughly mixed in the upper 5 cm of the soil (Van Wyk *et al.* 1988) in each pot. A non-inoculated control treatment was included.

Seeds of sorghum genotypes NK 283, PAN 8446 and PAN 8706W were surface disinfested and sterilized as previously described (Salas *et al.*, 1999). Five seeds were planted into each pot to a depth of 3 cm. The experiment was a factorial design consisting of three sorghum genotypes x four *Fusarium* spp. isolates and a control x three nitrogen applications rates replicated four times. The pots were laid out in a randomized block design and maintained in the glasshouse at 30 / 21°C day and night regime for six weeks. After six weeks three plants were removed from each pot and treated as described above. Crown and root rot severity was assessed on a 0-5 scale (Correll *et al.*, 1986). The experiment was conducted twice.

Soil amendments.

Three soil amendments were used at single rates as specified on the product label. These were mixed with steam sterilized soil in 250 ml pots at the following rates; steam sterilized soil: kraal manure (3:1), steam sterilized soil: commercial compost manure (3:1) and steam sterilized soil: chicken manure (10:1). The soil and amendment mixtures were inoculated with 2.5 g of ground oat seed inoculum prepared from isolates of each one of the *Fusarium* spp. isolates *F. equiseti*, *F. proliferatum*, *F. thapsinum*, and a control treatment consisting of uncolonized oat seeds. The inoculum was thoroughly mixed with the upper 5 cm of soil in pots (Van Wyk *et al.*, 1988).

Seeds of sorghum genotypes PAN 8446, PAN 8706W and NK 283 were surface disinfested and sterilized as described above (Salas *et al.*, 1999). Five seeds were planted per pot to a depth of 3 cm. Seeds were planted in direct contact with the

inoculum. The experiment was a factorial design with, three soil amendments x four *Fusarium* spp. and untreated control x three sorghum genotypes replicated four times. The pots were laid and maintained for six weeks in the glasshouse at the temperature of 30 / 21°C day and night regime and watered daily. Fertilization was as described above. After six weeks three plants were removed from each pot and treated as described above. Crown and root rot severity was assessed on a 0-5 scale (Correll *et al.*, 1986). The experiment was repeated.

Data analysis.

Data were analyzed using the general linear model (GLM) and ANOVA in SAS 9.1 (SAS Institute Cary NC). The data from repeated experiments were combined for analysis based on tests for homogeneity of variance. Treatment means were compared using the Fisher's least significant difference (LSD) with ($P \leq 0.05$). The ANOVA and GLM means from individual treatments were compared if interactions were significant ($P \leq 0.05$). Where interactions were not significant ($P \leq 0.05$) individuals treatments were combined to evaluate main effects.

RESULTS

Soil type.

Soil type, host genotype and *Fusarium* spp. and soil type x genotype x *Fusarium* spp. interactions significantly ($P \leq 0.001$) affected shoot and root mass and crown and root rot severity. Soil collected from Potchefstroom and Bloemfontein were suppressive to crown and root rot pathogens. Highest shoot and root mass was observed for PAN 8446 grown on soil collected from Potchefstroom and inoculated with *F. proliferatum* compared with the least for PAN 8706W grown on soil collected from Cedara inoculated with *F. thapsinum* (Table 6.1). Bethlehem and Cedara soils were most conducive to crown and root rot pathogens and resulted in greatest crown and root rot severity. Greatest crown and root rot severity ratings was recorded for NK 283 grown on soil collected from Cedara inoculated with *F. thapsinum* compared to lowest for PAN 8706W grown on soil collected from Bloemfontein inoculated with *F. thapsinum* (Table 6.1).

Genotype responses were observed in terms of shoot mass, root mass and crown and root rot severity. NK 283 was most susceptible to crown and root rot severity compared to PAN 8706W which showed a resistance response. PAN 8446 indicated an intermediate reaction as measured with the crown and root rot severity ratings (Table 6. 1). *F. proliferatum* was least aggressive and resulted in the highest shoot and root mass compared *F. equiseti* and *F. thapsinum* which were most aggressive as measured with the crown and root rot severity (Table 6.1).

Soil moisture.

The effects of *Fusarium* spp. on sorghum genotypes at different moisture levels were analyzed in terms of shoot and root mass and crown and root rot severity. *Fusarium* spp. and root zone moisture levels x genotype x *Fusarium* spp. interactions significantly ($P \leq 0.0001$) affected percent emergence. Percentage emergence was significantly ($P \leq 0.05$) reduced in plants inoculated with *F. solani* and exposed to 75 % root zone moisture level which resulted in less seed decay and rot and enhanced seed emergence. *F. equiseti* retarded seed germination in NK 283 and PAN 8446 exposed to 50 and 75% root zone moisture levels respectively (Table 6.2). Significant genotype and *Fusarium* spp. effects on shoot and root mass were not evident. Plant growth was enhanced at 50% root zone moisture levels and resulted in highest shoot and root mass on PAN 8706W inoculated with *F. proliferatum* compared to the lowest for NK 283 inoculated with *F. solani* and exposed to 25% root zone moisture level (Table 6.2).

Crown and root rot severity was significantly ($P \leq 0.0001$) affected by genotype, *Fusarium* spp., genotype x *Fusarium* spp., and genotype x root zone moisture level x *Fusarium* spp. interactions and ($P \leq 0.04$) root zone moisture level. Crown and root rot severity were favoured by 50% root zone moisture level. This resulted in greatest crown and root rot severity on NK 283 grown on soil inoculated with *F. thapsinum* compared to the lowest for PAN 8706W grown on soil inoculated with *F. equiseti* and exposed to 25% root zone moisture level (Table 6.2). Crown and root rot severity were associated with seedlings exposed to 50 and 75 % compared to 25 and 100% root zone moisture levels (Table 6.2). The sorghum genotype responses were observed in terms of percent emergence, fresh shoot mass, root mass and crown and root rot severity. Genotype PAN 8706W was least susceptible to crown and root rot severity pathogens

compared to the susceptible NK 283 and PAN 8446 which was intermediate (Table 6.2). There was a variable reaction with regard to the *Fusarium* spp. and sorghum genotypes exposed to the four root zone moisture levels as measured with crown and root rot severity (Table 6.2).

Soil pH and fertility.

There was a significant ($P \leq 0.001$) nitrogen and *Fusarium* spp. effect on shoot and root mass and a significant ($P \leq 0.001$) genotype x nitrogen x *Fusarium* spp. interaction on shoot mass. Shoot mass was significantly higher for PAN 8446 planted in soil treated at high rate of nitrogen and inoculated with *F. solani* compared to the least for PAN 8706W planted in soil treated with nitrogen at high rate applications inoculated with *F. proliferatum*. Highest root mass was recorded for PAN 8446 planted in soil treated with nitrogen at split rate application and inoculated with *F. solani* and was lowest when PAN 8446 was planted in soil treated with high rate of nitrogen and inoculated with *F. equiseti* (Table 6.3).

The effects of high nitrogen treatment on genotype were generally greater when compared with the other nitrogen treatments in terms of shoot and root mass for the genotypes tested. The analysis indicated a significant ($P \leq 0.0001$) genotype and *Fusarium* spp. effect but not their interactions on crown and root rot severity. High-N and Normal-N treated soil inoculated with *F. equiseti* resulted in significantly greater crown and root rot severity on genotype NK 283 and PAN 8446 when compared with the least for PAN 8706 grown on soil treated with split-N application and inoculated with *F. solani* (Table 6.3). PAN 8706W consistently displayed lowest crown and root rot severity when compared with NK 283 and PAN 8446 which were variable depending on the nitrogen soil treatment and *Fusarium* spp. used to inoculate the soil (Table 6.3).

Soil amendments.

There were significant ($P \leq 0.05$) genotype x soil amendment, ($P \leq 0.0001$) genotype x *Fusarium* spp., ($P \leq 0.01$) genotype x soil amendment and ($P \leq 0.01$) of genotype x *Fusarium* spp. x soil amendment interactions effects on shoot mass. Shoot mass was highest for PAN 8446 grown in soil amended with compost manure inoculated with *F. proliferatum* compared with least for NK 283 grown in soil amended

with kraal manure inoculated with *F. equiseti* (Tables 6. 4). Significant genotype and *Fusarium* spp. effects on root mass were not evident. However, soil amendment significantly ($P \leq 0.0001$) affected root mass. Root mass was highest and lowest for PAN 8446 grown in soil amended with compost and kraal manure inoculated with *F. proliferatum* and *F. equiseti* respectively (Table 6.4).

There was a significant ($P \leq 0.0001$) genotype and *Fusarium* spp., ($P \leq 0.01$) genotype x *Fusarium* spp. interaction, ($P \leq 0.01$) genotype x soil amendment interactions and ($P \leq 0.05$) genotype x *Fusarium* spp. x soil amendment interaction effect on crown and root rot severity. Crown and root rot severity was highest for NK 283 grown in soil amended with kraal manure inoculated with *F. equiseti* or *F. thapsinum* compared to least for PAN 8706W grown in soil amended with chicken manure inoculated with *F. proliferatum* (Table 6.4).

The reaction of the tested sorghum genotypes was variable. PAN 8706W consistently resulted in lowest crown and root rot severity when compared with the highest for NK 283 while PAN 8446 was intermediate (Table 6. 4). There were some variation in aggressiveness among the tested *Fusarium* spp. depending on the manure amendment and the sorghum genotypes grown in terms of plant growth, shoot and root mass and crown and root rot severity (Table 6.4). Addition of each of these materials resulted in reductions in crown and root rot severity. However, chicken manure adversely reduced crown and root rot severity compared with kraal manure. The reaction of compost manure was intermediate (Table 6. 4).

DISCUSSION

The aim of the study was to determine the relationship between the development and survival of *Fusarium* crown and root rot pathogens as influenced by variable soil characteristics. In the present study two soils showing suppressiveness to crown and root rot severity were collected from Potchefstroom and Bloemfontein. These soils had high clay content and loamy sand of fine texture respectively. Plant growth and development in terms of shoot and root mass were enhanced while the crown and root

rot severity was suppressed in these soils. Fravel, Stosz and Larkin (1996) found that soils showing suppressiveness to *Fusarium* wilt had high clay and organic content.

The effect of soil type on the control of crown rot caused by *Fusarium graminearum* (Schw.) was demonstrated with high levels of bio-control in silt loam soil compared to the poorest control observed in the sandy loam (Huang and Wong, 1998). Higher clay contents of finer textured soils are thought to protect bacteria from adverse fluctuations in physical soil conditions and enhance microbial competition or antagonism involved in the suppressiveness of these soils (Heijnen *et al.*, 1993; Hoper *et al.*, 1995; Latour *et al.*, 1996).

Organisms reported to be primarily responsible for disease suppression in suppressive soils were non pathogenic strains of *F. oxysporum* (Scher and Baker, 1980; Scher and Baker, 1982; Cook and Baker, 1983; Alabouvette, 1986; Alabouvette *et al.*, 1993, Larkin, Hopkins and Martin, 1993), species of *Trichoderma* (Marois and Mitchell, 1981; Locke, Marois and Papavizas, 1985; Sivan and Chet, 1987; 1989), other non pathogenic *Fusarium* spp. and fluorescent *Pseudomonas* spp. (Mazzola, 2002). The mechanism of disease suppression has mostly been attributed to microbial interactions (Shipton, Cook and Sitton, 1973; Shipton, 1975; Liu and Baker, 1980).

The present study demonstrated that low and excessive soil moisture content (25 and 100% MHC) suppressed shoot and root mass and was not conducive to crown and root rot pathogens compared to moderate 50 and 75% root zone moisture levels. Tu and Tan (2004) found that high soil moisture promoted seed rot and impeded seed germination and root rot severity was 3.01, 2.3 and 1.1 (on a 0-9 scale) for the 100, 70 and 25% moisture levels respectively. Wildermuth, McNamara and Quick (2001) found that low crown rot was observed on plants in pots that were watered and covered with a black plastic compared to high crown rot on plants that were watered and left uncovered.

Wildermuth *et al.* (2001) found that differences in crown rot ratings between cultivars were least in pots where surface soil was kept at field capacity. Dorrance *et al.* (2003) demonstrated that both low and excessive soil moisture levels had a negative impact on soybean seedlings based on reduced root weights when compared with moderate moisture zone levels. They found between these extremes that the ability of *R. solani* AG2-2111B isolates to cause seedling and root diseases in 'Rasenik' soybean

appeared to be only moderately related to soil moisture level. Similarly, Teo *et al.* (1988) found that soil moisture had little effect on infection and disease severity as measured by seedling emergence and percent disease at maturity. In contrast, Smit and McLaren (1997) observed nonlinear relationships between water stress and isolation frequency of *F. equiseti*, *F. moniliforme* and *F. oxysporum* suggesting that mild water stress may promote root colonization.

In the present study, the pH of the glasshouse soil used in the study was established at 5.8. This was within the range suggested by Mitchell and Musk (2004) that the soil pH (H₂O) above 5.8 should be maintained for the fields where sorghum is grown. Soil acidity predisposes sorghum to infection by soilborne seedling and root pathogens while root discoloration was greater at pH (KCl) less than 4.6 (McLaren, 2004). Nitrogen application above and at the recommended rate was found to enhance shoot and root growth and also favour disease development. The magnitude of increase in shoot and root growth was found to be related to the degree of genotype susceptibility to the *Fusarium* spp. used for inoculations. Nitrogen application had a greater impact on crown and root rot severity development on the intermediate PAN 8446 and susceptible NK 283 than on the resistant PAN 8706W genotype.

Long, Lee, and TeBeest (2000) found that rice cultivars responded differently to N-fertilization and that the most susceptible cultivars were at higher risk of epidemics than are more resistant cultivars. Nitrogen applied as a single application rate or above the recommended amounts (normal-N or High-N) respectively, significantly and differentially (by genotype) increased root rot severity over the split-N application treatment for each susceptible genotype. High nitrogen application increased rice blast development compared to the reduction when using split applications (Templeton, Wells and Johnston, 1970; Amin and Venkatorao, 1979; Kingslover, Barksdale, and Marchetti, 1984; Kurshner *et al.*, 1992; Ishiguro, 1994). Hagan (2004) found that the use of nitrogen fertilizers above those recommended for good turf vigour often increased the severity of *Pythium* blight and *Bipolaris* disease of turf and Bermuda grass.

The present study examined the effects of soil amendments on crown and root rot severity on sorghum. Incorporation of commercial compost to soil significantly enhanced plant growth in terms of shoot and root mass and decreased crown and root rot severity. Chicken and kraal manure were most effective in reducing crown and root

rot severity while compost was least effective. Results of the present study provided strong support to the concept that plant induced resistance conferred by soil suppressive systems such as composts could be the key component of the increased protection. Blok *et al.* (2000) found that the survival of *F. oxysporum* f. sp. *asparagi* was strongly reduced in amended soil. Mitchell and Alexander (1962) found that incorporation of readily metabolizable organic matter into flooded soil greatly accelerated the decline in the numbers of propagules of *Fusarium oxysporum* f sp. *conglutinans* compared to non-amended soil. Dissayanake and Hoy (1999) found that amendments with biosolids filter press cake and gin trash compost suppressed root rot of sugarcane and increased plant growth. LaMondia *et al.* (1999) found that soil amendments with spent mushroom compost reduced potato early dying disease severity and dramatically increased yields in their experiments. In addition, Lewis *et al.* (1992); Lumsden *et al.* (1983) found that application of composted municipal biosolids increased soil microbial activity and suppressed root diseases.

Elmer *et al.* (1995) found that spent mushroom compost amendments altered the mineral composition of roots, leaves and affected plant nutrition and also altered response to early dying of pathogens. Fliebbach, Martens and Reber (1994) found that soil amendments enhance soil microbial biomass and activity of beneficial microorganisms. Hoitink, Stone and Han (1997) confirmed these findings and reported that compost amended soils increased microbial densities in potato rhizospheres a factor which could also affect pathogen inoculum potential and result in general or specific suppression of diseases. These finding suggest that soil amendments with manure could be used to suppress or reduce the crown and root rot disease severity of grain sorghum.

The results demonstrated that *Fusarium* spp. survive over a wide range of soil types. The results confirm that soil type can influence the *Fusarium* population growth, development and survival as well as the level of biocontrol activity of some antagonists. The method used for evaluating the effect of soil moisture on plant growth and development of crown and root rot severity clearly distinguished sub-optimal, optimal and supra-optimal root zone moisture levels based on plant growth measurements. The availability of free moisture appeared not to be a limiting factor, as the crown and root rot severity occurred at all root zone moisture levels for all the *Fusarium* spp. evaluated. A well planned fertility program is essential to the production of healthy sorghum crops that produce optimum yields and maximize profits. Increasing the crop's ability to

resist disease infection through proper timing and application rates of fertilizers is critical. The prospects for control of Fusarium crown and root rot by incorporation of organic amendments may provide an alternative for chemical soil disinfection under conditions where other alternatives such as soil solarization or flooding are not effective or not feasible.

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Table 6.1 The effect of soil type on shoot and root mass and root rot severity of sorghum genotypes grown in the glasshouse

Soil source	<i>Fusarium</i> spp.	Shoot mass (g)			Root mass (g)			Root rot (0-5)		
		NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 706W	NK 283	PAN 8446	PAN 8706W
Potchefstroom	<i>F. equiseti</i>	10.07ab ¹	8.20c	9.33bc	9.70a	8.60bc	8.81bc	3.30bc	2.67bc	1.23c
	<i>F. proliferatum</i>	9.41b	21.62a	15.21ab	712ab	16.55a	13.10ab	3.33bc	2.20c	1.25c
	<i>F. solani</i>	9.66b	7.76cd	9.13bc	9.23a	7.91c	8.24bc	3.65b	3.30ab	2.33ab
	<i>F. thapsinum</i>	9.33b	4.20de	26.10a	7.70ab	2.80ef	23.00a	3.67b	3.00b	1.67bc
	Control	7.17c	5.66d	6.47c	6.32b	5.40d	5.21c	0.00d	0.00e	0.00d
Bloemfontein	<i>F. equiseti</i>	11.07a	9.12bc	10.42b	9.44a	6.78cd	8.01bc	3.66b	2.00c	2.00b
	<i>F. proliferatum</i>	8.67bc	10.71b	9.02bc	5.80bc	9.12b	5.84c	2.32c	2.21c	1.25c
	<i>F. solani</i>	8.06bc	8.42c	9.25bc	7.58ab	6.24cd	5.58c	2.66c	2.33c	2.00b
	<i>F. thapsinum</i>	9.86b	8.88c	6.35c	7.60ab	6.69cd	4.57cd	3.33bc	2.25c	1.05c
	Control	11.27a	12.20ab	10.90b	9.20a	11.11ab	9.72b	0.00d	0.00e	0.00d
Cedara	<i>F. equiseti</i>	2.88de	1.96fg	2.17de	1.93d	1.22f	1.00e	4.66a	3.33ab	1.67bc
	<i>F. proliferatum</i>	3.27d	2.20ef	2.36de	2.65cd	1.21f	1.63e	4.33ab	3.00b	1.20c
	<i>F. solani</i>	3.44d	1.69f	3.56d	3.28c	1.25f	3.20d	4.67a	3.33ab	1.33c
	<i>F. thapsinum</i>	5.21cd	2.13ef	1.23e	5.82bc	1.30f	0.80ef	5.00a	3.33ab	2.33b
	Control	1.13ef	1.20f	1.32e	0.71e	0.90g	0.79ef	0.00d	0.00e	1.33c
Bethlehem	<i>F. equiseti</i>	5.67cd	4.73de	4.70cd	3.80c	2.80ef	3.63d	4.15ab	3.67ab	3.33a
	<i>F. proliferatum</i>	2.50e	3.60e	3.73d	1.50d	4.00de	2.37de	3.30bc	3.65ab	2.33b
	<i>F. solani</i>	2.43e	3.13e	2.90de	3.70c	3.50e	2.26de	3.67b	3.66ab	3.00ab
	<i>F. thapsinum</i>	3.60d	2.16ef	3.17d	3.70c	2.00ef	2.86de	4.00ab	4.00a	3.30a
	Control	3.57d	5.43d	2.37de	2.33cd	4.20de	2.10de	0.00d	0.00e	0.00d

¹Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).

Table 6.2 Effect of root zone moisture on emergence, fresh shoot and root mass and crown and root rot severity of three sorghum genotypes inoculated with *Fusarium* isolates

Moisture (%)	<i>Fusarium</i> spp.	Emergence (%)			Shoot mass (g)			Root mass (g)			Root rot (0-5)		
		NK 283	PAN 8446	PAN 706W	NK 283	PAN 446	PAN 706W	NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W
25	<i>F. equiseti</i>	65.00bc ¹	65.00cd	75.00bc	3.88d	4.40bc	2.95d	3.15bc	3.13b	2.03c	3.75bc	3.75ab	1.00c
	<i>F. proliferatum</i>	75.00b	75.00b	80.00b	4.85cd	11.28a	3.88cd	3.60bc	7.08a	3.28bc	1.75e	2.00cd	1.25c
	<i>F. solani</i>	50.00c	85.00ab	65.00c	2.40de	8.23b	9.90bc	1.60d	3.33b	5.73b	2.50de	4.25a	1.33b
	<i>F. thapsinum</i>	80.00ab	80.00ab	55.00d	8.38b	4.55bc	3.63cd	3.65bc	2.83bc	2.35c	3.00d	2.75c	1.21c
	Control	70.00b	50.00d	80.00d	3.55d	1.93de	11.23ab	2.88c	1.63c	7.17a	0.00ef	0.00d	0.00d
50	<i>F. equiseti</i>	40.00d	55.00d	90.00a	2.85de	4.05bc	2.75d	2.28c	2.95bc	2.50c	3.25c	3.25b	2.25a
	<i>F. proliferatum</i>	80.00ab	65.00cd	75.00bc	4.10cd	3.63c	14.80a	7.08a	3.38b	7.78a	3.00d	2.50cd	1.50b
	<i>F. solani</i>	80.00ab	70.00c	80.00d	14.30a	4.00bc	10.00b	5.80ab	2.40bc	6.70ab	4.50ab	2.50cd	1.75ab
	<i>F. thapsinum</i>	80.00ab	80.00ab	80.00b	6.70bc	9.30ab	2.50d	4.00b	7.73a	1.80d	5.00a	2.50cd	1.50b
	Control	50.00c	60.00cd	90.00a	4.85cd	3.13cd	3.58cd	2.85c	2.70bc	3.45bc	0.00ef	0.00d	0.00d
75	<i>F. equiseti</i>	80.00ab	40.00e	60.00cd	2.85de	3.08cd	3.03cd	2.78c	2.55bc	2.50c	4.00b	3.25b	1.50b
	<i>F. proliferatum</i>	80.00ab	95.00a	85.00ab	6.23bc	3.13cd	3.13cd	4.80b	2.50bc	2.18c	3.75bc	3.25b	1.13c
	<i>F. solani</i>	100.00a	80.00ab	75.00bc	5.93c	4.03bc	2.93d	3.90bc	2.73bc	1.98d	4.50ab	2.50cd	1.75ab
	<i>F. thapsinum</i>	65.00bc	65.00cd	75.00bc	11.45ab	4.23bc	4.53c	7.74a	2.75bc	2.83c	4.00b	4.00a	2.25a
	Control	65.00bc	55.00d	70.00bc	5.23c	2.33d	2.98d	4.53b	2.63bc	1.80d	0.00ef	0.00d	0.00d
100	<i>F. equiseti</i>	80.00ab	65.00cd	55.00d	4.63cd	4.43bc	3.23cd	3.14bc	4.15b	2.85c	4.25ab	2.50cd	1.25bc
	<i>F. proliferatum</i>	75.00b	70.00c	54.00d	5.53c	3.38c	3.85cd	3.68bc	2.88bc	2.43c	3.50c	2.25cd	1.15c
	<i>F. solani</i>	80.00ab	75.00b	55.00b	4.38cd	3.53c	2.75d	3.35bc	3.05b	2.23c	4.25ab	2.75c	1.23c
	<i>F. thapsinum</i>	60.00bc	75.00b	60.00cd	6.58bc	4.95bc	4.18c	4.48b	3.45b	2.58c	2.50de	3.00bc	1.25bc
	Control	85.00ab	60.00cd	55.00b	5.00c	4.08bc	3.85bc	3.58bc	2.00bc	1.75d	0.00ef	0.00d	0.00d

¹Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).

Table 6.3 Effect of three fertilizer application and timing rates (normal application rate normal-N, high application rate high-N and split application rate split-N) on plant mass and crown and root rot severity

Nitrogen	<i>Fusarium</i> spp.	Shoot mass (g)			Root mass (g)			Root rot (0-5)		
		NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W
Normal	<i>F. equiseti</i>	42.43ab ¹	44.30ab	38.33b	13.37a	10.93bc	8.30b	5.00a	5.00a	2.67ab
	<i>F. proliferatum</i>	30.87e	45.57ab	39.27ab	5.83de	9.13c	9.73ab	4.00ab	4.67ab	3.33a
	<i>F. solani</i>	42.63ab	35.27cd	37.40bc	10.40bc	8.27cd	6.30c	3.33b	4.33ab	2.67ab
	<i>F. thapsinum</i>	43.43a	39.27bc	37.67bc	10.00bc	7.73d	8.93b	3.67b	4.33ab	2.00b
	Control	39.60c	37.70cd	31.40ef	11.40b	12.31ab	9.40ab	0.00d	0.09d	0.00e
High	<i>F. equiseti</i>	34.57de	31.27e	36.97c	7.47d	5.83de	10.03a	5.00a	4.00b	2.67ab
	<i>F. proliferatum</i>	37.87cd	39.87bc	31.21ef	11.57b	16.33a	10.08a	3.67b	4.00b	1.69bc
	<i>F. solani</i>	40.33bc	50.20a	40.33a	10.30bc	11.70b	8.80b	4.33ab	4.00b	2.33ab
	<i>F. thapsinum</i>	42.70ab	39.30bc	35.60cd	9.33c	12.10ab	7.80bc	3.67b	3.00c	2.00b
	Control	36.80d	34.53de	30.97f	12.53ab	10.01bc	9.73ab	0.08d	0.67d	0.00d
Split	<i>F. equiseti</i>	41.42b	39.91bc	32.12e	9.55c	11.20b	9.07ab	4.11ab	5.00a	2.56ab
	<i>F. proliferatum</i>	39.28c	38.90c	34.75d	8.60cd	7.50d	8.01b	3.78b	3.00c	1.88b
	<i>F. solani</i>	33.20de	36.60d	34.40d	8.75cd	16.60a	7.87bc	2.88bc	3.00c	1.67b
	<i>F. thapsinum</i>	40.21bc	36.80d	35.75cd	10.71bc	12.00ab	8.53b	3.39b	3.00c	1.81b
	Control	32.63de	39.85bc	33.30de	7.79d	7.74d	9.80ab	0.00d	0.00d	0.00c

¹Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05).

Table 6.4 The effect of compost, kraal and chicken manure on shoot mass, root mass and crown and root rot of sorghum

Amendment	<i>Fusarium</i> spp.	Shoot mass (g)			Root mass (g)			Root rot (0-5)		
		NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W
Chicken	<i>F. equiseti</i>	14.35e ¹	18.45cd	11.95e	4.43d	3.48d	2.45de	2.44ab	2.25bc	1.75ab
	<i>F. proliferatum</i>	31.58ab	15.80de	11.90e	7.63bc	5.15c	3.43d	2.75ab	2.25bc	0.75b
	<i>F. solani</i>	19.95e	14.92e	13.25de	5.40cd	3.45d	3.36d	2.17ab	2.67b	1.56ab
	<i>F. thapsinum</i>	34.53a	16.95d	11.35e	6.88c	4.30cd	2.68de	1.75b	1.75c	1.25ab
	Control	14.93ef	12.80ef	14.88d	4.68d	2.10de	3.32d	0.00c	0.00d	0.00b
Kraal	<i>F. equiseti</i>	7.33fg	23.55bc	25.63b	4.58d	2.44de	9.28b	3.50a	3.30a	2.00a
	<i>F. proliferatum</i>	9.98f	18.55cd	22.53c	4.35d	5.98c	8.25bc	3.25a	3.00ab	1.19ab
	<i>F. solani</i>	14.15ef	16.00d	17.02cd	4.23d	3.75d	6.18c	1.86b	1.94c	1.50ab
	<i>F. thapsinum</i>	19.18e	25.48b	25.03b	10.58ab	6.70bc	9.73b	3.50a	3.25ab	2.00a
	Control	20.13de	21.25c	28.08a	5.53cd	6.10bc	6.70c	0.07c	0.50d	0.75b
Compost	<i>F. equiseti</i>	24.18d	23.65bc	28.68a	5.13cd	11.48ab	4.83cd	3.25a	2.25bc	2.25a
	<i>F. proliferatum</i>	26.90c	35.68a	27.03ab	5.95cd	12.45a	11.25a	3.00a	3.00ab	2.00a
	<i>F. solani</i>	28.80bc	21.28c	24.24bc	8.59b	4.62cd	6.01c	2.25ab	2.42b	1.33ab
	<i>F. thapsinum</i>	30.78b	27.58ab	25.33b	11.13a	5.65c	10.08ab	3.25a	2.50b	1.25ab
	Control	25.58cd	25.33b	28.87a	6.75c	8.55b	8.10bc	0.00c	0.00d	0.00b

¹Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference

CHAPTER VII

THE EFFICACY OF SEED TREATMENT FUNGICIDES AND BIO- CONTROL ON FUSARIUM CROWN AND ROOT ROT OF GRAIN SORGHUM

ABSTRACT

Seed rot, pre-emergence and post-emergence damping-off and seedling blights are responsible for stand loss in sorghum (*Sorghum bicolor* (L.) Moench). A glasshouse experiment was conducted to evaluate four fungicides and bio-control seed treatments difenoconazole and metalaxyl-m (Dividend[®]), tebuconazole (Raxil[®]), *Trichoderma harzianum* (Tricho-Plus), tebuconazole (Ingwe[®]) and carboxin and thiram (Vitavax-Plus[®]) for control of *Fusarium* spp. diseases of sorghum. Treated seeds of three sorghum genotypes NK 283, SNK 3939 and PAN 8706W were planted in soil inoculated with ground oat seeds colonized by *F. equiseti*, *F. proliferatum*, *F. solani*, and *F. thapsinum*. The fungi reduced seed germination by more than 40 %. There were significant ($P \leq 0.05$) interactions between the effects of fungicide treatments, *Fusarium* spp. and sorghum genotypes on crown and root severity. Generally, results indicated that all four fungicides and the bio-control agent significantly improved shoot and root mass and decreased crown and root rot severity. *Trichoderma harzianum*, difenoconazole and metalaxyl-m and tebuconazole improved emergence, shoot and root mass. Results indicated that all four fungicides and the bio-control agent were effective against crown and root rot severity caused by *Fusarium* spp. However, carboxin and thiram (Vitavax-Plus[®]) and tebuconazole (Ingwe[®]) were generally more effective than tebuconazole (Raxil[®]), difenoconazole and metalaxyl – m (Dividend[®]) and *Trichoderma harzianum* (Tricho-Plus). Crown and root rot severity were significantly lower in the resistant genotype PAN 8706W compared with the susceptible SNK 3939. *F. equiseti* consistently caused a reduction in fresh shoot and root mass whereas shoot mass was highest in plants inoculated with *F. proliferatum*. Root mass was highest for plants inoculated with *F. solani*. However, *F. proliferatum* and *F. thapsinum* consistently caused the greatest reductions in root length and root mass. *F. equiseti* was the most aggressive isolate.

INTRODUCTION

Poor germination and seedling mortality due to seed-borne fungi reduce sorghum stands and replant at additional seed, fuel and other production costs is required. Several grain-borne fungi have been implicated as seedling pathogens of cereals. These include *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* spp. (Gulya, Martinson and Tiffang, 1979; Shurtleff, 1980; Martin and Johnston 1982; Fahim, Barakat and Aly 1983). Agrios (1997) suggested reducing primary inoculum (e.g. on seed, planting stock and in growing media) as a disease control option that can be achieved through the use of fungicides, heat or chemical treatments, disease free planting stock, sanitation and fumigation. Protectant and systemic seed treatments have been registered for wheat seed. Some are specific for certain seed or soil borne fungi while others have a wider spectrum and can be used singly or in combination (Ashley *et al.*, 2003).

Soil-borne fungi and seed treatment chemicals are affected by soil environment and planting dates (Piccinni, Shriver and Rush, 2001). Evaluation of the efficacy of seed-treatment fungicides would be most suitable done under controlled conditions and against specific pathogenic fungi. Seed treatment manufacturers typically perform evaluations of this type during the product development process, but results are barely published (McGee, 1988). Field tests provide crucial information about field performance of these fungicides, but do not provide information about their efficacy against specific fungi. However, where the history of disease development is well understood, chemicals might provide an efficient and economical control strategy (McGee, 1995).

Commercially produced seed of maize (*Zea mays* L.) is almost universally treated with a fungicide prior sale to protect the seed from fungal infection after planting, or to deter growth of seed borne fungal pathogens (Bergstrom, 1991; Agarwal and Sinclair, 1987). In most cases, fungicide treatment of maize seed improves emergence and yield compared to non treated seed (Mohan, Knott and Wilson, 1994; Munkvold and Carlton, 1996; Munkvold and Shriver, 2000). Until satisfactory resistant varieties are available, chemical control may be the only option to protect susceptible but high yielding varieties. Control of seed-borne and soil-borne pathogens in wheat

was successfully achieved through seed treatments with difenconazole, (Dividend) and tebuconazole and metalaxyl (Raxil) prior to planting (Ashley *et al.*, 2003). Barley, sorghum and wheat seeds were treated with carboxin and thiram (Vitavax 300) to protect the seedlings from *A. alternata* (Fr.:Fr) Keissl., *Apergillus flavus* Link and *F. oxysporum* Schlecht. Emend. Snyder & Hansen (Hassan, 2000).

Despite the increased interest in biocontrol there is very little published on techniques for isolating and screening potentially active biocontrol agents (Weller, Zhang, and Cook, 1985; Campbell, 1986; Hornby, 1990; Merriman and Russel, 1990). In recent years, plant health-promoting rhizobacteria (PHPR) have been identified as a biological control alternative to pesticide use since they suppress disease without negative effects on user, consumer or the environment (Linderman, 1986; Weller, 1988). These beneficial bacteria are found on many subterranean plant surfaces and thrive by consuming mainly plant derived nutrients. Beneficial bacteria are capable of suppressing micro-organisms or nematodes that cause root damage (Sikora, 1991).

Numerous research programs are investigating the biological control of root diseases of crop plants (Lynch, 1988). The potential of biological control agents to suppress diseases caused by *Fusarium* spp. has been studied in a number of crops (Davis, 1967; Cassini, Medawar and Cassini, 1985; Beale and Pitt, 1990; Mandel and Baker, 1991; Marois, 1993; Oyarzun *et al.*, 1994; Hervas, Trapero-Casas and Jimmenez-Diaz, 1995; Larkin, Hopkins and Martin, 1996). Sorghum seeds infected with *F. moniliforme* (*sensu lato*) were treated with pure culture of biocontrol agents *Pseudomonas fluorescens* CHA 0, *Trichoderma harzianum* Rifai and *Chaetomium globodum* Kunze and evaluated for percentage infection of *F. moniliforme*, seed germination, vigour index and field emergence (Raju *et al.*, 1999).

The objectives of the present study were to investigate the ability of fungicides and bio-control agents to control *Fusarium* crown and root rot in grain sorghum under glasshouse conditions.

MATERIALS AND METHODS

Efficacy of seed treatment fungicides

Oat seed inoculum. Inoculum was prepared by soaking 100g oat grains in a 250 ml bottle containing tap water overnight. Water was drained, and grain was autoclaved twice for 20 min at 121°C on each of two consecutive days. Each bottle was seeded with potato dextrose agar plugs of the *Fusarium* test isolates. *Fusarium equiseti* (Cda.) Sacc., *F. proliferatum* (Matsushima) Nirenberg, *F. solani* (Mart.) Appel & Wollenw. emend. Snyder & Hansen and *F. thapsinum* Klittich *et al.*, (1997), The bottles were maintained in a sporulation room at 25°C and shaken daily until seeds were completely colonized (approximately 21 days). The colonized seeds were removed from the bottles, spread on aluminium foil and air dried at room temperature (25°C) approximately 10 days (Clark, Hoy and Nelson, 1995; Wang and Jeffers, 2000; Dorrance *et al.*, 2003). The dry oat seeds were ground in a laboratory blender.

Fungicides were evaluated for efficacy on crown and root rot of sorghum in the glasshouse. Fungicides tested were carboxin/thiram (Vitavax[®] Plus FS) (carboxin, a.i. 200g/l + thiram, a.i. 200g/l, Uniroyal Chemical Company Inc. USA), Tebuconazole (Raxil[®] 015 ES) (250 g/l a.i. tebuconazole + 4g/l a.i. triflumuron) 200g/100 kg seed, Bayer (Pty) Ltd), Tebuconazole (Ingwe[®] 6FS) (60 g/l a.i. Cropmton Chemicals (Pty) Ltd), Difenoconazole/metalaxyl-m (Dividend[®] 030 FS) (300 ml /l a.i., Syngenta Crop Protection, Inc. USA), Bio-control *Trichoderma harzianum* Rifai (Tricho-Plus), 1 g / 1 litre of water and an un-inoculated control.

Sorghum seeds from each of the commercial genotypes NK 283, PAN 8446 and PAN 8706W with variable levels of resistance were surface disinfested by dipping seeds in 70 % ethanol for 30 seconds (Salas *et al.*, 1999). This was followed by surface sterilization in 0.05 % NaOCl for 2 minutes. Seeds were rinsed three times in sterile distilled water then blotting dry on a sterile paper towel. Sterilized seeds were treated with the test fungicides as a seed dressing in slurry form at the rate specified on the product label (Munkvold and O'Mara 2002). Five sorghum seeds were planted per pot to a depth of 3 cm. Percentage seed emergence was recorded five- seven days after emergence and expressed as percentage of total number of seeds that germinated over the planted seeds for the replicate pots.

Ground colonized oat seed inoculum prepared for each of the test isolates was used for inoculations and an un-inoculated control was included. Five grams of

inoculum was applied to 500 ml pots containing steam sterilized soil: peat (3:1) and thoroughly mixed in the upper 5 cm of the soil surface (Van Wyk *et al.* 1988). The experiment was a factorial design with six fungicide treatments (4 fungicides, 1 bio-control agent and control) x 3 sorghum genotypes x 4 *Fusarium* spp., replicated four times. Two weeks after emergence, seedlings were fertilized with limestone ammonium nitrate (LAN -28 % a.i) at a rate of 1 g per kg soil. Fertilization at the same rate was repeated every two weeks. Pots were maintained for a period of six weeks in the glasshouse at 30 / 21°C day and night regime and watered daily.

After six weeks, three plants were removed from each pot. Crowns and roots were rinsed in water to remove soil and blotted dry with a paper towel. Plants were air dried at room temperature (22 - 24°C) for 30 minutes before individual plants were cut at the crown and fresh shoot and root mass were measured. Crown and root rot severity was assessed on a 0-5 scale (Correll, Puhalla and Schneider, 1986) where; 0 = no root discoloration; 1 = slight vascular discoloration in root tissue, 2 = extensive vascular discoloration in root tissue, 3 = vascular discoloration in crown; 4 = extensive vascular discoloration in crown, 5 = crown completely necrotic. The experiment was conducted twice.

RESULTS

Efficacy of seed treatment fungicides

Seed emergence, root length, shoot mass, root mass and root rot were significantly ($P \leq 0.05$) affected by fungicide treatment. Fungicides significantly ($P \leq 0.05$) affected seed emergence. Soil infested with *F. solani* and *F. thapsinum* resulted in reduced emergence of seeds treated with tebuconazole on all sorghum genotypes evaluated with the greatest effect on NK 283 and PAN 8706W (Table 7.1). PAN 8446 treated with difenoconazole / metalaxyl-m (Dividend®) and tebuconazole and triflumuron (Raxil®) planted in soil infested with *F. equiseti* and *F. solani* respectively and NK 283 treated with carboxin/thiram planted in soil infested *F. proliferatum* resulted in highest seed germination. The greatest seed treatment effect on percent seed germination resulted on PAN 8706W treated with *T. harzianum* (Table 7.1).

Fungicide significantly ($P \leq 0.05$) affected shoot mass and ($P \leq 0.01$) affected root length and mass. Tebuconazole and triflumuron (Raxil®) was most effective in reducing seed colonization and decay and increasing root length for NK 283 and PAN 8446 planted in soil infested with *F. equiseti* and *F. proliferatum* respectively compared to PAN 8706W treated with tebuconazole (Ingwe®) planted in soil infested with *F. solani* (Table 7.1). Tebuconazole and triflumuron (Raxil®) enhanced plant growth in terms of shoot and root mass for NK 283 planted in soil infested with *F. proliferatum* and *F. thapsinum* respectively compared to least shoot and root mass for PAN 8706W treated with tebuconazole (Ingwe®) planted in soil infested with *F. solani* respectively (Table 7.1).

Crown and root rot severity was significantly ($P \leq 0.0001$) affected by genotype, ($P \leq 0.0001$) fungicides, ($P \leq 0.01$) *Fusarium* spp., ($P \leq 0.001$) genotype x fungicide interactions, ($P \leq 0.01$) genotype x *Fusarium* interactions, ($P \leq 0.005$) fungicide x *Fusarium* and ($P \leq 0.005$) genotype x fungicide x *Fusarium* interactions. All fungicides reduced the crown and root rot severity from 0.00 to 3.50 compared with the control (Table 7.1). NK 283 treated with difenoconazole / metalaxyl-m (Dividend®) planted in soil infested with *F. equiseti* resulted in greatest crown and root rot severity. The least crown and root rot severity rating was observed for PAN 8706W treated with difenoconazole / metalaxyl-m (Dividend®) and carboxin / thiram (Vitavax -Plus®), tebuconazole (Ingwe®), tebuconazole and triflumuron (Raxil®) planted in soil infested with *F. equiseti*, *F. proliferatum*, *F. solani* and *F. thapsinum* respectively (Table 7.1). *Fusarium* spp. differed in their level of aggressiveness which was dependent on genotype and the effect of the fungicide treatment (Table 7.1).

DISCUSSION

In the current study sorghum seeds treated with fungicides were planted in fungus infested soils. The method was found effective in evaluating the effect of seed treatments on *Fusarium* crown and root rot pathogen. Reliance on natural infection has often failed to produce sufficient infection pressure, and it is not possible to evaluate specific isolates of the fungus. Mao *et al.* (1997; 1998) successfully used this method to evaluate biological control agents as corn seed treatments, in potting medium infested with a combination of *Pythium* species and *Fusarium graminearum* (Schw.).

There were significant differences among the four fungicides and biocontrol in terms of seed emergence. Difenoconazole / metalaxyl-m (Dividend[®]), *T. harzianum* (Tricho-Plus), carboxin / thiram (Vitavax -Plus[®]), and tebuconazole and triflumuron (Raxil[®]) significantly increased seed emergence compared to tebuconazole (Ingwe[®]). Munkvold and Omara (2002) found that difenoconazole was most effective in reducing seed colonization and decay on maize seedling blight caused by *Fusarium* spp..

Wainwright, Jeitner, and Cazin-Bourguignon (1992), Halley *et al.* (1999) Mc Mullen *et al.* (1999) and Mesterhazy, Bartok and Lamper (2003) found that fungicides that contain tebuconazole as one of the active ingredients tended to be most effective against *Fusarium* ear disease of wheat. Broad spectrum systemic fungicide triadimefon and triadimenol seed dressings were effective against fungi associated with pre- and post emergence damping-off and seeding blight of sorghum (McLaren and Rijkenberg, 1989). *T. harzianum* was effective in reducing *F. moniliforme* and increasing seed germination, vigour index and field emergence of sorghum seeds (Raju *et al.*, 1999). Reid, Hausbeck and Kizilkaya (2002) found *T. harzianum* strain T-22 not effective in suppressing *Fusarium* crown and root rot of asparagus when a high rate of pathogen inoculum was used, indicating that the effectiveness of this product may be dependent on disease pressure.

Although fungicides successfully decreased crown and root rot severity levels but did not completely inhibit disease development. The present study demonstrated that the tested fungicide efficacy was higher on the more resistant than on the susceptible genotypes. Similarly, the virulence of the isolates was more aggressive on the susceptible than on the resistant genotypes. NK 283 was most susceptible and displayed greatest crown and root rot severity ratings compared to least for PAN 8706W and PAN 8446 was intermediate. The level of reduction in crown and root rot severity was dependent on the efficacy of the fungicides, the level of aggressiveness of the *Fusarium* isolates and genotype resistance. The present study demonstrated that the fungicides and bio-control agents were effective as seed dressings against crown and root rot severity of sorghum and could therefore be employed to combat this disease.

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Table 7. 1: The influence of fungicides and a biocontrol agent used as seed treatment on severity of crown and root rot severity of sorghum caused by *Fusarium* spp. in greenhouse tests 2004 \ 05.

Fungicide	<i>Fusarium</i> spp.	Emergence (%)			Root length (cm)			Shoot mass (g)			Root mass (g)			Root rot (0-5)		
		NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W	NK 283	PAN 8446	PAN 8706W
Dividend (Difconazole/metala xyl)	<i>F. equiseti</i>	80.00ab ¹	100.00a	40.00cd	60.30ab	61.30ab	70.90b	22.70ab	16.50bc	13.10cd	6.40ab	4.20ab	2.20bc	3.50a	3.00a	1.00b
	<i>F. proliferatum</i>	80.00ab	60.00bc	40.00cd	58.10b	62.12ab	72.11ab	20.10b	19.20ab	12.60d	4.90b	5.30a	2.20bc	2.00b	2.00b	1.00b
	<i>F. solani</i>	80.00ab	60.00bc	60.00b	61.00ab	60.20ab	56.00c	20.11b	15.10c	12.00d	4.70b	3.20bc	4.20ab	1.00c	3.00a	1.00b
	<i>F. thapsinum</i>	40.00c	60.00bc	60.00b	68.10ab	63.11ab	72.30ab	9.10ef	20.90a	18.30b	1.20cd	3.80cd	3.80b	2.00b	3.00a	1.00b
	Control	60.00bc	60.00	50.00c	57.31b	53.87b	46.81cd	14.82de	12.63d	12.15d	3.53bc	2.93	2.87bc	0.00	0.00d	0.00c
Ingwe (Tebuconazole)	<i>F. equiseti</i>	40.00c	60.00bc	60.00b	60.50ab	60.90ab	62.10bc	11.20e	12.00d	11.20de	2.40c	2.10cd	2.80bc	1.00c	2.00b	1.00b
	<i>F. proliferatum</i>	60.00bc	60.00bc	60.00b	57.00b	60.90ab	31.20d	18.21bc	15.90c	4.40f	3.60bc	3.90bc	1.10e	1.00c	1.00c	0.06c
	<i>F. solani</i>	20.00d	40.00c	20.00d	31.30c	32.90c	19.00e	3.30f	9.60e	1.70g	0.50d	2.60cd	0.20f	1.00c	1.00c	1.12b
	<i>F. thapsinum</i>	20.00d	40.00c	80.00ab	42.32bc	22.71d	25.30de	3.10f	5.50f	2.90fg	1.30cd	2.40cd	1.60cd	1.25c	1.00c	1.10b
	Control	70.00b	60.00	60.00b	60.23ab	55.00b	63.79bc	23.27a	11.47de	16.64c	4.53b	2.88cd	3.84b	0.00d	0.00d	0.00c
Raxil (Tebuconazole/triflurazone)	<i>F. equiseti</i>	40.00c	80.00ab	60.00b	75.00a	59.11b	65.00bc	15.10d	16.01bc	11.40de	3.00bc	3.70bc	2.60bc	2.00b	2.00b	1.00b
	<i>F. proliferatum</i>	80.00ab	20.00d	60.00b	60.92ab	76.00a	68.31bc	23.80a	12.40d	19.60ab	5.80b	2.30cd	4.10ab	2.00b	2.00b	1.00b
	<i>F. solani</i>	40.00c	100.00a	60.00b	59.13b	59.00b	67.05bc	14.89de	19.20ab	16.80c	3.90bc	4.30ab	3.70b	2.00b	2.00b	0.00c

	<i>F. thapsinum</i>	40.00c	60.00bc	60.00b	67.90ab	60.30ab	67.11bc	17.40c	16.90bc	21.60a	7.90a	5.10a	4.30ab	2.00b	1.00c	0.00c
	Control	40.00	60.00	60.00b	65.39ab	41.67bc	29.88de	17.09c	8.25ef	5.48ef	4.23b	2.04	1.16cd	0.00d	0.00d	0.00c
Vitavax	<i>F. equiseti</i>	40.00c	60.00bc	40.00cd	66.00ab	50.80b	63.00bc	11.80de	5.20f	8.50e	1.60cd	1.90d	1.10cd	2.00b	2.00b	0.00c
(Carboxin /thiram)	<i>F. proliferatum</i>	100.00a	80.00ab	40.00cd	60.22ab	55.20b	66.00bc	15.90d	13.50cd	11.80de	4.60b	4.00b	1.60cd	3.00a	2.00b	1.00b
	<i>F. solani</i>	40.00c	60.00bc	80.00ab	32.80c	62.20ab	60.70bc	5.90ef	9.90e	13.80cd	1.00cd	3.00c	5.00a	2.00b	2.00b	1.00b
	<i>F. thapsinum</i>	60.00bc	40.00c	40.00cc	62.21ab	66.90ab	60.70bc	17.10c	11.90de	8.10e	3.60bc	2.50cd	1.50cd	2.00b	1.00c	1.00b
	Control	60.00bc	60.00	80.00ab	59.48b	50.01b	57.83c	20.27b	12.00d	11.73de	4.49b	2.98	2.70bc	0.00d	0.00d	0.00
<i>Tricho-p</i> ²	<i>F. equiseti</i>	60.00bc	60.00bc	60.00b	59.80b	50.80b	59.81c	16.10cd	5.20f	5.20ef	4.60b	1.90d	1.90cd	3.00a	3.25a	2.00a
	<i>F. proliferatum</i>	40.00c	80.00ab	100.00a	60.21ab	55.23b	74.60a	15.90d	13.50cd	13.71cd	4.60b	4.00b	2.32bc	2.00b	3.00a	1.00b
	<i>F. solani</i>	80.00ab	60.00bc	100.00a	59.12b	62.20ab	58.30c	11.70e	9.90e	17.00bc	2.80c	3.00c	3.50b	3.00a	2.00b	1.00b
	<i>F. thapsinum</i>	40.00c	40.00c	100.00a	60.10ab	22.10d	66.90b	17.50c	5.30f	11.90de	3.50bc	2.20cd	2.50bc	2.00b	2.00b	1.00b
	Control	65.00b	66.00b	80.00ab	49.27bc	65.03ab	65.53bc	20.18b	20.97a	19.85ab	4.38b	4.53ab	4.58ab	0.03d	0.00d	0.00c

¹ Means within a parameter followed by different letters differ significantly according to Fisher's protected least significant difference (LSD) test (P<0.05)

² *Trichoderma harzianum* (Tricho-plus)

SUMMARY

This study investigated the interaction between grain sorghum and *Fusarium* spp. associated with the crop in field soils and on sorghum roots as causal agents of crown and root rot. The effect of disease on plant growth and development was investigated as was the efficiency of various inoculation techniques on disease severity. Factors affecting disease susceptibility such as plant age, soil type, soil moisture, soil pH, soil fertility and possible chemical and bio-control control tactics were also investigated.

Fusarium spp. isolated from sorghum roots and field soils at Cedara (Kwazulu-Natal province), Bethlehem (Free State province) and Potchefstroom (North West province) indicated that *F. oxysporum*, *F. solani*, *F. verticillioides* and *F. thapsinum* were the most frequently recovered species. Other *Fusarium* spp. recovered were *F. equiseti*, *F. nygamai*, *F. pseudonygamai*, *F. proliferatum*, *F. subglutinans* and *F. polyphialidicum*. The most aggressive spp. were *F. equiseti*, *F. thapsinum* and *F. solani* while *F. proliferatum*, *F. subglutinans*, *F. Verticillioides* and *F. nygamai* were moderately aggressive and *F. oxysporum*, *F. polyphialidicum* and *F. pseudonygamai* least aggressive.

Population densities of *Fusarium* spp. in field soils and on sorghum roots were affected by genotype resistance and initial inoculum. Population densities were higher for susceptible than for resistant sorghum genotypes. A study on the effect of *Fusarium* spp. on plant growth and development indicated that shoot and root mass did not always correspond with the severity of the disease.

Various inoculation techniques for determining susceptibility of sorghum genotypes to crown and root rot and the virulence of *Fusarium* species were investigated. Wounding the crown and roots and inoculating them with ground colonized oat seeds and drenching soil with a conidial suspension were both very effective. An inoculum concentration of 1×10^6 conidia per ml consistently reproduced the disease on inoculated sorghum plants. Crown and root rot severity increased and plant mass decreased with an increase in inoculum concentration. Sorghum genotypes differed in their level of resistance in accordance with the inoculation techniques used.

Plant age was shown to affect resistance with two and four-week-old plants being more susceptible than six-week-old plants. Sources of partial resistance to *Fusarium* crown and root rot were present in some genotypes. Although immunity to *Fusarium* crown and root rot was not found, *Trichoderma harzianum* induced systemic resistance in sorghum through the reduction of crown and root rot severity.

Fusarium spp. can survive in a wide range of soil types. Certain soils were suppressive while others were more conducive to crown and root rot development. Soil moisture studies indicated that *Fusarium* spp. causing crown and root rot can survive over a wide range of soil moisture levels ranging between 25 and 100 percent. Low 25% and high 100% moisture levels were suppressive to crown and root rot severity compared to conducive at 50% and 75%.

Soil fertility studies indicated that nitrogen applied at normal and high rates significantly and differentially (by genotype) increased crown and root rot severity. Soil amendments also significantly reduced crown and root rot severity with chicken manure being most effective.

Studies on the efficacy of fungicides indicated that four fungicides (carboxin / thiram (Vitavax[®] Plus FS), tebuconazole/triflumuron (Raxil[®] 015 ES), tebuconazole, (Ingwe[®] 6FS), difenoconazole / metalaxyl-m (Dividend[®] 030 FS) and a bio-control agent (*Trichoderma harzianum*) significantly reduced colonization of seedlings. Fungicides also improved plant growth and development by increasing the shoot mass, root mass and enhancing root health. Major *Fusarium* spp. responsible for crown and root rot were *F. equiseti*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. pseudonygamai*, *F. thapsinum*, *F. solani*, *F. subglutinans*, and *F. verticillioides* based on the isolation frequencies and pathogenicity tests results.

The present study revealed the wide occurrence and distribution of *Fusarium* spp. associated with crown and root rot of sorghum in South Africa. It is hoped that these findings may motivate more research on variation in virulence of these *Fusarium* spp. Secondly work on the defense mechanisms present in the sorghum genotypes widely grown in South Africa need to be evaluated in relation to crown and root rot development. The most useful parameters for assessing the disease would be crown and root rot severity and root mass.

OPSOMMING

Die betrokke studie het die interaksie tussen graansorghum en verskeie grond- en wortelgedraagde *Fusarium* spp., wat as die oorsaak van kroon- en wortelvrot van die gewas beskou word, ondersoek. Die invloed van siekte op plantegroei en ontwikkeling is ondersoek, asook die doeltreffendheid van verskeie inokulasietegnieke op die ergheidsgraad van die siekte. Faktore wat siekteweerstand beïnvloed, soos plantouderdom, grondtipe, grondvog, grond pH, grondvrugbaarheid en moontlike chemiese en biologiese beheertaktieke, is ook ondersoek.

Fusarium spp. wat vanuit sorghumwortels en geassosieerde grondmonsters vanaf Cedara (KwaZulu-Natal), Bethlehem (Vrystaat) en Potchefstroom (Noordwes) geïsoleer is het aangetoon dat *F. oxysporum*, *F. solani*, *F. Verticillioides* en *F. thapsinum* die mees algemeen teenwoordige spesies was. Ander *Fusarium* spp. uit grond herwin was *F. equiseti*, *F. nygamai*, *F. pseudonygamai*, *F. proliferatum*, *F. subglutinans* en *F. polyphialidicum*. Die mees aggressiewe spesies was *F. equiseti*, *F. thapsinum* en *F. Solani*, terwyl *F. proliferatum*, *F. subglutinans*, *F. Verticillioides* en *F. nygamai* minder aggressief was en *F. oxysporum*, *F. polyphialidicum* en *F. pseudonygamai* die minste aggressief.

Populasiedigthede van *Fusarium* spp. in grond en op sorghumwortels is deur genotipe-weerstand en aanvanklike hoeveelheid inokulum in die grond beïnvloed. Populasiedigthede was hoër vir vatbare as vir weerstandbiedende sorghum genotipes. 'n Studie op die effek van *Fusarium* spp. op plantegroei en ontwikkeling het aangetoon dat stingel- en wortelmasse nie voortdurend met siektegraad gekorreleer was nie.

Verskeie inokulasietegnieke om die vatbaarheid van sorghum genotipes teen kroon- en wortelvrot te bepaal, asook die virulensie van sekere *Fusarium* spp., is ondersoek. Verwonding van die kroon en wortels, gevolg deur inokulasie met gemaalde gekoloniseerde hawersaad en die deurdrenking van plantgrondmedia met 'n spoorsuspensie, was albei effektief. 'n Inokulumkonsentrasie van 1×10^6 konidia per ml het voortdurend die siekte op geïnokuleerde sorghumplante geïniseer. Die graad van kroon- en wortelvrot het toegeneem en plantmasse het afgeneem met toenemende

inokulumkonsentrasie. Sorghum genotipes het verskil in hul vlak van weerstand na aanleiding van die inokulasietegniek wat gebruik is.

Dit is bewys dat plantouderdom siekteweerstand beïnvloed en dat twee- en vier-week-oue plante meer vatbaar is as ses-week-oue plante. Bronne van gedeeltelike weerstand teen *Fusarium* kroon en wortelvrot was in sommige genotipes teenwoordig. Alhoewel immuniteit teen die siektes nie gevind is nie, het *Trichoderma harzianum* sistemiese weerstand geïnduseer deur 'n afname in die intensiteit van die siektes.

Fusarium spp. kan in 'n wye verskeidenheid grondsoorte oorleef. Sekere gronde was onderdrukkend teenoor kroon en wortelvrot, terwyl ander die ontwikkeling van die siekte bevoordeel het. Grondvog studies het aangetoon dat *Fusarium* spp. wat kroon en wortelvrot veroorsaak oor 'n wye verskeidenheid grondvogvlakke tussen 25-100% kan oorleef. Baie lae (25%) en baie hoë (100%) vogvlakke het die intensiteit van kroon en wortelvrot onderdruk in vergelyking met middelmatige grondvogvlakke van 50-75% wat dit laat toeneem het.

Grondvrugbaarheidstudies het aangetoon dat stikstofvoeding teen normale en hoë peile die intensiteit van kroon- en wortelvrot beduidend en differensieël (volgens genotipe) laat toeneem het. Grondtoevoegings het kroon- en wortelvrot beduidend laat afneem, met hoendermis as die mees effektiefste middel.

Studies op die doeltreffendheid van swamdoders het aangetoon dat vier fungisiedes, naamlik carboxin/thiram (Vitavax[®] Plus FS), tebuconazole/triflumuron (Raxil[®] 015 ES), tebuconazole, (Ingwe[®] 6FS), difenoconazole/metalaxyl-m (Dividend[®] 030 FS) en 'n bio-beheer agent (*Trichoderma harzianum*) die kolonisasie van saailinge aansienlik verminder het. Fungisiedes het ook plantegroei en -ontwikkeling bevorder deur stingel- en wortelmasse te vermeerder en wortelgesondheid in die algemeen te verbeter.