THE RESISTANCE OF GRAIN SORGHUM TO THE ROOT ROT PATHOGEN COMPLEX

by

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DECLARATION

I hereby declare that this dissertation submitted by me for the degree of *Magister Scientiae* in Plant Pathology at the University of the Free State is entirely my own work and has not previously been submitted by me at other higher education institutions. I further more cede all copyright of this dissertation to the University of the Free State.

DANELLE VAN ROOYEN

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PREFACE

This dissertation consists of four chapters, including a literature review. The main focus of this study was to evaluate resistance of sorghum to root- and- mesocotyl rot pathogens. Biotic causes of the disease complex were elucidated and the potential of biological control was examined.

The first chapter is a literature review of sorghum root rot, starting with a general introduction into the history and origin of sorghum and its uses, as well as pathogens associated with root rot and the role that certain factors such as temperature and moisture play in the occurrence of sorghum root rot. Different control measures, such as cultural, chemical and biological were discussed as well as host resistance mechanisms that prevent the pathogen from colonizing the plant tissue.

Chapter 2 addresses the pathogen; the isolation of the potential pathogen from field sorghum, the examination of pathogenicity on a white tan and red purple sorghum cultivar grown in the greenhouse, through the measurement of root rot severity and the measurement of ergosterol analysis to indicate the ability of the test fungi to colonize the root tissue. Sequencing was used to identify the isolated test fungi.

In Chapter 3 general resistance of sorghum cultivars was assessed in a naturally infested field by measuring root rot and plant length, extracting and measuring the phenolic content of susceptible and resistant sorghum cultivar roots and testing the inhibition effect of root extracts from these sorghum cultivars against the growth of the selected test fungi.

The objective of Chapter 4 was to examine the biological control of sorghum root rot through the addition of *Trichoderma* spp. to seed and soil. The inhibition abilities of *Trichoderma* spp. were indicated through inhibition of test fungi in dual culture, as well as the production of volatile and non-volatile substances. Colonization of the sorghum root by *Trichoderma* spp. *in vitro* was also measured, as well as the inhibition of root rot

in the greenhouse and the potential of the *Trichoderma* spp. to colonize and survive in the rhizosphere of sorghum roots.

Typical symptoms of root rot of sorghum (Courtesy of Prof. N. W. McLaren)

CHAPTER 1

A REVIEW OF ROOT ROT OF SORGHUM

1.0 INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench), a member of the grass family *Graminea*, is a hardy plant able to grow under a variety of field conditions, and which, together with maize, barley, wheat, rice and sugarcane, forms part of the world's feed and food production chain for animal and human consumption (Du Plessis, 2008; Jwa *et al*., 2006).

It is believed that sorghum was one of the first grasses cultivated for grain usage in the early civilizations of the Mediterranean region. A carving representing a field of sorghum was found in King Sennacherib's palace at Nineveh, the ancient capital of Assyria, on the banks of the Tigris River (704-681 B.C.). Records can be found of sorghum at the beginning of the Christian era in India and China and it is believed that sorghum has been grown since the reign of the pharaohs in Egypt. In Africa wild or cultivated sorghums have most likely been used as food for several thousand years (Tarr, 1962).

India was first believed to be the origin of sorghum, but it is now considered to have originated in Africa. The great diversity of sorghum types in Kardofan, a province of Sudan, suggests this region to be the origin of sorghum and from there it was distributed to Egypt and subsequently towards Arabia, India and China in the Far East (Tarr, 1962; Doggett, 1970).

Annual sorghum production in the world has doubled since 1962 and global yield currently stands at over 60 million tons from a cultivation area of 46 million ha in 2006 (Tarr, 1962; Dicko *et al.,* 2006). In South Africa the annual production of sorghum

varies from 100 000 t to 180 000 t with the Free State and Mpumalanga Provinces being the principle production areas (Du Plessis, 2008).

Although the commercial uses of sorghum may change over time, it is estimated that over 35% of all sorghum grown is used for human consumption. The remainder of the sorghum produced is used for alcohol production, animal feed and industrial products (Awika and Rooney, 2004). Sorghum is especially grown as a staple food for many rural communities in areas where maize cannot serve as food security (Du Plessis, 2008). This is due to sorghum's ability to adapt to drier and hardened conditions.

Sorghum out-yields other crops under a variety of environmental conditions and is therefore more cost-effective to cultivate (Awika and Rooney, 2004). Sorghum is primarily grown in dry and hot areas that are normally too dry for other cereal crops (Dicko *et al.,* 2006). Rainfall of 400 mm in drier areas to 800 mm in wetter areas is sufficient for sorghum cultivation. The drought tolerance effect seen with sorghum can be attributed to certain physical and physiological characteristics. These include (Du Plessis, 2008):

- the efficient absorption of water through the well-developed and branched root system and the limited transpiration through the small leaf areas
- the ability of the leaves to fold up efficiently during hot and dry conditions
- the thin waxy layer that covers the epidermis of the leaf and protects the plant from desiccation
- the stomata close quickly to limit the loss of water
- the ability of sorghum to enter a dormant stage when conditions become unfavourable and to resume growth when the situation changes.

Temperature plays an important role in the yield, growth and flowering stage of sorghum. The base temperature for germination is from 7 - 10°C. Sorghum usually requires high temperatures for germination and growth, but extremely high temperatures cause a reduction in yield by delaying the initiation of flowering and the development of flower

primordia. Temperatures from 20 - 30°C with a frost-free period of 120 - 140 days are required for optimal growth and yield potential (Du Plessis, 2008). At a lower maximum temperature, for instance 20°C, sorghum can be grown without a striking effect on the yield or growth. However, when temperature drops to below freezing, the survival of the plants depends on the age of the plants. Younger plants between one and three weeks can recover after exposure to -5°C. Below -7°C the plants die. Older plants are less tolerant to very low temperatures and will die at 0°C (Du Plessis, 2008).

Sorghum is mostly grown on low potential, shallow soils that contain a high clay content. Sandy soil results in poor sorghum growth and a clay content of 10 - 30% is necessary for optimal growth. Sorghum is more tolerant of alkaline salts compared to other crops and can be cultivated in soils with a pH_{H20} ranging from 5.5 to 8.5. Compared to maize, sorghum can better tolerate short periods of water logging (Du Plessis, 2008).

Pathogen or pest attacks lead to crop losses that are estimated at 30% for sorghum annually (Chandrashekar and Satyanarayana, 2006). Management of pathogen or pest attacks in order to enhance food quality and the amount of sorghum produced is therefore important. Yield losses as a result of root pathogens can be as much as 25% of the annual production. The extensive root system and the ability of sorghum to tolerate dry conditions allow sorghum to endure a certain level of root loss due to root rot (Tarr, 1962). For this reason obvious aerial symptoms are not always evident. Root rot pathogens destroy the root structure and volume and can lead to lodging of the plants.

This literature study gives an overview of the pathogens associated with root rot of sorghum, followed by management strategies that may give rise to healthier, more resistant plants, whether natural occurring, for instance biological control and inherent resistance or introduced by cultivation practices and thereby increasing yield.

2.0 SORGHUM ROOT ROT ETIOLOGY AND SYMPTOMS

Root rot of sorghum is caused by soilborne fungi, including *Fusarium* spp., *Pythium* spp., *Macrophomina phaseolina*, *Colletotrichum graminicola* and *Periconia circinata* (Mughogho, 1984). Root rot is generally associated with a complex of these and other fungi and colonization of tissues depends on environmental factors that favour a particular pathogen at a certain time as well as the degree of host predisposition (Mughogho and Pande, 1984).

Root rot fungi can be distributed through rain, agricultural equipment, wind and animals (insects in particular) and survive in plants, soil or plant debris either as spores, hyphae or resting structures (Waniska *et al*., 2002). Germination of resting cultures or spores is stimulated by root and seed exudates (Idris *et al.*, 2008) and the pathogens gain access to the roots through natural root wounds or injuries caused by machinery, insects or other causes (Claflin, 2000). Primary infection starts in the cortex tissues and spreads towards the vascular tissues of the root (Zummo, 1984).

2.1 *Root rots caused by* **Fusarium** *spp.*

F. moniliforme J. Sheld (*Sensu late*) originally described in 1904 and regarded as one of the major *Fusarium* spp. that cause root rot, was recently reclassified and the species most commonly found on sorghum has been renamed *F. thapsinum* Klittich, Leslie, Nelson, *et.,* Marasas (Claflin, 2000). In greenhouse and field experiments conducted by Tesso *et al*. (2010) *F. thapsinum* was most virulent on sorghum, compared to the other *Fusarium* spp. tested. These included *F. andiyazi* and *F. verticillioides.*

Studies have demonstrated that *Fusarium* spp. infect sorghum rootlets without the development of obvious symptoms until plants reach maturity (Giorda *et al.*, 1995). At maturity, discolouration of the roots occurs and infected sorghum plants show a reduction in plant growth with poor grain fill and concomitant yield loss when disease is severe.

Severe damage to the roots can result in decreased water absorption, nutrient uptake (Claflin, 2000) and loss of anchorage through the destruction of older roots (Zummo, 1984). This can lead to plants being easily uprooted, lower grain yield, a reduction in drought tolerance and eventually plant death (Zummo, 1984).

Lesions caused by *Fusarium* spp. can differ in size, from small circular spots scattered over the roots or stripes that extend over most of the root surface. These are usually red to purple in colour (Claflin, 2000) depending on the host genotype, although light brown or black discolouration may be associated with tan plant types. The infection may cause a breakage of the lateral roots from the main root system and infected roots generally lack root hairs (McLaren, 2002). Degeneration of the vascular bundles and pith are rarely caused by *Fusarium* spp. on roots, but may be caused by secondary or opportunistic organisms that are usually present and can cause the disintegration of tissues (Claflin, 2000), e.g. *Periconia* spp. and other *Fusarium* spp. (Odvody and Forbes, 1984). When disintegration of the inner tissues occurs, all that remains is a dry hollow shell that lacks any form of structural integrity (Trimboli, 1983).

The inoculum source of *Fusarium* spp. associated with root rot of sorghum can be either seedborne, airborne or soilborne. Conidia cannot survive for longer than three months and survival of the pathogen requires plant debris and favourable growth conditions. The longest survival on maize debris under field conditions has been reported to be two years (Claflin, 2000). Studies have shown that no loss in viability of conidia occurred when *F. thapsinum* was stored for six months at -16°C (Claflin, 2000).

Variation between isolates of the same *Fusarium* spp. complicates pathogenicity as indicated by McLaren (1987) where e.g. *F. oxysporum* resulted in significant differences between primary root and mesocotyl discolouration which ranged from 2.3 - 12.2% and from 3.7 - 26.5% respectively, depending on the isolate used in pathogenicity studies.

2.2 *Pythium root rot*

The two major causal organisms of *Pythium* root rot of sorghum are *P. arrhenomanes* Drechsl. (1936) and *P. graminicola* Subramanian (1928) (cited by Odvody, 2000). *P. arrhenomanes*, reported in 1937, was the first *Pythium* spp. to be associated with sorghum root rot and was initially mistaken to be the cause of Milo disease in Texas and other regions of the United States. Subsequently, *Periconia circinata* was identified as the pathogen responsible for Milo disease of sorghum (Odvody and Dunkle, 1984).

In 1971 and 1972 severe root rot of sorghum was reported in the High Plains of northern Texas and the causal fungus was subsequently identified as *P. graminicola* (Odvody, 2000). Other *Pythium* spp. isolated from insect-damaged roots and stalks of sorghum are *P. periplocum* Drechs and *P. myriotylum*, with only the latter proven to be pathogenic, although its distribution seems to be limited (Odvody, 2000).

The sorghum pathogenic strains of *Pythium* spp. are most likely to survive in the soil as oospores and the idea of saprophytic growth as a survival mechanism is generally dismissed due to *Pythium* spp. being poor competitors that colonize tissues only in the absence of other organisms or in the presence of organisms that have been inactivated by environmental conditions (Odvody and Forbes, 1984). The oospores in the soil are triggered by root and seed exudates of sorghum to germinate by either producing a germ tube or zoospores that encyst and then germinate, after which the pathogen enters the host cells and tissues (Odvody and Forbes, 1984). An increase in sorghum's susceptibility to *Pythium* spp. in cold, wet soil is a result of slower germination rate, delayed emergence and a reduction in root growth (Forbes *et al*., 1986).

Darkened or blackening roots with the formation of sunken red-brown to blackish lesions are typical symptoms associated with root rot of sorghum caused by *Pythium* spp. Occasionally a tanned lesion or root can be observed when root death occurs. Greater discolouration of lesions and roots can arise when colonization by *Fusarium* spp. follows infection by *Pythium* spp. (Odvody, 2000).

2.3 *Charcoal root rot*

Charcoal root rot, caused by *Macrophomina phaseolina* (Tassi) Gold is one of the most frequently found root and stalk rot disease of sorghum (Mughogho and Pande, 1984). It has been detected in all ecological areas where sorghum is cultivated, including the tropics, sub-tropics and temperate areas. Environmental conditions, such as hot and dry conditions are largely responsible for the predisposition of host plants to charcoal rot (Jordan *et al.,* 1984) and despite inoculum being present, the disease can be widespread or localized in some seasons or even absent in others. The origin of the name of this disease is due to the charcoal appearance of infected areas where vascular bundles become covered with numerous tiny black microsclerotia of the pathogen (Mughogho and Pande, 1984). *M. phaseolina* can also infect seedlings under moist and high temperature conditions and cause seedling blight or damping-off of seedlings (Mughogho and Pande, 1984).

M. phaseolina has a wide geographical distribution with a wide host range that includes more than 284 plant species (Farr *et al.,* 1995), although genetic variation has been found between native and agricultural species (Saleh *et al.,* 2010). *M. phaseolina* is generally found in warmer areas on a wide range of hosts and is associated with damping-off, seedling blight, leaf spotting and root rot, as well as additional rotting of fruits, stems and other plant organs. It was first reported in the 1930's in India to be the cause of seedling blight and hollow stem of sorghum and was identified on maize the same time in the United States and can now be found on other plant species as well. *M. phaseolina* has a wide host range that includes sorghum, beans, potatoes, legumes, tomato, cotton, tobacco, etc. (Tarr, 1962).

M. phaseolina is a root-inhabiting pathogen with little or no saprophytic growth in either infected plant cells or in the soil. In the absence of a host, the pathogen survives as micro-sclerotia in diseased root and stem debris or in the soil following decay of the host plant in which they were produced. Micro-sclerotia therefore are a primary source of

inoculum (Mughogho and Pande, 1984) which germinate after being stimulated by sorghum root exudates.

A wide range of symptoms are associated with charcoal rot on susceptible cultivars and these include root rot, soft stalks, yield loss and reduced grain quality. This is mainly due to stunted plants, smaller stalks with premature drying, poorly developed panicles and reduced crop stands as a result of seedling blight and these in turn cause low quality grain due to infected and destroyed stalks and lodging of the plants (Mughogho and Pande, 1984).

Lodging, being the most prominent symptom as plants mature, occurs as a result of damage and weakening of the stalk after disintegration of the pith and cortex by the pathogen, resulting in the lignified fibrovascular bundles becoming suspended as separate strands in the hollow stalk. Lodged plants lead to complete yield loss where there is dependence on mechanical harvesting and losses due to termites or other pests where panicles from lodged plants remain on the soil for various periods before the grain is manually harvested (Mughogho and Pande, 1984).

2.4 *Periconia root rot*

Periconia root rot, also known as Milo disease of sorghum, is caused by the fungus *Periconia circinata* (Mang.) Sacc. and was first detected in 1924 in Texas (Chillicothe) and two years later in Kansas (Garden City) (*sensu* Odvody and Dunkle, 1984). The causal pathogen was only identified twenty-three years later in 1947 by Leukel and Pollack (Odvody and Dunkle, 1984).

Roots of infected seedlings have water-soaked, reddish discolourations of the cortical and vascular tissues. Towards maturity and as the disease develops, smaller roots are destroyed and larger roots turn dark red or brown. Subsequently, symptoms spread to the canopy, where leaf symptoms may be observed. Leaves wilt, droop and become slightly

rolled. More mature leaves turn yellow with the tips and margins dried and necrotic. Younger leaves are the last to become discoloured and die. Susceptible sorghum cultivars are usually stunted and often die with a poorly filled head or without producing a head (Odvody and Dunkle, 1984).

Leukel (1948, cited by Odvody and Dunkle, 1984) suggested that toxin production plays a role in disease development and thirteen years later in 1961 Scheffer and Pringle (*sensu* Odvody and Dunkle, 1984) revealed the production of a host-specific toxin, which only has an effect on susceptible cultivars.

2.5 *Colletotrichum root rot*

Colletotrichum graminicola (Ces.) Wilson is one of the most economically important vascular stalk rot pathogens found on maize (*Zea mays*) and can cause root rot, crown rot, seedling blight, stalk rot, leaf blight or top dieback, with the latter occurring when the stalk tissues are invaded above the ear (Wicklow *et al.,* 2009).

After colonization of roots by *C. graminicola,* the pathogen forms specialized infection and survival structures, such as hyphodia, hyphae and microsclerotia and from here the canopy stems and leaves are systemically colonized, with the pathogen being restricted to individual vascular bundles. Despite the xylem cells being colonized by hyphae, no blockage of the vascular system occurs and wilting normally associated with vascular disease, seemed to elude the plants (Sukno *et al*., 2008). Symptoms associated with stalk infection include water-soaked discolouration of rind tissue in the lower internodes, with black discolouration to sunken lesions covering the stalk rind. These are due to a large number of immature acervuli (Wicklow *et al.*, 2009). Beneath these lesions, a brownish black discolouration of the pith tissue can be observed that leads to lodging of the plants or stalk breakage and pith tissues disintegrate (White, 1999).

Studies conducted by Wicklow *et al.,* (2009) showed that *C. graminicola* produces monorden and monocillins and it was suggested that these metabolites play a role in the infection process by inhibiting the hypersensitive response in maize and by inhibiting the growth of other pathogenic fungi, such as *Stenocarpella maydis*, although this effect is also dependable on the specific fungi being inhibited because these metabolites had no effect on *Fusarium graminearum*.

2.6 *Other fungi*

Other fungi that have been associated with sorghum root rot include: *Nigrospora* spp., *Alternaria* spp., *Acremonium* spp., *Rhizoctonia* spp., *Epicoccum* spp., *Erwinia* spp., *Sclerotium rolfsii* and *Phoma sorghina* (Tarr, 1962; Reed *et al.,* 1983; Mughogho, 1984; Zummo, 1984). *Exserohilum pedicellatum* has also been isolated from maize roots (Flett, 2007) and other root colonizers include *Phoma* spp., *Curvularia* spp. (Hugo, 1995) and sometimes even *Stenocarpella maydis* (Flett, 2007).

3.0 ENVIRONMENTAL INFLUENCES ON ROOT ROT AND THEIR MANIPULATION IN MANAGEMENT STRATEGIES OF SORGHUM ROOT ROT.

The biotic and abiotic environment in which the sorghum plants grow, determines to a large extent, the maintenance of disease resistance or predisposition to infection by pathogens. As a result disease incidence and severity depend on environmental adaptation of the specific host and pathogen. McLaren (2002) showed that the effect of genotype, environment and the G x E interaction were 15.1%, 70.5% and 9.19% on root rot severity, respectively indicating that environmental factors are a primary driving variable in root rot epidemiology.

Stress factors, such as plant population density and weeds that increase competition for moisture and nutrients, as well as drought or moisture stress conditions and high insect populations may predispose the host to infection. Cultural practices that alleviate both biotic and abiotic environmental stresses are important considerations in the management of root rots. This will lead to good, sustained, balanced soil fertility and healthier and stronger plants (Claflin, 2000).

Changing the planting date to reduce the risk of disease favourable weather, during critical plant growth stages, is suggested. Root rot is generally a disease of maturing tissue, with the roots being at their most vulnerable in growth stages between week six and week eight (McLaren, 2004).

3.1 *Soil environment*

3.1.1 *Temperature*

Temperature plays an important role in the yield, growth and flowering stage of sorghum. Sorghum usually requires high temperatures for germination and growth, although extremely high temperatures cause a reduction in yield by delaying the initiation of flowering and the development of flower primordia. Temperatures from 20 - 30°C with a frost-free period of 120 - 140 days are required for optimal growth and yield potential. The base temperature for germination is from 7 - 10°C (Du Plessis, 2008).

Soil temperature can also either encourage or delay the infection of sorghum by certain pathogenic fungi. Low soil temperatures favour infection by *Fusarium* spp. while high soil temperatures favour infection by *Macrophomina phaseolina* (Mughogho and Pande, 1984). As a result these authors suggested that row width and population density be taken into account, as a narrow row width and higher population density leads to a denser canopy which results in lower soil temperature which will favour the infection by *Fusarium* spp.

3.1.2 *Moisture*

Field observations indicate that basal stalk rot and root rot usually occur in crops that develop under near-optimal or optimal conditions between planting and flowering, but are then subjected to moisture stress (Trimboli, 1983). The severity of root rot caused by *Fusarium* spp. appears to increase with cool, wet weather conditions following a dry, hot period or can be stress-induced during the blooming period until the hard dough stages of growth (Claflin, 2000). Crop losses due to infection by *Macrophomina phaseolina* are higher where prolonged drought period and higher temperatures prevail in cultivated fields (Mihail, 1989).

In cool, wet soils, sorghum becomes more susceptible to *Pythium* spp. because of slower seed germination, reduced root growth and delayed emergence (Forbes *et al*., 1986). Root infection of sorghum on the High Plains of Texas may occur throughout the growing season, but is particularly observed during boot stage or soon thereafter, when numerous adventitious roots are being produced in irrigated fields with high levels of soil moisture and high soil temperatures. After the last irrigation is given when plants reach maturity, hot and dry conditions usually follow and this results in leaf and plant death caused by *Pythium* spp. (Odvody, 2000). Conversely, severe basal rot and root rot caused by *Fusarium* spp. were not detected in irrigated or dry-land sorghum plants with a sufficient amount of soil moisture from sowing to maturity (Trimboli, 1983). This was only observed where plants were grown under sufficient water availability, followed by severe moisture stress after flowering, with a quick rewetting.

3.1.3 *Mulching*

The addition of mulches to soil often leads to an increase in soil moisture and decreased soil temperature. Studies conducted by Tilander and Bonzi (1997) indicated that the addition of mulches consisting of neem, compost, acacia and grass significantly reduced the soil temperature, with compost being the poorest reducer. Low soil temperatures

reduce the risk of root infections of sorghum by *Macrophomina phaseolina* (Mughogho and Pande, 1984). Neem, acacia and grass mulches preserved more water and the neem and acacia also retained a higher soil humidity compared to the compost additions. This was seen with both soil layers tested, i.e. a depth of 0 cm - 5 cm and 5 cm - 20 cm. Maintenance of soil moisture in the deeper soil layers increased availability to plant roots as this reduces drought stress which in turn decreases crop losses due to infection by *M. phaseolina* (Mihail, 1989).

3.2 *Cultural practices*

3.2.1 *Stubble management through tillage practices*

Residue management implies the transformation of crop residues back into organic carbon in the soil. The decomposition of residue can differ with crop type, amount of residue, the depth at which the residue is located, allelopathic interactions between the soil occupants and time (Bailey and Lazarovits, 2003). Tillage plays a major role in stubble management. Reduced or no-tillage practices can enhance water uptake by reducing water runoff, decreasing the occurrence of wind and water erosion, reducing soil crusting and is more economical due to the elimination of the extensive use of mechanical maintenance and fuel inputs (Mannering and Fenster, 1983). The selection of tillage strategies has the potential to change inoculum potentials of pathogens and their survival in the soil (Bailey and Lazarovits, 2003).

Flett (2007) reported that rotation and tillage practices are only effective against diseases where pathogens are dependent on surface stubble retention for survival, for instance *Stenocarpella maydis* that causes crown, stalk and ear rot on maize. The occurrence of *S. maydis* was reduced where the maize stubble was buried with conventional and reduced tillage, compared with no-tillage practices. In pathogens such as *Fusarium subglutinans*, where the survival of the pathogen is not subject to where the stubble is situated either in

or above the soil, neither conventional nor reduced tillage reduced the disease incidence (Flett, 2007).

Residue also plays a role in root rot of maize, as demonstrated by Govaerts *et al.,* (2007), where root rot was more severe on the primary roots when residue was present, compared to when it was removed. Stover removal and ploughing in of maize residue into soil decreased drainage of the soil, which in turn lead to higher seedling infections by *Pythium* spp. due to water-logging under high rainfall conditions, an environment that favours *Pythium* spp. (Medvecky *et al.,* 2007).

On the other hand, reduced or no-tillage practices lead to increased surface stubble retention that enhances microbial communities in the soil, both those that are pathogenic and increase yield losses and those that are advantageous and antagonistic to pathogens. No-tillage practices can also change the soil environment of the microbiota by causing higher soil temperature and moisture in the upper layer and this can favour certain plant pathogens (Janvier *et al*., 2007). The influence of reduced tillage on a variety of pathogens is mainly dependent on the life cycles and survival strategies of the pathogen involved (Govaerts *et al.,* 2007).

No-tillage systems are associated with a number of root rot pathogens. These include Rhizoctonia root rot and bare patch caused by *Rhizoctonia solani,* damping-off and root rot caused by *P. aphanidermatum,* Fusarium crown, foot and root rot caused by *F. culmorum* and *F. pseudograminearum* and other *Fusarium* spp. (Govaerts *et al.,* 2007). Under no-tillage systems, in an experiment conducted by Govaerts *et al.* (2007), root rot of maize was higher on the primary and secondary roots, compared with conventional tillage practices. Tillage practices can change the environment of the pathogen, by altering the soil properties, the pH levels of the soil, nutrients that can alter soil fertility and the distribution of the pathogen by displacing them to less favourable conditions.

Flett (2007) suggested that tillage and rotation could affect sorghum root rot, but that other factors are involved that affect this interaction, resulting in inconsistent control being obtained. More information about the root rot complexes and the interaction with stubble management is needed before a conclusion can be drawn (Flett, 2007).

3.2.2 *Crop rotation*

The uninterrupted cropping with the same susceptible plant hosts for several seasons leads to the formation of specific plant pathogenic communities that contribute to yield loss in crops (Janvier *et al*., 2007). The formation of these pathogenic communities can be reduced by crop rotation with unrelated plant species. Crop rotation improves soil structure and the organic matter content of soils, which in turn has a positive effect by reducing disease occurrence caused by soilborne pathogens. Crop rotation is an ancient cultural practice and the first known indication of crop rotation was a poem by Virgil, the great Roman poet, in 30 - 37 BC (Flett, 2007).

The effectiveness of crop rotation is dependent on a number of factors including the host preference and the adaptability of the pathogen. Genetic variation between *Macrophomina phaseolina* isolates allows them to colonize different plant hosts (Saleh *et al.,* 2010) and therefore crop rotation can effectively suppress colonization of species-specific *M. phaseolina* by altering the pathogen population structure. Greater colonization of maize plants was observed when the field in which the maize was cultivated, had previously been infested with *M. phaseolina* isolates specific to maize, rather than isolates specific to sorghum, soybean or cotton (Su *et al.,* 2001).

In contrast, *Cochliobolus sativus* is a soilborne pathogen that causes common root rot of cereals and the survival abilities of *C. sativus* ensure that this pathogen is mainly unaffected by crop rotation. These survival abilities include the survival of its spores in the soil for up to 20 months, the ability of this pathogen to survive saprophytically in the soil and the ability of this pathogen to sporulate on several different hosts, including barley, wheat, triticale, oat, canary seed and wheat grass species (Bailey and Lazarovits, 2003).

3.2.3 *Composting*

Compost is the final product of aerobic biodegradation of organic matter and displays noticeable disease suppression properties (Hoitink *et al.,* 1993). Organic matter in the soil enhances the activity of beneficial organisms that suppress pathogens (Lewis and Papavizas, 1975).

Compost application can control plant diseases through different mechanisms such as the production of antibiotics by beneficial microbiota, competition for nutrients and the activation of disease-resistance genes in plants. Compost improves the structure and moisture retention of soil, supplies nutrients to plants and has been shown to possess suppressiveness towards soilborne pathogens (Hoitink and Fahy, 1986).

The efficacy of composts in the suppression of root rots depends on the composition of the compost. Compost with peat-based growing medium with crab/shrimp shell chitin, increases the growth of cucumber in the absence of root pathogens. In treatments where the root and stem rot pathogen, *Fusarium oxysporum* f. sp. *radicis-cucumerinum,* was present, a higher disease incidence occurred with composting compared to the absence of composts. This was attributed to the breakdown of chitin that releases ammonia which enhances susceptibility of the cucumber to root and stem rots (Rose *et al.,* 2003). It is therefore important to know the content of the compost used, the chemical processes involved in the breakdown and the products that are formed and released, so that an informed decision can be made about the type of compost to use in order to control diseases in plants.

The addition of compost can have a significant effect on the nutrient content of soil. In studies conducted by Tilander and Bonzi (1997), significantly higher nitrogen content was observed in plots treated with neem leaves than compost or grass. This could have been a result of the neem leaves having a higher nutrient content and release rate.

3.3 *Chemical environment*

3.3.1 *pH*

The infection of sorghum by seedling and root pathogens, such as *Fusarium* spp., *Colletotrichum graminicola, Macrophomina phaseolina, Phoma sorghina* and *Alternaria* spp. can be influenced by soil acidity. An increase in mesocotyl and primary root discolouring of sorghum seedlings and secondary root discolouring of more mature plants was observed with a decrease in pH with a significant increase at $pH_{(KCl)}$ less than 4.6 (McLaren, 2004). Soil acidity also reduces plant growth as a result of induced toxicities by Al and by inhibiting the absorption of essential nutrients (McLaren, 2004).

Of 55 soil samples collected by Kobayashi and Komada (1995) in Japan, 5 were suppressive to Fusarium wilt of cucumber caused by *F. oxysporum* f.sp. *cucumerinum*, and 8 were suppressive to Fusarium wilt of *Phaseolus vulgaris* caused by *F. solani* f.sp. *phaseoli*. There was a close relationship between disease severity and soil pH. Most of the soils suppressive to cucumber Fusarium wilt had a higher pH than the non-suppressive soils. However, suppressive soils to *P. vulgaris* Fusarium wilt had lower pH's, and in these acid soils spore germination was inhibited. Cucumber Fusarium wilt was almost completely suppressed at pH 8.0 while *P. vulgaris* root rot was suppressed at pH 4.0. These results indicate that the effect of pH on root rots is both crop and pathogen species specific.

3.3.2 *Fertilization*

Organic compounds are secreted by plants and these stimulate the germination of fungal spores on the root- and leaf surfaces. Alternately plants resist this intrusion by producing chemical barriers for example phenols, O_2-radicals and hydrogen peroxides against the pathogen attack. Nutrients are critical in the production of these chemical substances, especially micronutrients such as copper, zinc, iron and manganese. Micronutrients

generally act as inhibitors, catalysts and co-factors, whereas macronutrients such as nitrogen, phosphorus, calcium, magnesium, potassium and sulphur are involved in structural, osmotic, compositional and conformational function (Jordan *et al.,* 1984).

Some plants impose physical barriers against pathogenic attack. Silicon is deposited in the epidermal cell layer and copper stimulates increased lignifications to fight off any attack. A leakage of sugar onto the plant surfaces often results when a copper deficiency occurs. This stimulates pathogen germination (Valentine and Kleinert, 2006).

The infection by *Pythium arrhenomanes* and *P. graminicola* of roots of wheat and barley, were greater in soils lacking phosphate (Waller, 1979). Zinc can decrease the severity of *Rhizoctonia* root rot, but the required amount is phytotoxic. An important element for soilborne pathogens is iron and a way to depress them is to deprive them of this important element (Valentine and Kleinert, 2006). *Fusarium* spp. root and stalk rot severity can be reduced if the ratio of potassium and nitrogen is 1:1 because a high nitrogen level together with a low potassium level can increase *Fusarium* spp. root and stalk rot of sorghum and maize (Claflin, 2000). Nitrate and ammonium forms of nitrogen applied to the soil can decrease the incidence of disease. Some pathogens can only utilize one form of nitrogen, where others can utilize both NO_3-N and NH_4-N. *Cochliobolus sativus* is dependent on the NO_3-N form and disease potential is reduced when NH_4-N are applied (Bailey and Lazarovits, 2003).

3.3.3 *Chemical control*

Chemicals used to control pathogens usually have one or more active ingredients that influence the growth or life-cycle of the pathogen. Chemicals are used by farmers to enhance the quality and yield of crops and decrease any potential crop losses in the field or during storage (Abawi and Widmer, 2000). In 1994 the global chemical market was estimated at US$ 5.4 billion (Hamlen *et al.,* 1997). The cost of developing a new pesticide was approximately $30,000,000 in 1990 (Chet, 1990).

Chemical control of plant diseases has more disadvantages than advantages. Not only is it unaffordable for many developing countries (Idris *et al.,* 2007), but some chemicals are also phytotoxic, lead to resistance-build up in pathogens and can alter the microbial community, especially when a broad spectrum chemical is used.

Fungicidal seed treatments are effective against seed- and soilborne pathogenic fungi that cause seedling diseases and seed rots of sorghum. It is unclear whether seed treatments are effective in adult sorghum plants (Williams and Nickel, 1984). Attempts have been made in Ethiopia to control root rot caused by *Fusarium* spp. in sorghum using benomyl, but without success (Idris *et al.*, 2007). This was mainly because of phytotoxicity towards sorghum (Benhamou, 1992).

The benzimidazole fungicidal groups are particularly effective against sorghum diseases caused by *Fusarium* spp. *Rhizoctonia* spp. and *Colletotrichum* spp. (Williams and Nickel, 1984). Thiram administered as seed treatment controlled *Fusarium* spp., *Pythium* spp. and *Rhizoctonia* spp. effectively in sorghum fields. Thiram also displayed effective control of *Fusarium* spp., *Curvularia* spp., *Alternaria* spp., *Phoma* spp., *Verticillium* spp. and *Cladosporium* spp. (Williams and Nickel, 1984).

Metalaxyl can effectively control *Pythium ultimum* on sorghum (Hwang *et al*., 2001; Taylor *et al*., 2002 as cited by Idris *et al.,* 2008), but when metalaxyl was applied in Ethiopia's Alemaya areas with cooler and wetter soils, control of *P. ultimum* root rot was unsuccessful. Some reductions in disease were recorded at high dosages, but the efficacy of metalaxyl was quickly lost (Idris *et al.*, 2008). A recent outbreak of sorghum downy mildew, caused by the obligate oomycete *Peronosclerospora sorghi* has indicated a metalaxyl-resistant variant which illustrated the ability of this group of pathogens to adapt to metalaxyl (Perumal *et al.,* 2006) and this may also be the reason for reduced Pythium root control here.

3.4 *Biotic environment*

The negative environmental effects resulting from chemical control have initiated a search for alternative control measures. The solution, proven by many research studies, could lie in the soil surrounding the roots, the rhizosphere. The interest in biological control of diseases has grown remarkably and the microbial bio-pesticide market has grown from $20 million in 1975 to $268 million in 2005 (Leipoldt, 2007). Fungi are a very diverse group of organisms, with about 230,000 species dispersed in each ecosystem with only a few of these species displaying biological control properties. The possibility of using antagonistic microorganisms to control pathogenic fungi has received considerable attention (Chet, 1990). Unfortunately there are always risks and certain concerns when introducing a new organism into an already established environment.

One of these concerns is the non-target effects the introduced biological control agent (BCA) has on other organisms apart from the target organism, whether either direct or indirect by a chain of events mediated by the BCA such as the disruption of microbial processes or the disruption of carbon, nitrogen or phosphorus cycles (Winding *et al.,* 2004). Another concern is that the target organism could develop resistance to the introduced BCA (Compant *et al*., 2005), therefore countering the biological effect. After all the effort and money input the main risk lies in the newly introduced BCA's ability to multiply and survive in its new environment, as its prime mode of action remains the competition for food and space.

3.4.1 *Biological control of sorghum root rot*

A number of biological control agents are available on the market, and these include commercial solutions from both bacterial and fungal genera, including *Streptomyces* spp., *Pseudomonas* spp., *Agrobacterium* spp., *Bacillus* spp. bacterial strains and fungal strains of *Gliocladium* spp., *Trichoderma* spp., *Ampelomyces* spp., *Candida* spp. and *Coniothyrium* spp. (Vinale *et al.,* 2008). *Trichoderma* species are usually considered soil

borne organisms associated with the roots of plants (Bailey *et al.,* 2008) and in 1932 R. Weinding demonstrated the effectiveness of *Trichoderma* spp. as a biological control agent against pathogens such as *Rhizoctonia solani* (Chet, 1990).

Greenhouse tests conducted by Al-Jedabi (2009) demonstrated that sorghum root rot caused by *F. oxysporum* was successfully controlled by *T. harzianum* and *T. viride* by up to 80%, compared to the control that displayed 100% root rot, with the majority plants either stunted or dead. This can probably be attributed to the ability of *T. harzianum* and *T. viride* to colonize the roots of sorghum effectively and inhibit the infection of roots by *F. oxysporum* (Al-Jedabi 2009).

4.0 ROOT ROT RESISTANCE IN SORGHUM

Plants may appear to be easy, immobile targets that are vulnerable to attack by microorganisms, but they have defence mechanisms that can be activated for protection against pathogen invasion. The hypersensitive reaction is one of the most efficient host defence systems against pathogens and/or stresses and is correlated with metabolic alterations which obstruct further penetration of tissues by pathogens or alleviate stress. This includes an assortment of novel proteins and secondary metabolites (Jwa *et al*., 2006).

Although the presence of all kinds of plant metabolites was only recently discovered, humans have been utilizing them for economical gain and health issues throughout history without even knowing it. Dyes for clothing, medicines and even poisons for arrows that aided food gathering were all collected from plants (Waterman and Mole, 1994).

Secondary metabolites are normally referred to as metabolites produced that are not required for normal growth and development. These metabolites allow the plant to survive and persist under certain conditions and can give the plant unique colours,

poisons or aromas and other compounds that can either deter or attract other organisms that give them a fair chance in nature to compete and survive (Stern, 2003).

4.1 *Phenols*

Phenolic compounds mainly consist of an aromatic ring that contains a variety of substituent groups, for instance carboxyl, methoxyl and hydroxyl groups and sometimes other non-aromatic ring structures (Waterman and Mole, 1994; Salisbury and Ross, 1992). It is important to state that not all hydroxyl groups are phenolic. Some that bond to non-aromatic ring structures or non-cyclic structures, for instance cholesterol and ethanol, do not have the properties of a phenol (Waterman and Mole, 1994).

Phenolic compounds are found in all sorghum cultivars, with only the concentration differing. Three major classes can be distinguished in sorghum, ie. flavonoids, tannins and phenolic acids (Hahn *et al.*, 1984). These compounds are used to classify sorghum due to their effect on the appearance, colour and nutritional quality of sorghum (Hahn *et al.,* 1984).

Studies have shown that phenolic compounds are associated with plant resistance to pathogenic attack. The accumulation of phenols (possibly flavones) in the surrounding tissues of lesions caused by *Colletotrichum graminicola* differed between resistant and hypersensitive-resistant maize. These phenolic compounds were toxic to the fungus and this suggested that phenols were involved in the resistance of maize towards *C. graminicola* (Hammerschmidt and Nicholson (1977). Phenolic compounds extracted from grape seeds (Baydar *et al.,* 2004) also had inhibitory effects on *Bacillus subtilis*, *B. brevis*, *B. cereus*, *B. megaterium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Enterobacter aerogenes* and other bacteria. The main component in the grape seed extract appeared to be gallic acid. In sorghum, a relationship between total sugars and phenols in sorghum roots and the first internodes and *M. phaseolina* infection was recorded with resistant genotypes having 2 - 3 times higher levels than susceptible ones

(Anahosur and Naik, 1985; Patil *et al.,*1985). Measurement of sugars and phenol levels could assist in identifying sources of resistance.

4.2 *Proteins*

Studies have divided PR proteins into 17 families based on serological relationships, sequence of amino acids and biological activities (Jwa *et al*., 2006). The most outstanding PR proteins are the chitinases, belonging to the PR3, 4, 8 and 11 families that play a major role in defence due to their ability to restrict the growth of many fungi and accumulate around hyphae of the fungi (Jwa *et al*., 2006). Two mechanisms in which chitinase operate against pathogen attack include the discharge of pathogen cell wall fragments containing oligosaccharides, which in turn induce defence responses in plants and by interfering with the synthesis of pathogen cell wall polysaccharides including β-1,3-glucans and chitin (Huang and Backhouse, 2006).

In sorghum seedlings a high diversity of chitinase can be found, and this includes forms naturally occurring in the seedlings and others induced by pathogenic attack (Huang and Backhouse, 2006). Studies performed by Huang and Backhouse (2006) showed that infection by *Fusarium thapsinum* and *F. proliferatum* increased the levels of chitinase activity, particularly in the roots of sorghum and disease expression was a factor of the extent of chitinase activity.

Three proteins of 18, 26 and 30 kDa were identified in sorghum that affected the hyphae growth of *Fusarium* spp. The 18 kDa protein resulted in sloughing of cell wall polysaccharides, whereas 26 and 30 kDa proteins cause leakages of cytoplasmic fluids (Sunitha and Chandrashekhar, 1994 as cited by Waniska *et al*., 2002), however intense studies of anti-fungal proteins in sorghum are still required.

5.0 CONCLUSION

Sorghum has been around for thousands of years and is well-adapted to dry and hot conditions as a result of its morphology. Because of this drought-resistant factor, sorghum is more economical to cultivate compared to other crops, especially in rural communities that rely on sorghum as a staple food.

Soilborne pathogens contribute to yield loss and the soilborne diseases that occur are usually the result of interactions among the pathogens, sorghum and all the biotic and abiotic factors in their respective environments. When one or more of these factors becomes unstable, for instance as a result of human interference, an opportunity for the pathogen to cause disease is created. A good understanding of all the processes involved between the sorghum plant, microbiota found in the soil and the cultural practices that can assist or hinder these interactions is required for controlling or managing soilborne diseases caused by plant pathogens. Certain agricultural practices can alter the soil environment, indirectly or directly, thereby creating conditions that are less favourable for pathogens to survive and attack plants or by just moving them about and in the process reduce the incidence of disease.

Chemical control is not always effective in controlling soilborne pathogens and because of its negative impact on the environment, for instance, loss of non-target beneficial organisms, groundwater pollution, and the development of resistant pathogens (Dubey *et al.,* 2007), other control options are needed. For these reasons biological control is preferred whereby pathogens are managed by making use of the natural suppressiveness of soil to disease. Natural suppressiveness is usually the result of microbiota that compete against pathogens for living space and nutrients. But perhaps more important is to know which agricultural practices reduce or enhance the disease suppressiveness of soils and applying them. Recognizing and understanding the dynamics of the soil environment in which root rot occurs, is therefore a very important factor in controlling this sorghum disease.

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CHAPTER 2

PATHOGENS ASSOCIATED WITH SORGHUM ROOT ROT

Abstract: Sorghum root rot has been associated with *Fusarium* spp., *Pythium* spp., *Macrophomina phaseolina*, *Colletotrichum graminicola* and *Periconia circinata* and a number of other fungi. A study was conducted to identify fungi from sorghum roots in South Africa. Plants were randomly collected from sorghum plots at Cedara, Betlehem, Klerksdorp, Greytown, Senekal and Winterton for the isolation of potential pathogens. Sixty cultures representing colony and growth variation with *Fusarium oxysporum* and 41 cultures representing other root colonizers, were selected for pathogenicity tests on two cultivars, PAN 8420 and PAN 8706W in the greenhouse. Root rot severity was assessed as the percentage root discolouration and ranged from 21.83 - 37.56% in PAN 8420 and 15.26 - 30.05% in PAN 8706W due to *F. oxysporum*, while other fungi tested yielded root rot severities in the range of 17.70 - 57.72% in PAN 8420 and 22.00 - 45.33% in PAN 8706W. Ergosterol, extracted as an indicator of fungal colonization of roots, ranged from 0.00011 - 0.00637 µg/g in PAN 8420 and 0.00008 - 0.00465 µg/g in PAN 8706W with *Fusarium oxysporum* isolates and 0.00031 - 0.00818 µg/g in PAN 8420 and 0.00066 - 0.01366 µg/g in PAN 8706W with the other fungal isolates tested. These results also indicated that some isolates were able to colonize the root tissue effectively but without producing visible symptoms on the roots or causing growth reductions. The test fungi were identified through sequencing and included *Fusarium* spp., *Alternaria* spp., *Phoma* spp., *Acremonium strictum*, *Curvularia trifolii* and *Colletotrichum capsici*.

Keywords: sorghum, root rot, *Fusarium oxysporum*, pathogenicity, ergosterol

2.0 INTRODUCTION

Little is known about sorghum root rot due to the absence of obvious symptoms in the aerial canopy. It is for this reason that little attention has been given to sorghum roots

and the seriousness of the disease only becomes apparent when damage to the roots is so severe that wilting or premature death of the plant occurs (Tarr, 1962).

Many severe diseases of sorghum are caused by fungi and the principle organisms associated with root rot of sorghum include the soilborne fungi, *Fusarium* spp., *Pythium* spp., *Macrophomina phaseolina*, *Colletotrichum graminicola* and *Periconia circinata* (Mughogho, 1984). Root rot is usually the effect of a combination of these (Tarr, 1962) and other pathogens such as *Rhizoctonia* spp., *Alternaria* spp., *Acremonium* spp., *Sclerotium rolfsii*, *Erwinia* spp., *Epicoccum* spp., and *Nigrospora* spp. (Reed *et al.,* 1983). The nature of the complex depends on the host and/or the environment that determine which pathogens occur by favouring a particular pathogen at a specific time or host growth stage. An example is *Fusarium* spp. vs. *M. phaseolina*, where low soil temperatures favour the infection of roots with *Fusarium* spp. and high soil temperatures favour infection with *M. phaseolina* (Mughogho and Pande, 1984).

The sizes of lesions associated with root rot pathogens can differ, from small circular spots covering the root surface or stripes extending over most of the root surface. These lesions are usually purple to red in colour, depending on the host genotype, although light brown or black discolourations may also occur (Claflin, 2000).

Sorghum rootlets become infected by *Fusarium* spp. before plants reach maturity but only show signs of infection after maturity is reached (Giorda *et al.*, 1995). Subsequently, discolouration of the roots and a reduction in plant growth occur, followed by poor grain fill that finally result in yield loss.

The absence of visible symptoms when infection is initiated often results in visual ratings of plant tissue not sufficiently indicating the degree of fungal invasion, even in two adjacent and similar samples. Ergosterol analysis has been applied as a measure of pathogen infection. Ergosterol is a stable ester molecule commonly found in conidia and mycelial fragments of microscopic fungi. Analysis of this molecule is mainly used to determine colonization of cereals and soils by fungi. Ergosterol degrades when exposed

to UV light, but can last up to two months when protected from light (Robine *et al*., 2005).

In this study root rot of sorghum was examined to identify fungi associated with the disease and to determine whether ergosterol accumulation could be used as a reliable measure of root rot colonization by the pathogen.

2.1 MATERIALS AND METHODS

2.1.1 *Fungal isolation and identification*

2.1.1.1 *Fungal isolation*

Sorghum plants were randomly selected from experimental plots at Cedara (previously planted to sorghum), Cedara (not previously planted to sorghum), Bethlehem (Courtesy of ARC-Grain Crops Institute, Potchefstroom), Klerksdorp, Greytown (Courtesy of Pannar Seed Co.), and commercial fields at Senekal and Winterton. The roots were carefully removed from the soil and washed in running tap water to remove adhering soil. Roots were cut into 1 cm segments. These were surface sterilized for 3 min in sodium hypochlorite, rinsed three times with sterilized water and five segments were placed onto Petri dishes containing half strength malt extract agar (BioLab) and PCNB agar (Leslie and Summerell, 2006). Ten to 15 Petri dishes of each medium were used per sample. Petri dishes were incubated near UV light at 25°C. After 2 - 4 days fungal colonies were transferred to, and maintained on potato dextrose agar (PDA, BioLab) (Dubey *et al.,* 2007).

2.1.1.2 *Fungal Identification*

2.1.1.2.1 Single hyphal tip cultures

Pure cultures were obtained from single hyphal tips. Hyphal tips were cut from 1 - 3 day-old cultures (depending on growth rate of fungi) on PDA (BioLab), using a stereomicroscope and placed on fresh PDA. The colonies were grown near UV light until the whole Petri dish was covered in thick mycelium. The mycelium was scraped off the culture and placed in a 2 ml Eppendorff tube and kept frozen at -80°C until further use.

2.1.1.2.2 CTAB DNA extraction

Freeze dried fungal hyphae were ground into a fine powder on a Tissue-lyzer (TissueLyserII). CTAB buffer (750 µl) was added to each sample and tubes were incubated for an hour at 65°C. Chloroform/isoamylalcohol (24:1 v/v) (500 µl) was added and the suspension was mixed well. The samples were centrifuged at 12000 g for 10 min at 4°C and the aqueous phase was removed and added to 500 µl isopropanol in a clean, 1.5 ml eppendorff tube to precipitate the DNA present. These were incubated for 20 min at room temperature and centrifuged at 12000 g for 10 min at 4°C. The supernatant was discarded using a water jet-pump. The pellet was washed by the addition of 500 µl ice-cold 70% (v/v) ethanol and incubated for 20 min at room temperature. The samples were centrifuged at 12000 g for 5 min at 4°C and all liquid was removed and the pellets were air-dried for one hour at room temperature. The pellets were resuspended in 200 µl TE buffer and left overnight at 4°C.

RNase A (10 mg/ml) (2 µl) was added to each sample and incubated at 37°C for two hours. DNA was extracted with 20 µl 7.5 M ammonium acetate and 200 µl chloroform/isoamylalcohol (24:1 v/v) and mixed well. These were centrifuged at 12000

g for 10 min at 4°C and the aqueous phase was removed and added to 500 µl ice-cold 100% (v/v) ethanol to precipitate the DNA present overnight at -20°C.

The samples were centrifuged at 12000 g for 15 min at 4°C. Any liquid was removed carefully (some of the pellets could become loose) and the pellets were washed twice with 500 µl ice-cold 70% (v/v) ethanol by centrifuging them at 12000 g for 10 min at 4°C. All liquid was removed and the pellets were air-dried until all the ethanol had evaporated. The pellets were dissolved in 50 µl TE at 37°C for two hours and stored in the freezer at -20°C.

2.1.1.2.3 PCR reaction

The concentrations of the DNA extractions were calculated from the appearance of the DNA on agarose gel viewed under UV light and dilutions were made so that each sample contained the same amount of DNA of approximately 4 ng/µl. Polymerase chain reaction (PCR) was conducted with each sample. The DNA was amplified using: 10 ng template, 10 pmol of each primer and 1 x concentration of KAPA ready mix (KAPA Biosystems). Firstly the Ef 1 and Ef 2 primer set, to amplify elongation factor gene, were added to the sample. The reaction conditions were as follows: An initial denaturation step at 94°C for 1 min, followed by 30 cycles of 94°C for 30 sec, 61.2°C for 15 sec, 72°C for 1 min. A final elongation step at 72°C for 5 min was included.

The products were observed on agarose gel. The samples that did not amplify with the Ef primer set were redone with ITS 1 and ITS 4 primer set, to amplify internal transcribed spacer gene, under the same reaction conditions as mentioned above and the amplified products observed on agarose gel.

This PCR product was amplified using ABI PRISM® Big Dye® Terminator V 3.1 Cycle Sequence Kit (Applied Biosystems). This consisted of ca 20 ng template, 3.2 pmol primer, 1 x sequence buffer and 1/8 sequence ready reaction mix (final concentration).

The conditions of the sequence reactions were as follows: An initial denaturation step at 96°C for 1 min, followed by 25 cycles of 96°C for 10 sec, an annealing step for 5 sec (variable temperature for each primer used: 61.8°C for Ef 1, 58.3°C for Ef 2, 56°C for ITS 1 and 58.3°C for ITS 4) and 60°C for 4 min.

To clean up the sequencing PCR product, the products were placed in a 96-well plate and the following were added: 10 µl water, 5 µl EDTA and 60 µl 100% ethanol (ice-cold). The plate was sealed with an ultraclear film and touch-vortexed briefly. The plate was incubated at -20°C for 5 min and centrifuged for 80 min at 4°C and 3000 g. The film was removed and plate was placed upside down on a paper towel to remove the supernatant. The plate was centrifuged upside down very quickly at 4°C and 150 g to remove all excess liquids. The PCR product were washed by adding 200 µl 70% ethanol (ice-cold) to each well and the film was replaced and centrifuged for 20 min at 4°C and 3000 g. The film was removed and the plate was turned upside down on a paper towel to remove the supernatant. The plate was centrifuged upside down at 4°C and 150 g for just a few seconds. The product were washed again by the addition of 200 µl 70% ethanol (ice-cold) to each well and centrifuged for 20 min at 4°C and 3000 g. The film was removed and plate was placed upside down on a paper towel. The plate was centrifuged upside down very briefly. The plate was heated in a cycler for 30-60 sec at 95°C with the lid of the cycler left open. A new film was placed on the plate.

2.1.1.2.4 *Sequencing*

The plate was send to the department of Microbiology at the University of the Free State for sequencing. The sequences were received and the reverse sequences were turned around with http://bioinformatics.org/sms/rev_comp.html. The forward and reverse compliment sequences were compared by aligning them with http://www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html. Any mistakes were repaired and the samples were blast against a GenBank and identified using http://isolate.fusariumdb.org/index.php and http://blast.ncbi.nlm.nih.gov/Blast.cgi

2.1.2 *Pathogenicity tests:* **Fusarium oxysporum**

2.1.2.1 *Inoculum production*

Sixty cultures of *F. oxysporum* grown on PDA (BioLab), from an initial collection of approximately 400 isolates from sorghum roots, were selected based on variation in growth characteristics and culture morphology. Two hundred grams of commercial sorghum grain in 300 ml distilled water in glass jars were autoclaved for 20 min on two consecutive days. Three 2 x 3 cm blocks were cut from PDA cultures of the test fungi and aseptically placed in the bottles containing the sorghum grain. Bottles were maintained at 25 °C. The bottles were hand shaken every week, to ensure even growth of the isolates on the grain. Four weeks later the inoculum was air dried on the laboratory bench for a week and ground to a fine powder (Idris *et al.,* 2007).

2.1.2.2 *Greenhouse tests*

Ninety g of inoculum and 3 ℓ steam pasteurized soil were mixed to give a 3% inoculum ratio. The soil was divided into six 500 g pots per inoculum treatment and the pots were placed in the greenhouse at a 25/22°C regime. PAN 8706W (white tan cultivar) and PAN 8420 (red cultivar) seeds were heat sterilized 8 times in water at 60°C for 2 min. This also served to remove the commercial fungicide treatment. Seeds were pre-germinated for three days on water agar (BioLab) and eight seeds of each cultivar were planted into three pots per treatment. Pots were watered daily with tap water. Six weeks after planting plants were assessed for root rot development. The plants were carefully removed from pots and washed with running tap water, to recover as much of the root system as possible. Root rot severity was visually assessed for percentage root discolouration and root volume was measured using water displacement (McLaren, 2002). Data were analysed using NCSS (Hintze, 2001) by means of Analysis of Variance and means separation was done using Fischer's LSD ($P<0.05$).

2.1.3 *Pathogenicity tests: Miscellaneous root isolates*

2.1.3.1 *Inoculum production*

Forty-one cultures representing all fungi isolated from sorghum roots were selected for pathogenicity testing. The above method was followed, except that 200 g wheat seeds where used instead of sorghum seeds for inoculum production.

2.1.3.2 *Greenhouse tests*

The above method of soil inoculation, planting and root rot assessment was used.

2.1.4 *Ergosterol quantification*

Ergosterol was extracted from the roots of PAN 8706W and PAN 8420 as a component of pathogenicity tests. The roots were ground into a fine powder in liquid nitrogen. The amount of powder recovered was recorded. Ergosterol was determined using the modifed method of Jambunathan *et al.* (1991). Ten g of powder of each test sample was added to 50 ml of methanol and mixed with a magnetic stirrer in a 100 ml beaker for 30 minutes. Where the recovered root mass was less than 10 g the entire sample was used. The mixture was allowed to settle and 25 ml of the clear extract was added to a 50 ml test tube with a screw cap, containing 3 g of potassium hydroxide (KOH). The mixture was stirred in a vortex mixer (Vortex Genie 2, Scientific Industries) to dissolve the KOH. Ten milliliter of n-hexane was added and the mixture was incubated for 30 min in a water bath at 75°C and allowed to cool to room temperature. During cooling test tubes were covered with foil to prevent ergosterol degradation as ergosterol is light-sensitive and degrades when exposed to UV light (Robine *et al*., 2005). Five ml distilled water was added, mixed in a vortex mixer and the hyperthermal reaction was cooled to room temperature. The upper hexane layer was transferred to a glass test tube. Ten milliliter

n-hexane was added to the remaining aliquot in the screw-cap test tube and mixed well. The hexane layer was again removed and added to the earlier aliquot. This step was repeated. The hexane extract was evaporated in the glass test tube until dry in a water bath at 75°C. The residue was resuspended in 5 ml methanol and filtered through 0.45 µl syringe filter. The filtrate was analyzed on a Shimadzu high performance liquid chromatograph (HPLC) with a SIL-20A auto sampler. The extract was loaded onto a reverse phase column (C18 125 A 10 µm particle size, 150 x 4.6 mm) at 50°C. The mobile phase was methanol:water (96:4) at a flow rate of 1.2 ml/min. Standard ergosterol (Sigma) was used to calibrate the equipment. Ergosterol was determined from the peak area at a retention time of approximately 7 min.

2.2 RESULTS

2.2.1 *Fungal isolation and identification*

Sequencing confirmed the identification of most of the fungi isolated from sorghum roots (Table 2.1). Twelve isolates appear to be unmapped species while five isolates could only be partially identified. These data suggest that many fungi on sorghum roots are new species that require further study and analysis. Fully identified isolates included *Acremonium strictum, Alternaria alternata, Arthrinium phaeospermum, Colletotrichum capsici, Curvularia trifolii, Epicoccum nigrum, Fusarium equiseti, F. oxysporum, F. solani, F. temperatum, Gibberella thapsina* (*F. thapsinum*), *Penicillium verruculosum, Periconia macrospinosa, Phoma macrostoma, Phoma sorghina* and *Peyronellaea pomorum (Phoma pomorum)*. Sequencing also confirmed that all the selected isolates in the *F. oxysporum* pathogenicity study were correctly identified.

2.2.2 *Pathogenicity tests:* **Fusarium oxysporum**

Control pots yielded a relative high root rot severity based on visual estimates (27.67% in PAN 8420 and 20.61% in PAN 8706W), suggesting that the level of soil pasteurization may not have been sufficient to suppress or eliminate soil fungal activity or that other factors may have been involved. This may have confounded the pathogenic activity of the test fungi. Root rot caused by *F. oxysporum* isolates did not differ significantly ($P<0.05$), even though root rot did occur on all the sorghum roots, within the range of 21.83 (PCed9-2) - 37.56% (CedNie 7-2) in PAN 8420 and 15.26 (Kl26-1) - 30.05% (CedNie27-1) in PAN 8706W (Table 2.2). Compared to the control, the lower root rot caused by isolate PCed9-2 on PAN 8420 and Kl26-1 on PAN 8706W may also suggests that some isolates are capable of suppressing root rot development. There was a significant ($P<0.05$) difference between the two sorghum cultivars tested, which indicated that the cultivar PAN 8706W is more resistant to root rot caused by *F. oxysporum* than PAN 8420. No significant interaction was recorded between the cultivars and the isolates.

As with root rot, mesocotyl rot was high in the control pots. Mesocotyl rot ranged from 49.61 (PCed9-2) - 91.33% (Grey6-5) in PAN 8420 and 21.89 (Kl8-1) - 72.89% (Grey25-3) in PAN 8706W (Table 2.2) and significant ($P<0.05$) differences between isolates were recorded. This implies that mesocotyl tissues are more susceptible to colonization by *F. oxysporum* than the root tissues. A significant ($P<0.05$) difference between the two sorghum cultivars was recorded, which indicate that the mesocotyl tissues of PAN 8706W is more resistant to infection by *F. oxysporum* than those of PAN 8420. The Cultivar x Isolate interaction was also significant ($P<0.05$) indicating that mesocotyl tissues in the cultivars differ in susceptibility to the different isolates.

Significant ($P<0.05$) differences in shoot growth was recorded, which ranged from 13.20 (Control) - 29.17 cm (CedNie5-1) in PAN 8420 and 13.54 (Control) - 32.81 cm (Kl25-3) in PAN 8706W (Table 2.3). Control plants yielded the smallest shoot growth, suggesting growth stimulation by infection with *F. oxysporum*. A significant interaction was

observed between isolates and cultivar, suggesting that cultivars differ in the extent to which growth is induced by isolates. Similarly a significant (P<0.05) difference in root growth with isolates was recorded which ranged from 9.49 (CedNie7-2) - 15.46 cm (Kl6-1) in PAN 8420 and 9.34 (Grey6-5) - 17.04 cm (Sen10-1) in PAN 8706W. This suggested that different isolates of the same species could have different growth stimulation or inhibition effects.

Regression analysis did not reveal a relationship between root or mesocotyl rot severity and plant growth parameters, suggesting that visual estimates of physical damage to roots is not directly related to a reduction in plant growth.

2.2.3 *Pathogenicity tests: Miscellaneous root isolates*

All the isolates tested had a significant effect on root rot which ranged from 17.70 Control) - 57.72% (Bet9-3 - unidentified) in PAN 8420 and from 22.00 (1/2Ced10-1, unidentified) - 45.33% (Bet9-3 - unidentified) in PAN 8706W (Table 2.4). There was a significant (P<0.05) difference between the two sorghum cultivars tested, indicating that cultivar PAN 8706W is more resistant to root rot caused by the test pathogens than PAN 8420. Significant interactions between cultivars and isolates were recorded, indicating that the cultivars reacted differently to different isolates tested.

Significant differences were recorded with mesocotyl rot, which ranged from 62.42% in the Control - 99.00% with both PBet40-1 (*Alternaria alternata*) and 1/2Ced1-2 (*Curvularia trifolii*) in PAN 8420 (Table 2.4) and from 62.03% with Wint9-2 (*Fusarium temperatum*) - 95.50% with 1/2Ced12-4 (*Phoma macrostoma*) in PAN 8706W (Table 2.4). The high levels of rot in mesocotyl tissues indicate greater susceptibility to rot than root tissues. Mesocotyl tissue of PAN 8420 was more susceptible than mesocotyl tissue of PAN 8706W with a significant (P<0.05) difference between cultivars. There was a significant Cultivar x Isolate interaction indicating that mesocotyl tissue differs in susceptibility to the different isolates.

A significant difference was observed between shoot growth associated with infection by the respective test isolates and this varied from 17.61 cm (Control) - 47.13 cm (Kl20-1B, unidentified) in PAN 8420 (Table 2.5) and from 22.80 cm (1/2Ced10-1 - unidentified) - 56.08 cm (Bet9-3 - unidentified) in PAN 8706W (Table 2.5). A significant ($P<0.05$) interaction was observed between isolates and cultivar, suggesting that cultivars differ in induced or reduced growth caused by the isolates. Similarly root growth was significantly ($P<0.05$) affected by isolates within the range 7.52 cm (Control) - 25.92 cm (PBet40-1 - *Alternaria alternata*) in PAN 8420 (Table 2.5) and from 7.30 cm (1/2Ced10-1 - unidentified) - 18.33 cm (Sen14-2 - unidentified) in PAN 8706W (Table 2.5). Results indicated different effects by the different species of isolates by either stimulating or reducing the growth of plants. It is noteworthy that isolates identified as *Curvularia trifolii* resulted in no emergence of plants, hereby indicating its ability to prevent seed germination, emergence or plant growth. An exception was isolate 1/2Ced1-2 on PAN 8420 which suggests isolate differences as well as Cultivar x Isolate interactions.

Significant ($P<0.05$) differences were recorded with root volume as a result of infection, which ranged from 0.07 (1/2Ced10-1 - unidentified) - 2.48 ml (Sen14-1 - *Colletotrichum capsici*) for PAN 8420 and 0.07 (1/2Ced10-1 - unidentified) - 1.98 ml (Sen25-1-*Fusarium solani*) for PAN 8706W.

2.2.4 *Ergosterol quantification*

Significant ($P<0.05$) differences were recorded between ergosterol levels in roots infected by the respective *F. oxysporum* isolates which ranged from 0.00011 (Control) - 0.00637 µg/g (Grey6-5) in PAN 8420 and from 0.00008 (Wint20-1) - 0.00465 µg/g (Kl25-3) in PAN 8706W (Table 2.6), hereby suggesting that cultivar PAN 8706W is more root rot resistant due to the inhibition of successful colonization of *Fusarium oxysporum* root rot isolates.

Significant (P<0.05) differences were recorded between ergosterol levels in roots infected by miscellaneous root isolates which ranged from 0.00031 µg/g (1/2Ced10-1 - unidentified) - 0.00818 µg/g (Bet26-4 - *Acremonium strictum*) in PAN 8420 (Table 2.7) and from 0.00066 µg/g (1/2Ced10-1 - unidentified) - 0.01366 µg/g (Bet26-4 - *Acremonium strictum*) in PAN 8706W (Table 2.7), suggesting that cultivar PAN 8420 is more root rot resistant when it comes to colonization by root rot fungal isolates.

2.3 DISCUSSION

Soilborne fungi associated with sorghum seedling and root rots of sorghum include *Colletotrichum graminicola*, *Macrophomina phaseolina*, *Periconia circinata*, *Pythium* spp. and *Fusarium* spp. that may occur in complexes with one another or other pathogens including *Alternaria* spp., *Sclerotium rolfsii*, *Acremonium* spp., *Rhizoctonia* spp., *Nigrospora* spp., *Erwinia* spp., *Phoma sorghina* and *Epicoccum* spp. (Tarr, 1962; Reed *et al.,* 1983; Mughogho, 1984; Zummo, 1984). *F. solani* and *F. equiseti* have been isolated from diseased root and stalk tissue of sorghum (Claflin, 2000), although *F. equiseti* has also been identified as a plant growth-promoting fungus and suppressive organism against crown and root rot of some crops (Horinouchi *et al.,* 2008). *Penicillium* spp. are able to cause seed damage and reduce seedling emergence of maize (Chambers, 1987) while anthracnose stalk rot is caused by *Colletotrichum graminicola* (Wicklow *et al.,* 2009). *Acremonium strictum* is a particularly destructive isolate that has been previously isolated from stems and other parts of wilted sorghum plants, where it colonizes the plants systemically (Bandyopadhyay, 1987). Although many of these species were identified in the current study, fungi such as *C. graminicola* and *M. phaseolina* were not recorded. Many species not previously recorded on sorghum were isolated, including *C. capsici* and *Curvularia trifolii*.

The numerous unidentified fungal species from sorghum roots is a concern, particularly in view of the high levels of root rot recorded in pathogenicity trials with a number of these isolates. The implication is that much of the etiology of sorghum root rot requires

elucidation. Concern, too lies in the species identification, for example *Curvularia lunata* that is regarded as a common colonizer of sorghum was not recorded but a number of isolates of *C. trifolii* were positively identified. Falloon (1975) showed that *C. trifolii* causes severe disease on turf grasses. Similarly, *Colletotrichum graminicola*, supposedly common on sorghum roots, was not recorded but *C. capsici* was found in the current study. These apparent anomalies raise the question as to whether previous studies have incorrectly identified the pathogens or whether the local etiology differs from that reported in the literature. Nonetheless, these data suggest that the entire etiology of sorghum root rot locally needs to be revisited and that the assumptions based on previous studies may not be valid under local sorghum production systems.

In pathogenicity trials, root rot differed significantly ($P<0.05$) on both cultivars tested (Table 2.2; Table 2.4). Both cultivars showed root rot symptoms when infected with *Fusarium oxysporum*, but analyses of variance ($P<0.05$) indicated that isolates did not differ significantly from one another. Infection levels with these isolates were also lower than those caused by isolates in the "miscellaneous root isolates" trial and the importance in the root rot complex remains questionable. Studies conducted by Tesso *et al.* (2010) indicated that *Fusarium thapsinum* was the most virulent pathogen among all the *Fusarium* spp. tested in field and greenhouse studies. Isolate Kl17-1 (*Gibberella thapsina*) had the highest root rot severity of 34.45% compared to other *Fusarium* spp tested. In the current study Bet9-3 (unidentified) had the highest root rot severity on both cultivars tested and was also among the highest root ergosterol levels recorded on PAN 8420. This add further weight to the suggestion that many unidentified fungi may be responsible for root rot and may in fact be more important than the so-called "known" pathogens. The high level of damping-off recorded with *C. trifolii* in the current study where no emergence was recorded, is an indication that pathogens not previously associated with roots of sorghum, may have severe consequences. Chambers (1987) found that seedling emergence of maize was only 95.2% when maize was planted in pots infested with *C. brachyspora* and 89.3% in pots with *C. clavata* and this is a fungal group that may warrant further investigation on the consequences of these on root health and plant production.

The evaluation of root rot in the current study was to some extent confounded by the high level of visible root discoloration in control pots. However, when ergosterol analyses were considered, control treatments yielded the lowest ergosterol content in roots and thus questioned the reliability of root discolouration as a root rot evaluation criterion. Fungal infection of sorghum and stress elicit the production of 3-deoxyanthocyanidins, an anthocyanin-related compound, which serve as phytoalexins (Nicholson and Hammerschmidt, 1992; Snyder and Nicholson, 1990; Snyder *et al.,* 1991). These compounds are responsible for the pigmentations of infected tissues in sorghum. This extent of pigmentation is dependent on the elicitor, i.e. the pathogen or stress factor. Hence the question needs to be raised as to whether the measured response to pathogens in the current study using root discolouration, is a suitable measure of pathogenicity and thus colonization of roots or whether the observed discolourations are an indication of host resistance response to infection by the specific isolates.

Egosterol is an indicator of fungal biomass and has been used successfully in the quantification of colonization of sorghum grain by grain mold pathogens (Jambunathan *et al.,* 1991). The significant differences recorded with ergosterol as an indicator of root colonization, does in fact indicate significant ($P<0.05$) differences in the ability of *F. oxysporum* isolates to colonize roots, which is contrary to the conclusion when recording pathogenicity as a factor of root discolouration. Similarly, the significant Isolate x Cultivar interaction with ergosterol further supports the notion of differential colonization of roots. This is supported by findings of McLaren (1987), where pathogenicity was complicated by variation between isolates of the same species of *F. oxysporum* that resulted in significant differences between primary root and mesocotyl discolouration.

Isolate B26-4 (*Acremonium strictum*) is another case in point. This isolate only caused 28.83 and 26.89% root discolouration on PAN 8420 and PAN8706W respectively, significantly lower than many of the other isolates tested, but resulted in the highest ergosterol content. The contrast between root colonization as indicated by root ergosterol content and root discolouration may be due to a suppressed phytoalexin response as opposed to reduced disease, hence reduced root discolouration. The latter result also

corresponds with the finding of Bandypadhyay (1987) who found this pathogen to be serious cause of root and stalk rot and wilting in sorghum. These data indicate that the criteria used to assess root rots in sorghum need to be re-evaluated but also that ergosterol may be a better indicator of a pathogen's ability to colonize roots.

In this study plant growth parameters associated with inoculation by the different fungi differed significantly ($P<0.05$) (Table 2.3; Table 2.5). These varied from growth suppression to growth stimulation. Growth variations were however, not related to either root or mesocotyl discolouration or root ergosterol content suggesting that host growth is dictated by more than host tissue colonization or physical damage. Fungal metabolites may be involved. De Melo and Piccinin (1999) found that culture filtrates of *F. oxysporum,* when added to plant growth medium could be used to identify plants with resistance to the pathogen based on plant growth inhibition. Phytohormones have been associated with strains of *F. culmorum* (Manka, 1980; Mitter *et al.,* 2001) which may have a growth stimulating effect. Jaroszuk-Scisel *et al.* (2008) recorded different responses in rye with isolates of *F. culmorum* where one isolate stimulated growth and the other suppressed growth. Fungal metabolite effects on plant growth were not considered here and may be another area that needs elucidation.

2.4 CONCLUSION

Root rot is caused by a complex of soilborne fungi, including *Pythium* spp., *Fusarium* spp., *Colletotrichum graminicola*, *Macrophomina phaseolina* and *Periconia circinata* as well as a range of fungi that still need to be identified. In this study pathogenicity tests were conducted to determine potential pathogens associated with root rot diseases in sorghum. However, results are inconclusive but have highlighted many areas that need to be addressed to improve the assessment of pathogenicity. Ergosterol analysis as an indication of total fungal biomass in roots, may be a useful tool to quantify the colonization ability of isolates. However, additional criteria such as the inclusion of fungal metabolites and their effect on host growth should also be considered.

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Table 2.1 Identification of fungi isolated from sorghum roots randomly collected at seven localities in South Africa.

Fusarium oxysporum isolates						
Isolate	Isolate no.	Primer Set	Identification	Accession no.	E-Value	% Identity
1/2Bet16-1	1	Ef	*Fusarium oxysporum*	FD_01267_EF-1a	0	100%
PBet40-2	2	Ef	*Fusarium oxysporum*	GU165958.1	0	99%
Bet3-2	3	Ef	*Fusarium oxysporum*	FD_01268_EF-1a	0	100%
Bet5-2	4	Ef	*Fusarium oxysporum*	FD_01267_EF-1a	0	100%
Bet8-1	5	Ef	*Fusarium oxysporum*	FD_01267_EF-1a	0	100%
Bet13-2	6	Ef	*Fusarium oxysporum*	HM770735.1	0	99%
Bet14-2	7	Ef	*Fusarium oxysporum*	FD_00801_EF-1a	0	99%
Bet16-1	8	Ef	*Fusarium oxysporum*	HM770735.1	0	99%
Bet19-3	9	Ef	*Fusarium oxysporum*	HM770735.1	0	98%
Bet23-5	10	Ef	*Fusarium oxysporum*	HM770735.1	0	99%
Bet30-3	11	Ef	*Fusarium oxysporum*	FJ985393.1	3.00E-144	96%
PCed9-2	12	Ef	*Fusarium oxysporum*	GU199328.1	0	88%
CedNie4-1	13	Ef	*Fusarium oxysporum*	GU165951.1	0	100%
CedNie4-2	14	Ef	*Fusarium oxysporum*	GU165951.1	0	99%
CedNie5-1	15	Ef	*Fusarium oxysporum*	GU165951.1	0	100%
CedNie7-2	16	Ef	*Fusarium oxysporum*	FJ664911.1	0	92%
CedNie9-1	17	Ef	*Fusarium oxysporum*	DQ220098.1	0	100%
CedNie18-2	18	Ef	*Fusarium oxysporum*	EU313535.1	0	99%
CedNie 22-5	19	Ef	*Fusarium oxysporum*	GU165958.1	0	99%
CedNie25-3	20	Ef	*Fusarium oxysporum*	JN167177.1	4.00E-168	99%
CedNie26-1	21	Ef	*Fusarium oxysporum*	FJ985375.1	0	100%
CedNie27-1	22	Ef	*Fusarium oxysporum*	GU165951.1	0	100%
Ced27-4	23	Ef	*Fusarium oxysporum*	GU165958.1	0	99%
Grey6-5	24	Ef	*Fusarium oxysporum*	HQ114270.1	0	100%
Grey10-2	25	Ef	*Fusarium oxysporum*	GU165958.1	0	100%

Table 2.1 Continued

Fusarium oxysporum isolates						
Isolate	Isolate no.	Primer Set	Identification	Accession no.	E-Value	% Identity
Grey10-4	26	Ef	*Fusarium oxysporum*	GU165958.1	0	100%
Grey15-2	27	Ef	*Fusarium oxysporum*	GU250616.1	0	100%
Grey18-3	28		Unidentified			
Grey21-2	29	Ef	*Fusarium oxysporum*	HM770735.1	0	99%
Grey22-2	30	ITS	*Fusarium oxysporum*	HQ379658.1	0	99%
Grey22-5	31	Ef	*Fusarium oxysporum*	GU165958.1	0	99%
Grey23-3	32	Ef	*Fusarium oxysporum*	GU165958.1	0	99%
Grey25-3	33		Unidentified			
Grey27-4	34	Ef	*Fusarium oxysporum*	GU165958.1	0	99%
Grey27-5	35	Ef	*Fusarium oxysporum*	GU250616.1	0	100%
Kl6-1	36	Ef	*Fusarium oxysporum*	GU165958.1	0	100%
Kl8-1	37	Ef	*Fusarium oxysporum*	HM770735.1	0	99%
Kl20-1A	38	Ef	*Fusarium oxysporum*	HQ114270.1	0	100%
Kl25-3	39	Ef	*Fusarium oxysporum*	HQ114270.1	0	100%
Kl26-1	40	Ef	*Fusarium oxysporum*	DQ837696.1	0	99%
Kl26-2	41	Ef	*Fusarium oxysporum*	GU165958.1	0	100%
Sen2-1	42	Ef	*Fusarium oxysporum*	FN645739.1	1.00E-132	99%
Sen6-2	43	Ef	*Fusarium oxysporum*	HM770735.1	0	99%
Sen10-1	44	Ef	*Fusarium oxysporum*	HQ667161.1	0	96%
Sen11-1	45	Ef	*Fusarium oxysporum*	HM770735.1	0	99%
Sen12-1	46	Ef	*Fusarium oxysporum*	HM770735.1	4.00E-178	99%
Sen13-3	47	Ef	*Fusarium oxysporum*	GU165951.1	4.00E-143	96%
Sen22-3	48	Ef	*Fusarium oxysporum*	GU165951.1	0	99%
Sen25-2B	49	Ef	*Fusarium oxysporum*	GU165958.1	0	100%
Sen27-2	50	Ef	*Fusarium oxysporum*	GU165951.1	0	99%

Table 2.1 Continued

Fusarium oxysporum isolates						
Isolate	Isolate no.	Primer Set	Identification	Accession no.	E-Value	% Identity
Sen26-3	51	Ef	*Fusarium oxysporum*	HM770735.1	0	99%
Sen27-3	52	Ef	*Fusarium oxysporum*	HM770735.1	0	99%
Wint14-1	53	Ef	*Fusarium oxysporum*	HM770735.1	0	99%
Wint14-2	54	Ef	*Fusarium oxysporum*	GU250616.1	0	99%
Wint17-1	55	Ef	*Fusarium oxysporum*	HQ114270.1	0	99%
Wint17-2	56	Ef	*Fusarium oxysporum*	GU250593.1	0	100%
Wint19-1	57	Ef	*Fusarium oxysporum*	HQ114270.1	0	100%
Wint20-1	58	Ef	*Fusarium oxysporum*	EU313535.1	0	99%
Wint23-1	59	Ef	*Fusarium oxysporum*	GU250598.1	0	100%
Wint24-1	60	Ef	*Fusarium oxysporum*	GU250598.1	0	100%

Table 2.1 Continued

Miscellaneous isolates						
Isolate	Isolate no.	Primer Set	Identification	Accession no.	E-Value	% Identity
1/2Bet36-2	61	ITS	*Alternaria* sp.	FJ708614.1	0	95%
PBet39-1	62		Unidentified			
PBet39-2	63		Unidentified			
PBet40-1	64	ITS	*Alternaria alternata*	JF835906.1	0	100%
Bet1-2	65	Ef	*Fusarium solani*	FN689827.1	0	99%
Bet4-1	66	ITS	*Fusarium equiseti*	FR872729.1	0	100%
Bet8-2	67		Unidentified			
Bet9-3	68		Unidentified			
Bet26-4	69	ITS	*Acremonium strictum*	HM052811.1	4.00E-112	99%
Bet27-1	70	ITS	*Fusarium equiseti*	FR872729.1	0	100%
Bet28-1A	71	ITS	*Peyronellaea pomorum*	JN003244.1	0	100%
Bet28-1B	72	ITS	*Alternaria alternata*	JF835906.1	0	100%
Bet30-2	73		Unidentified			
1/2Ced1-2	74	ITS	*Curvularia trifolii*	JN712459.1	0	100%
1/2Ced3-2	75	ITS	Dothideales	HQ608008.1	0	100%
1/2Ced10-1	76		Unidentified			
1/2Ced12-4	77	ITS	*Phoma macrostoma*	DQ474111.1	0	100%
1/2Ced33-4	78	ITS	*Curvularia trifolii*	JN712458.1	0	100%
CedNie6-2	79	ITS	*Epicoccum nigrum*	JF311935.1	0	99%
CedNie6-4	80	ITS	Dothideomycete	EU680531.1	0	95%
CedNie20-4	81	ITS	*Curvularia trifolii*	JN712459.1	0	99%
CedNie21-1	82	Ef	*Fusarium oxysporum*	FJ664911.1	0	99%
Grey19-3	83	Ef	*Gibberella thapsina*	FJ603507.1	0	99%
Kl16-2	84	ITS	*Penicillium verruculosum*	HQ608025.1	0	99%
Kl17-1	85	Ef	*Gibberella thapsina*	GU116586.1	0	99%

Table 2.1 Continued

Miscellaneous isolates						
Isolate	Isolate no.	Primer Set	Identification	Accession no.	E-Value	% Identity
Kl 20-1B	86		Unidentified			
Kl 28-1	87	ITS	*Periconia macrospinosa*	GU586854.1	4.00E-153	100%
Sen 8-2	88	ITS	*Arthrinium phaeospermum*	HM222950.1	0	99%
Sen 14-1	89	ITS	*Colletotrichum capsici*	JF796244.1	0	100%
Sen 14-2	90		Unidentified			
Sen 17-5	91	ITS	*Colletotrichum capsici*	JF796244.1	0	100%
Sen 25-1	92	Ef	*Fusarium solani*	FN689826.1	0	99%
Sen 27-4	93	ITS	*Fusarium oxysporum*	GU934524.1	0	99%
Wint 5-5	94	ITS	*Fusarium equiseti*	HQ176430.1	0	99%
Wint 6-4	95		Unidentified			
Wint 7-2	96	ITS	*Fusarium* sp.	JN207347.1	0	99%
Wint 7-4	97		Unidentified			
Wint 8-4	98	ITS	*Phoma sorghina*	FJ427070.1	0	100%
Wint 9-2	99	Ef	*Fusarium temperatum*	HM067690.1	0	99%
Wint 10-5	100	ITS	*Fusarium* sp.	U61695.1	0	100%
Wint 21-1	101	Ef	*Fusarium temperatum*	HM067690.1	0	99%

Table 2.1 Continued

Trichoderma spp. isolates						
Isolate	Isolate no.	Primer Set	Identification	Accession no.	E-Value	% Identity
1/2Bethlehem 13-1	1		*Trichoderma* sp.			
Bethlehem 18-1	2		*Trichoderma* sp.			
Ced 3-1	3	ITS	*Trichoderma hamatum*	HQ292851.1	5.00E-139	92%
Ced 3-2	4	ITS	*Trichoderma hamatum*	HQ608116.1	0	99%
Ced nie 8-1	5		*Trichoderma* sp.			
Ced nie 10-3	6	ITS	*Hypocrea lixii (Trichoderma harzianum)*	JN228898.1	0	98%
Grey 9-6	7	ITS	*Trichoderma spirale*	FJ442622	0	99%
Kl 4-2	8		*Trichoderma* sp.			
Kl 8-3	9		*Trichoderma* sp.			
Kl 23-1	10	ITS	*Hypocrea virens* (*Trichoderma virens*)	JN228907.1	0	100%
Kl 30-1	11		*Trichoderma* sp.			
Sen 2-2	12	ITS	*Hypocrea lixii (Trichoderma harzianum)*	JF923806.1	0	98%
Sen 2-5	13	ITS	*Hypocrea lixii (Trichoderma harzianum)*	JN157752.1	2.00E-140	100%
Sen 16-2	14	ITS	*Hypocrea lixii (Trichoderma harzianum)*	JN157754.1	1.00E-117	100%
Sen 16-4	15	ITS	*Hypocrea lixii (Trichoderma harzianum)*	JF923802.1	0	100%
Wint 4-2	16	ITS	*Hypocrea lixii (Trichoderma harzianum)*	FJ442252.1	0	99%
Wint 16-1	17	ITS	*Hypocrea lixii (Trichoderma harzianum)*	JF923802.1	0	99%
Wint 21-2	18	ITS	*Hypocrea lixii (Trichoderma harzianum)*	HQ647325.1	0	100%
Wint 22-2	19	ITS	*Hypocrea lixii (Trichoderma harzianum)*	HQ596939	0	99%

Table 2.2 Root- and mesocotyl rot on sorghum cultivars PAN 8420 and PAN 8706W inoculated in the greenhouse with *F. oxysporum* isolates from seven localities in South Africa.

Fungi	PAN 8420			PAN 8706W		
	Root rot severity (%)	Mesocotyl rot (%)	**Mean**	Root rot severity (%)	Mesocotyl rot (%)	**Mean**
Control	27.67	67.61	**47.64**	20.61	32.61	**26.61**
1/2Bet16-1	31.17	63.06	**47.11**	23.89	47.83	**35.86**
P Bet40-2	32.50	72.67	**52.59**	19.72	58.17	**38.94**
Bet3-2	33.11	82.00	**57.55**	26.06	55.17	**40.61**
Bet5-2	33.83	64.28	**49.06**	27.89	56.72	**42.31**
Bet8-1	32.83	69.28	**51.06**	28.83	67.95	**48.39**
Bet13-2	34.78	84.44	**59.61**	27.78	61.39	**44.59**
Bet14-2	34.78	81.72	**58.25**	28.72	64.72	**46.72**
Bet16-1	30.50	70.28	**50.39**	26.11	57.11	**41.61**
Bet19-3	34.89	85.28	**60.09**	23.84	50.83	**37.34**
Bet23-5	29.95	72.61	**51.28**	24.56	56.78	**40.67**
Bet30-3	35.17	89.50	**62.34**	18.39	61.11	**39.75**
PCed9-2	21.83	49.61	**35.72**	21.11	42.89	**32.00**
CedNie4-1	33.17	90.44	**61.81**	29.83	69.17	**49.50**
CedNie4-2	27.61	54.22	**40.92**	25.39	66.83	**46.11**
CedNie5-1	35.61	77.33	**56.47**	27.52	65.62	**46.57**
CedNie7-2	37.56	88.83	**63.20**	27.33	52.94	**40.14**

LSD (P<0.05) Root rot: *F. oxysporum* isolate = n/s; Cultivar = 0.10; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD (P<0.05) Mesocotyl rot: *F. oxysporum* isolate = 6.67; Cultivar = 0.22; *F. oxysporum* isolate x Cultivar interaction = 0.66

Table 2.2 Continued

Fungi	PAN 8420			PAN 8706W		
	Root rot severity (%)	Mesocotyl rot (%)	**Mean**	Root rot severity (%)	Mesocotyl rot (%)	**Mean**
CedNie9-1	33.95	68.45	**51.20**	27.17	48.11	**37.64**
CedNie18-2	34.22	84.06	**59.14**	17.34	42.63	**29.99**
CedNie22-5	27.00	87.11	**57.06**	25.94	57.89	**41.92**
CedNie25-3	31.22	79.75	**55.49**	23.22	47.00	**35.11**
CedNie26-1	29.45	83.39	**56.42**	27.11	60.50	**43.81**
CedNie27-1	29.72	85.00	**57.36**	30.05	43.72	**36.89**
Ced27-4	33.06	76.11	**54.59**	19.30	49.44	**34.37**
Grey6-5	36.72	91.33	**64.03**	22.19	45.00	**33.60**
Grey10-2	34.11	84.00	**59.06**	17.50	41.22	**29.36**
Grey10-4	33.11	89.11	**61.11**	24.94	60.00	**42.47**
Grey15-2	33.61	68.11	**50.86**	28.11	45.72	**36.92**
Grey18-3	25.81	86.60	**56.21**	27.72	44.45	**36.09**
Grey21-2	31.83	83.28	**57.56**	24.66	52.78	**38.72**
Grey22-2	28.11	72.95	**50.53**	20.00	39.11	**29.56**
Grey22-5	35.89	89.89	**62.89**	27.61	43.17	**35.39**
Grey23-3	25.67	65.00	**45.33**	24.67	52.11	**38.39**
Grey25-3	33.28	87.33	**60.31**	23.72	72.89	**48.31**

LSD ($P<0.05$) Root rot: *F. oxysporum* isolate = n/s; Cultivar = 0.10; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD ($P<0.05$) Mesocotyl rot: *F. oxysporum* isolate = 6.67; Cultivar = 0.22; *F. oxysporum* isolate x Cultivar interaction = 0.66

Table 2.2 Continued

Fungi	PAN 8420			PAN 8706W		
	Root rot severity (%)	Mesocotyl rot (%)	**Mean**	Root rot severity (%)	Mesocotyl rot (%)	**Mean**
Grey27-4	25.94	81.28	**53.61**	21.44	48.06	**34.75**
Grey27-5	35.67	80.55	**58.11**	17.94	35.06	**26.50**
Kl6-1	32.56	78.17	**55.36**	15.39	44.83	**30.11**
Kl8-1	26.05	75.72	**50.89**	18.00	21.89	**19.95**
Kl20-1A	25.05	80.17	**52.61**	17.89	26.72	**22.31**
Kl25-3	31.17	83.78	**57.48**	25.67	52.28	**38.97**
Kl26-1	33.06	84.67	**58.86**	15.26	33.00	**24.13**
Kl26-2	35.22	69.56	**52.39**	23.72	42.72	**33.22**
Sen2-1	33.11	88.72	**60.92**	18.00	42.34	**30.17**
Sen6-2	34.39	84.06	**59.22**	23.78	51.78	**37.78**
Sen10-1	33.61	85.00	**59.31**	23.39	52.72	**38.06**
Sen11-1	28.57	62.68	**45.63**	18.33	39.67	**29.00**
Sen12-1	36.11	89.28	**62.70**	18.29	34.10	**26.20**
Sen13-3	34.94	85.38	**60.16**	25.61	50.61	**38.11**
Sen22-3	34.28	79.38	**56.83**	25.63	59.28	**42.46**
Sen25-2B	33.06	80.50	**56.78**	25.00	57.84	**41.42**
Sen27-2	36.94	74.83	**55.89**	28.50	52.61	**40.56**

LSD ($P<0.05$) Root rot: *F. oxysporum* isolate = n/s; Cultivar = 0.10; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD ($P<0.05$) Mesocotyl rot: *F. oxysporum* isolate = 6.67; Cultivar = 0.22; *F. oxysporum* isolate x Cultivar interaction = 0.66

Table 2.2 Continued

Fungi	PAN 8420			PAN 8706W		
	Root rot severity (%)	Mesocotyl rot (%)	**Mean**	Root rot severity (%)	Mesocotyl rot (%)	**Mean**
Sen26-3	32.28	75.95	**54.11**	19.05	37.44	**28.25**
Sen27-3	28.54	77.66	**53.10**	24.94	65.67	**45.31**
Wint14-1	33.61	67.45	**50.53**	26.55	57.17	**41.86**
Wint14-2	36.06	87.72	**61.89**	21.56	37.22	**29.39**
Wint17-1	30.05	62.39	**46.22**	27.78	48.17	**37.97**
Wint17-2	27.05	58.72	**42.89**	17.94	47.89	**32.92**
Wint19-1	28.94	84.28	**56.61**	25.95	43.78	**34.86**
Wint20-1	34.33	85.78	**60.06**	22.22	46.67	**34.44**
Wint23-1	33.50	72.78	**53.14**	22.67	59.95	**41.31**
Wint24-1	29.94	84.78	**57.36**	27.50	56.17	**41.83**
Mean	**31.93**	**78.13**	**55.03**	**23.56**	**50.15**	**36.85**

LSD ($P<0.05$) Root rot: *F. oxysporum* isolate = n/s; Cultivar = 0.10; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD ($P<0.05$) Mesocotyl rot: *F. oxysporum* isolate = 6.67; Cultivar = 0.22; *F. oxysporum* isolate x Cultivar interaction = 0.66

Table 2.3 Root, mesocotyl and stover length and root volume of sorghum cultivars PAN 8420 and PAN 8706W inoculated in the greenhouse with *F. oxysporum* isolates from seven localities in South Africa.

Fungi	PAN 8420					PAN 8706W				
	Root length (cm)	Mesocotyl length (cm)	Stover length (cm)	Root volume (ml)	**Mean**	Root length (cm)	Mesocotyl length (cm)	Stover length (cm)	Root volume (ml)	**Mean**
Control	11.69	1.03	13.20	0.55	**6.62**	13.74	0.89	13.54	0.83	**7.25**
1/2Bet16-1	12.09	0.84	17.83	3.26	**8.51**	13.12	1.13	19.88	1.53	**8.92**
P Bet40-2	14.15	1.55	28.49	0.82	**11.25**	14.99	0.84	28.70	1.81	**11.59**
Bet3-2	14.26	1.18	26.91	1.60	**10.99**	12.14	1.12	27.76	1.34	**10.59**
Bet5-2	13.59	1.04	21.82	1.58	**9.51**	12.34	0.97	21.63	1.10	**9.01**
Bet8-1	12.41	1.00	19.65	2.06	**8.78**	11.73	1.03	15.56	0.89	**7.30**
Bet13-2	11.79	1.39	18.28	0.84	**8.08**	11.88	1.11	24.35	2.21	**9.89**
Bet14-2	12.60	1.41	25.05	0.68	**9.93**	12.78	1.11	24.14	1.11	**9.79**
Bet16-1	13.55	1.12	21.79	1.61	**9.52**	15.21	1.26	22.70	0.66	**9.96**
Bet19-3	12.41	1.35	26.60	1.78	**10.53**	14.06	0.96	24.62	1.86	**10.38**
Bet23-5	12.19	1.27	23.05	0.94	**9.36**	13.18	1.02	22.63	1.44	**9.57**
Bet30-3	12.25	1.34	28.76	1.57	**10.98**	13.65	1.10	28.91	2.91	**11.64**
PCed9-2	13.48	0.89	23.50	2.40	**10.07**	14.26	1.34	21.35	1.09	**9.51**
CedNie4-1	10.94	1.17	23.34	1.90	**9.34**	11.23	1.16	23.62	0.84	**9.21**
CedNie4-2	13.26	1.01	21.41	1.65	**9.33**	15.50	1.53	22.77	1.55	**10.34**
CedNie5-1	13.14	0.94	29.17	1.48	**11.18**	14.72	1.24	28.58	2.51	**11.76**
CedNie7-2	9.49	1.25	13.97	0.60	**6.33**	13.47	1.06	17.20	1.12	**8.21**

LSD ($P<0.05$) Root length: *F. oxysporum* isolate = 0.82; Cultivar = 0.03; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD ($P<0.05$) Mesocotyl length: *F. oxysporum* isolate = n/s; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD ($P<0.05$) Stover length: *F. oxysporum* isolate = 1.12; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = 2.22

LSD ($P<0.05$) Root volume: *F. oxysporum* isolate = 0.44; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = n/s

Table 2.3 Continued

Fungi	PAN 8420					PAN 8706W				
	Root length (cm)	Mesocotyl length (cm)	Stover length (cm)	Root volume (ml)	**Mean**	Root length (cm)	Mesocotyl length (cm)	Stover length (cm)	Root volume (ml)	**Mean**
CedNie9-1	12.91	1.24	25.41	2.28	**10.46**	13.67	1.15	18.44	1.50	**8.69**
CedNie18-2	12.89	1.15	23.23	0.96	**9.56**	15.58	1.54	24.08	3.33	**11.13**
CedNie22-5	12.77	1.51	25.93	2.29	**10.62**	14.28	1.26	21.39	1.60	**9.63**
CedNie25-3	11.51	1.36	26.68	1.21	**10.19**	10.81	1.03	22.18	0.89	**8.73**
CedNie26-1	12.64	1.08	20.08	1.49	**8.83**	13.85	1.43	20.58	2.14	**9.50**
CedNie27-1	10.45	1.49	15.70	1.68	**7.33**	13.00	1.13	16.32	0.63	**7.77**
Ced 7-4	12.51	1.09	18.34	1.90	**8.46**	13.77	1.54	20.05	1.93	**9.32**
Grey 6-5	12.17	1.17	25.49	1.02	**9.96**	9.34	0.83	19.86	1.47	**7.88**
Grey10-2	10.47	1.20	20.94	1.39	**8.50**	13.22	0.72	18.36	1.59	**8.47**
Grey10-4	12.80	1.15	20.54	1.02	**8.88**	12.75	1.18	21.62	0.83	**9.09**
Grey15-2	13.04	1.37	18.27	1.58	**8.56**	13.49	1.34	18.71	1.13	**8.67**
Grey18-3	12.81	1.27	19.86	2.27	**9.05**	12.24	1.03	19.16	1.15	**8.39**
Grey21-2	14.06	1.30	27.30	2.69	**11.34**	14.71	1.67	26.90	3.14	**11.60**
Grey22-2	11.23	1.44	14.73	1.55	**7.24**	13.22	1.04	20.20	2.63	**9.27**
Grey22-5	13.13	1.76	20.43	1.18	**9.13**	12.33	1.21	21.39	1.13	**9.02**
Grey23-3	13.12	1.06	21.33	2.30	**9.45**	14.31	1.44	21.59	1.40	**9.68**
Grey25-3	11.91	1.23	21.20	1.11	**8.86**	10.97	0.94	18.29	1.29	**7.87**

LSD ($P<0.05$) Root length: *F. oxysporum* isolate = 0.82; Cultivar = 0.03; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD ($P<0.05$) Mesocotyl length: *F. oxysporum* isolate = n/s; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD ($P<0.05$) Stover length: *F. oxysporum* isolate = 1.12; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = 2.22

LSD ($P<0.05$) Root volume: *F. oxysporum* isolate = 0.44; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = n/s

Table 2.3 Continued

Fungi	PAN 8420					PAN 8706W				
	Root length (cm)	Mesocotyl length (cm)	Stover length (cm)	Root volume (ml)	**Mean**	Root length (cm)	Mesocotyl length (cm)	Stover length (cm)	Root volume (ml)	**Mean**
Grey27-4	11.07	0.93	20.14	1.61	**8.44**	11.46	0.93	18.32	1.03	**7.93**
Grey27-5	11.84	1.16	20.37	0.81	**8.55**	14.88	0.87	21.79	2.54	**10.02**
Kl6-1	15.46	1.15	26.44	2.01	**11.27**	14.10	1.23	22.88	1.75	**9.99**
Kl8-1	12.17	1.13	23.83	2.09	**9.81**	13.32	1.11	24.03	1.58	**10.01**
Kl20-1A	11.04	1.05	24.56	1.72	**9.59**	12.92	1.02	23.61	1.01	**9.64**
Kl25-3	13.55	1.47	28.49	2.31	**11.45**	15.69	1.08	32.81	0.83	**12.60**
Kl26-1	12.18	1.32	26.14	0.76	**10.10**	13.51	1.20	20.53	1.67	**9.23**
Kl26-2	13.31	1.30	21.89	1.65	**9.54**	13.08	1.10	16.35	0.22	**7.69**
Sen2-1	11.15	1.04	21.87	1.77	**8.96**	14.57	0.96	21.91	2.37	**9.95**
Sen6-2	13.89	1.31	25.20	1.50	**10.48**	15.12	0.89	25.05	1.62	**10.67**
Sen10-1	12.59	1.22	26.30	1.74	**10.46**	17.04	1.17	25.23	1.21	**11.16**
Sen11-1	13.56	1.23	24.02	2.16	**10.24**	15.19	1.12	23.15	2.00	**10.37**
Sen12-1	11.77	1.10	18.52	0.41	**7.95**	12.33	1.10	16.78	0.79	**7.75**
Sen13-3	12.97	0.93	22.48	1.25	**9.41**	14.13	1.23	28.84	0.58	**11.20**
Sen22-3	12.40	1.21	24.18	1.35	**9.78**	14.86	1.13	23.50	2.94	**10.61**
Sen25-2B	12.56	1.32	21.15	0.59	**8.90**	12.51	1.12	24.44	1.68	**9.94**
Sen27-2	12.39	0.91	17.57	0.59	**7.87**	13.98	0.95	18.84	1.60	**8.84**

LSD (P<0.05) Root length: *F. oxysporum* isolate = 0.82; Cultivar = 0.03; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD (P<0.05) Mesocotyl length: *F. oxysporum* isolate = n/s; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD (P<0.05) Stover length: *F. oxysporum* isolate = 1.12; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = 2.22

LSD (P<0.05) Root volume: *F. oxysporum* isolate = 0.44; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = n/s

Table 2.3 Continued

Fungi	PAN 8420					PAN 8706W				
	Root length (cm)	Mesocotyl length (cm)	Stover length (cm)	Root volume (ml)	**Mean**	Root length (cm)	Mesocotyl length (cm)	Stover length (cm)	Root volume (ml)	**Mean**
Sen26-3	13.34	1.13	20.67	0.73	**8.97**	13.13	0.78	21.89	1.99	**9.45**
Sen27-3	13.31	1.04	27.27	2.16	**10.94**	13.68	1.34	23.32	1.41	**9.94**
Wint14-1	11.10	0.84	24.62	1.62	**9.54**	12.22	1.11	24.40	1.33	**9.76**
Wint14-2	13.49	1.71	16.91	0.70	**8.20**	13.09	1.09	15.05	0.63	**7.47**
Wint17-1	10.99	1.29	22.65	0.64	**8.89**	12.18	1.12	22.14	0.97	**9.11**
Wint17-2	12.61	0.97	24.64	1.47	**9.92**	11.58	1.30	20.68	1.58	**8.79**
Wint19-1	9.98	1.19	23.11	1.76	**9.01**	11.70	0.80	22.54	1.27	**9.08**
Wint20-1	12.08	1.47	18.82	0.84	**8.30**	14.11	0.92	17.49	1.14	**8.42**
Wint23-1	11.62	1.11	24.38	1.27	**9.60**	13.36	1.26	21.01	1.09	**9.18**
Wint24-1	10.29	1.48	19.83	1.48	**8.27**	14.32	1.20	23.12	1.32	**9.99**
Mean	**10.99**	**1.26**	**16.51**	**1.01**	**7.44**	**14.03**	**1.05**	**18.33**	**1.08**	**8.62**

LSD (P<0.05) Root length: *F. oxysporum* isolate = 0.82; Cultivar = 0.03; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD (P<0.05) Mesocotyl length: *F. oxysporum* isolate = n/s; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = n/s

LSD (P<0.05) Stover length: *F. oxysporum* isolate = 1.12; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = 2.22

LSD (P<0.05) Root volume: *F. oxysporum* isolate = 0.44; Cultivar = n/s; *F. oxysporum* isolate x Cultivar interaction = n/s

Table 2.4 Root- and mesocotyl rot on sorghum cultivars PAN 8420 and PAN 8706W inoculated in the greenhouse with miscellaneous sorghum root isolates from seven localities in South Africa.

Fungi	Isolate	PAN 8420			PAN 8706W		
		Root rot severity (%)	Mesocotyl rot (%)	**Mean**	Root rot severity (%)	Mesocotyl rot (%)	**Mean**
Control		17.70	62.42	**40.06**	22.95	64.50	**43.73**
1/2Bet36-2	*Alternaria* sp.	46.55	96.94	**71.75**	34.05	81.44	**57.75**
PBet39-1	Unidentified	38.00	95.61	**66.81**	28.05	78.67	**53.36**
PBet39-2	Unidentified	34.17	85.00	**59.58**	29.83	80.11	**54.97**
PBet40-1	*Alternaria alternata*	31.83	99.00	**65.42**	29.50	86.00	**57.75**
Bet1-2	*Fusarium solani*	30.78	92.83	**61.81**	28.61	93.61	**61.11**
Bet4-1	*Fusarium equiseti*	29.55	94.56	**62.06**	28.11	83.83	**55.97**
Bet8-2	Unidentified	30.55	94.00	**62.28**	27.61	87.72	**57.67**
Bet9-3	Unidentified	57.72	93.56	**75.64**	45.33	89.89	**67.61**
Bet26-4	*Acremonium strictum*	28.83	98.39	**63.61**	26.89	83.61	**55.25**
Bet27-1	*Fusarium equiseti*	31.17	93.39	**62.28**	27.72	69.78	**48.75**
Bet28-1A	*Peyronellaea pomorum*	32.67	97.89	**65.28**	27.50	85.00	**56.25**
Bet28-1B	*Alternaria alternata*	31.06	98.39	**64.72**	27.11	91.44	**59.28**
Bet30-2	Unidentified	50.50	96.89	**73.70**	32.61	88.44	**60.53**
1/2Ced1-2	*Curvularia trifolii*	30.33	99.00	**64.67**	0.00	0.00	**0.00**
1/2Ced3-2	Dothideales	26.39	92.45	**59.42**	26.14	83.50	**54.82**
1/2Ced10-1	Unidentified	19.00	66.00	**42.50**	22.00	65.33	**43.67**

LSD ($P<0.05$) Root rot: Root isolate = 1.86; Cultivar = 0.09; Root isolate x Cultivar interaction = 3.73

LSD ($P<0.05$) Mesocotyl rot: Root isolate = 6.06; Cultivar = 0.30; Root isolate x Cultivar interaction = 0.91

Table 2.4 Continued

Fungi	Isolate	PAN 8420			PAN 8706W		
		Root rot severity (%)	Mesocotyl rot (%)	**Mean**	Root rot severity (%)	Mesocotyl rot (%)	**Mean**
1/2Ced12-4	*Phoma macrostoma*	26.72	94.17	**60.44**	27.11	95.50	**61.31**
1/2Ced33-4	*Curvularia trifolii*	0.00	0.00	**0.00**	0.00	0.00	**0.00**
CedNie6-2	*Epicoccum nigrum*	29.22	95.94	**62.58**	26.11	86.94	**56.53**
CedNie6-4	Dothideomycete	30.83	98.17	**64.50**	27.89	91.00	**59.44**
CedNie20-4	*Curvularia trifolii*	0.00	0.00	**0.00**	0.00	0.00	**0.00**
CedNie21-1	*Fusarium oxysporum*	29.61	97.56	**63.58**	26.44	63.94	**45.19**
Grey19-3	*Gibberella thapsina*	29.41	97.32	**63.37**	24.05	71.39	**47.72**
Kl16-2	*Penicillium verruculosum*	31.83	98.39	**65.11**	26.56	78.28	**52.42**
Kl17-1	*Gibberella thapsina*	34.45	97.72	**66.09**	24.94	83.96	**54.45**
Kl20-1B	Unidentified	28.50	95.78	**62.14**	24.61	86.11	**55.36**
Kl28-1	*Periconia macrospinosa*	31.78	98.50	**65.14**	27.67	92.67	**60.17**
Sen8-2	*Arthrinium phaeospermum*	29.45	94.83	**62.14**	27.65	70.70	**49.18**
Sen14-1	*Colletotrichum capsici*	28.83	91.94	**60.39**	25.50	87.50	**56.50**
Sen14-2	Unidentified	31.44	97.56	**64.50**	27.67	91.22	**59.44**
Sen17-5	*Colletotrichum capsici*	27.50	91.72	**59.61**	26.17	73.00	**49.58**
Sen25-1	*Fusarium solani*	29.28	93.89	**61.59**	26.33	91.22	**58.78**
Sen27-4	*Fusarium oxysporum*	33.50	98.44	**65.97**	27.33	81.05	**54.19**

LSD ($P<0.05$) Root rot: Root isolate = 1.86; Cultivar = 0.09; Root isolate x Cultivar interaction = 3.73

LSD ($P<0.05$) Mesocotyl rot: Root isolate = 6.06; Cultivar = 0.30; Root isolate x Cultivar interaction = 0.91

Table 2.4 Continued

Fungi	Isolate	PAN 8420			PAN 8706W		
		Root rot severity (%)	Mesocotyl rot (%)	**Mean**	Root rot severity (%)	Mesocotyl rot (%)	**Mean**
Wint5-5	*Fusarium equiseti*	31.78	97.28	**64.53**	26.17	72.78	**49.47**
Wint6-4	Unidentified	30.44	97.56	**64.00**	25.89	89.17	**57.53**
Wint7-2	*Fusarium sp.*	28.95	96.56	**62.75**	26.39	81.39	**53.89**
Wint7-4	Unidentified	29.61	92.61	**61.11**	26.67	65.67	**46.17**
Wint8-4	*Phoma sorghina*	30.39	98.00	**64.19**	27.56	87.00	**57.28**
Wint9-2	*Fusarium temperatum*	28.17	93.22	**60.70**	25.17	62.03	**43.60**
Wint10-5	*Fusarium sp.*	33.56	96.56	**65.06**	28.39	77.17	**52.78**
Wint21-1	*Fusarium temperatum*	30.72	93.85	**62.28**	27.78	74.17	**50.97**
Mean		**30.07**	**89.62**	**59.84**	**25.57**	**75.40**	**50.49**

LSD ($P<0.05$) Root rot: Root isolate = 1.86; Cultivar = 0.09; Root isolate x Cultivar interaction = 3.73

LSD ($P<0.05$) Mesocotyl rot: Root isolate = 6.06; Cultivar = 0.30; Root isolate x Cultivar interaction = 0.91

Table 2.5 Root, mesocotyl and stover length and root volume of sorghum cultivars PAN 8420 and PAN 8706W inoculated in the greenhouse with miscellaneous root isolates from seven localities in South Africa.

	PAN 8420					PAN 8706W				
Fungi	Root L (cm)	Mesocotyl L (cm)	Leaf L (cm)	Root volume (ml)	**Mean**	Root L (cm)	Mesocotyl L (cm)	Leaf L (cm)	Root volume (ml)	**Mean**
Control	7.52	0.86	17.61	1.71	**6.92**	13.76	1.01	33.62	0.37	**12.19**
1/2Bet36-2	12.29	1.08	28.17	0.14	**10.42**	11.76	1.01	31.63	0.16	**11.14**
PBet39-1	12.51	1.41	33.62	0.68	**12.06**	14.41	1.13	40.14	0.20	**13.97**
PBet39-2	16.39	0.98	37.58	1.85	**14.20**	15.42	1.00	46.78	0.61	**15.95**
PBet40-1	25.92	1.29	33.31	0.64	**15.29**	15.25	1.05	47.53	0.67	**16.13**
Bet1-2	23.79	1.11	35.11	1.84	**15.46**	16.60	1.20	45.88	1.26	**16.24**
Bet4-1	13.62	1.16	40.56	0.54	**13.97**	15.07	1.01	40.37	1.00	**14.36**
Bet8-2	14.25	0.94	32.25	1.22	**12.17**	14.96	0.96	40.25	0.60	**14.19**
Bet9-3	13.47	1.15	45.59	0.80	**15.25**	15.22	1.08	56.08	0.95	**18.34**
Bet26-4	16.18	1.23	46.17	1.50	**16.27**	14.59	1.05	41.91	0.68	**14.56**
Bet27-1	12.80	1.02	35.24	0.23	**12.32**	15.11	1.00	39.89	1.29	**14.32**
Bet28-1A	17.29	1.00	46.35	1.50	**16.54**	14.08	1.03	44.67	1.39	**15.29**
Bet28-1B	12.54	1.17	43.71	0.93	**14.59**	14.01	1.21	44.84	1.39	**15.36**
Bet30-2	18.36	1.14	46.35	1.82	**16.92**	14.07	1.04	43.81	1.16	**15.02**
1/2Ced1-2	15.10	0.97	41.33	0.17	**14.39**	0.00	0.00	0.00	0.00	**0.00**
1/2Ced3-2	15.08	1.41	43.12	1.87	**15.37**	15.77	1.10	51.43	1.22	**17.38**
1/2Ced10-1	7.77	0.67	36.10	0.07	**11.15**	7.30	0.50	22.80	0.07	**7.67**

LSD (P<0.05) Root length: Root isolate = 1.71; Cultivar = n/s; Root isolate x Cultivar interaction = n/s

LSD (P<0.05) Mesocotyl length: Root isolate = n/s; Cultivar = n/s; Root isolate x Cultivar interaction = n/s

LSD (P<0.05) Stover length: Root isolate = 3.1; Cultivar = 0.16; Root isolate x Cultivar interaction = 6.20

LSD (P<0.05) Root volume: Root isolate = 0.39; Cultivar = n/s; Root isolate x Cultivar interaction = n/s

Table 2.5 Continued

Fungi	PAN 8420					PAN 8706W				
	Root L (cm)	Mesocotyl L (cm)	Leaf L (cm)	Root volume (ml)	**Mean**	Root L (cm)	Mesocotyl L (cm)	Leaf L (cm)	Root volume (ml)	**Mean**
1/2Ced12-4	13.55	1.16	45.88	0.42	**15.25**	12.92	1.06	50.28	1.70	**16.49**
1/2Ced33-4	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
CedNie6-2	14.56	1.23	45.54	0.34	**15.42**	15.97	1.12	50.20	0.10	**16.85**
CedNie6-4	14.84	1.27	42.22	0.67	**14.75**	12.98	1.11	46.51	1.37	**15.49**
CedNie20-4	0.00	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00	**0.00**
CedNie21-1	13.16	1.22	43.95	1.38	**14.93**	16.90	1.06	50.03	1.08	**17.27**
Grey19-3	14.06	1.33	32.91	1.46	**12.44**	15.21	1.14	49.33	1.20	**16.72**
Kl16-2	15.67	1.51	42.80	1.19	**15.29**	13.28	1.14	37.66	0.78	**13.22**
Kl17-1	14.94	1.38	43.49	0.85	**15.17**	15.01	1.09	49.58	0.92	**16.65**
Kl20-1B	14.31	1.32	47.13	0.28	**15.76**	15.31	1.21	49.95	1.43	**16.97**
Kl28-1	13.42	1.37	42.67	0.88	**14.59**	12.72	1.21	35.91	1.05	**12.72**
Sen8-2	14.50	1.28	41.05	0.65	**14.37**	13.19	0.99	45.92	1.16	**15.32**
Sen14-1	14.57	1.23	35.82	2.48	**13.53**	11.96	1.02	30.56	1.42	**11.24**
Sen14-2	15.22	1.20	42.46	1.30	**15.04**	18.33	1.06	48.12	0.94	**17.11**
Sen17-5	13.34	1.13	39.56	1.63	**13.92**	13.47	1.05	40.04	1.02	**13.90**
Sen25-1	15.09	1.21	38.80	2.05	**14.29**	14.62	1.28	46.23	1.98	**16.03**
Sen27-4	16.23	1.27	39.88	0.85	**14.56**	16.70	1.10	41.30	1.77	**15.22**

LSD ($P<0.05$) Root length: Root isolate = 1.71; Cultivar = n/s; Root isolate x Cultivar interaction = n/s

($P<0.05$) Mesocotyl length: Root isolate = n/s; Cultivar = n/s; Root isolate x Cultivar interaction = n/s

LSD ($P<0.05$) Stover length: Root isolate = 3.1; Cultivar = 0.16; Root isolate x Cultivar interaction = 6.20

LSD ($P<0.05$) Root volume: Root isolate = 0.39; Cultivar = n/s; Root isolate x Cultivar interaction = n/s

Table 2.5 Continued

Fungi	PAN 8420					PAN 8706W				
	Root L (cm)	Mesocotyl L (cm)	Leaf L (cm)	Root volume (ml)	**Mean**	Root L (cm)	Mesocotyl L (cm)	Leaf L (cm)	Root volume (ml)	**Mean**
Wint5-5	15.10	1.38	41.40	1.27	**14.79**	17.02	1.02	46.82	1.78	**16.66**
Wint6-4	14.27	1.02	38.27	0.86	**13.61**	17.20	1.33	40.90	0.87	**15.07**
Wint7-2	13.06	1.21	40.46	1.27	**14.00**	15.23	0.89	40.67	1.74	**14.63**
Wint7-4	14.60	1.19	42.39	0.94	**14.78**	16.32	0.99	42.79	0.72	**15.20**
Wint8-4	15.90	1.27	46.28	1.90	**16.34**	16.70	1.05	45.19	1.04	**16.00**
Wint9-2	15.29	1.11	40.54	1.68	**14.66**	16.54	1.04	39.96	1.93	**14.87**
Wint10-5	14.15	1.17	45.18	0.65	**15.28**	16.56	0.88	47.19	0.56	**16.30**
Wint21-1	16.41	1.22	40.84	1.05	**14.88**	15.72	1.00	42.87	1.42	**15.25**
Mean	**14.07**	**1.12**	**38.14**	**1.04**	**13.59**	**13.74**	**0.98**	**40.23**	**0.98**	**13.98**

LSD ($P<0.05$) Root length: Root isolate = 1.71; Cultivar = n/s; Root isolate x Cultivar interaction = n/s

($P<0.05$) Mesocotyl length: Root isolate = n/s; Cultivar = n/s; Root isolate x Cultivar interaction = n/s

LSD ($P<0.05$) Stover length: Root isolate = 3.1; Cultivar = 0.16; Root isolate x Cultivar interaction = 6.20

LSD ($P<0.05$) Root volume: Root isolate = 0.39; Cultivar = n/s; Root isolate x Cultivar interaction = n/s

Table 2.6: Ergosterol recorded in root tissues of sorghum cultivars PAN 8420 and PAN 8706W inoculated in the greenhouse with *F. oxysporum* isolates from seven localities in South Africa.

Fungi	Ergosterol content (µg/g)		**Mean**
	PAN 8420	PAN 8706W	
Control	0.00011	0.00038	**0.00024**
1/2Bet16-1	0.00046	0.00118	**0.00082**
PBet40-2	0.00243	0.00211	**0.00227**
Bet3-2	0.00453	0.00145	**0.00299**
Bet5-2	0.00012	0.00104	**0.00058**
Bet8-1	0.00119	0.00034	**0.00077**
Bet13-2	0.00186	0.00057	**0.00121**
Bet14-2	0.00095	0.00135	**0.00115**
Bet16-1	0.00226	0.00199	**0.00213**
Bet19-3	0.00330	0.00079	**0.00204**
Bet23-5	0.00045	0.00046	**0.00045**
Bet30-3	0.00169	0.00054	**0.00111**
PCed9-2	0.00088	0.00073	**0.00081**
CedNie4-1	0.00172	0.00132	**0.00152**
CedNie4-2	0.00141	0.00370	**0.00255**
CedNie5-1	0.00220	0.00288	**0.00254**
CedNie7-2	0.00159	0.00048	**0.00104**
CedNie9-1	0.00083	0.00039	**0.00061**
CedNie18-2	0.00324	0.00212	**0.00268**
CedNie22-5	0.00206	0.00050	**0.00128**
CedNie25-3	0.00082	0.00059	**0.00071**
CedNie26-1	0.00053	0.00027	**0.00040**
CedNie27-1	0.00166	0.00021	**0.00093**
Ced27-4	0.00068	0.00099	**0.00083**
Grey 6-5	0.00637	0.00230	**0.00434**
Grey10-2	0.00079	0.00274	**0.00177**
Grey10-4	0.00466	0.00034	**0.00250**
Grey15-2	0.00056	0.00335	**0.00195**
Grey18-3	0.00159	0.00247	**0.00203**
Grey21-2	0.00163	0.00265	**0.00214**
Grey22-2	0.00111	0.00068	**0.00089**

LSD ($P<0.05$): Root isolate = 0.0028; Cultivar = 0.000513; Root isolate x Cultivar interaction = n/s

Table 2.6 Continued

Fungi	Ergosterol content (μg/g)		**Mean**
	PAN 8420	PAN 8706W	
Grey22-5	0.00043	0.00051	**0.00047**
Grey23-3	0.00118	0.00065	**0.00092**
Grey25-3	0.00038	0.00040	**0.00039**
Grey27-4	0.00229	0.00111	**0.00170**
Grey27-5	0.00122	0.00111	**0.00117**
Kl6-1	0.00112	0.00041	**0.00077**
Kl8-1	0.00063	0.00115	**0.00089**
Kl20-1A	0.00067	0.00057	**0.00062**
Kl25-3	0.00419	0.00465	**0.00442**
Kl26-1	0.00369	0.00365	**0.00367**
Kl26-2	0.00106	0.00043	**0.00075**
Sen2-1	0.00093	0.00076	**0.00084**
Sen6-2	0.00111	0.00018	**0.00064**
Sen10-1	0.00114	0.00191	**0.00152**
Sen11-1	0.00164	0.00224	**0.00194**
Sen12-1	0.00024	0.00090	**0.00057**
Sen13-3	0.00415	0.00138	**0.00276**
Sen22-3	0.00030	0.00095	**0.00062**
Sen25-2B	0.00172	0.00123	**0.00148**
Sen27-2	0.00074	0.00167	**0.00120**
Sen26-3	0.00072	0.00040	**0.00056**
Sen27-3	0.00048	0.00128	**0.00088**
Wint14-1	0.00341	0.00182	**0.00261**
Wint14-2	0.00071	0.00027	**0.00049**
Wint17-1	0.00252	0.00034	**0.00143**
Wint17-2	0.00317	0.00063	**0.00190**
Wint19-1	0.00188	0.00292	**0.00240**
Wint20-1	0.00119	0.00008	**0.00064**
Wint23-1	0.00115	0.00037	**0.00076**
Wint24-1	0.00439	0.00247	**0.00343**
Mean	**0.00167**	**0.00127**	**0.00147**

LSD ($P<0.05$): Root isolate = 0.0028; Cultivar = 0.000513; Root isolate x Cultivar interaction = n/s

Table 2.7 Ergosterol recorded in sorghum roots of sorghum cultivars PAN 8420 and PAN 8706W inoculated in the greenhouse with miscellaneous root isolates from seven localities in South Africa.

Fungi	Isolate	Ergosterol content (µg/g)		**Mean**
		PAN 8420	PAN 8706W	
Control		0.00615	0.01041	**0.00828**
1/2Bet36-2	*Alternaria* sp.	0.00488	0.00945	**0.00717**
PBet39-1	Unidentified	0.00701	0.00672	**0.00686**
PBet39-2	Unidentified	0.00370	0.00325	**0.00348**
PBet40-1	*Alternaria alternata*	0.00321	0.00975	**0.00648**
Bet1-2	*Fusarium solani*	0.00312	0.00480	**0.00396**
Bet4-1	*Fusarium equiseti*	0.00117	0.01009	**0.00563**
Bet8-2	Unidentified	0.00227	0.00311	**0.00269**
Bet9-3	Unidentified	0.00673	0.00403	**0.00538**
Bet26-4	*Acremonium strictum*	0.00818	0.01366	**0.01092**
Bet27-1	*Fusarium equiseti*	0.00270	0.00513	**0.00392**
Bet28-1A	*Peyronellaea pomorum*	0.00447	0.00424	**0.00436**
Bet28-1B	*Alternaria alternata*	0.00217	0.00156	**0.00186**
Bet30-2	Unidentified	0.00514	0.00573	**0.00543**
1/2Ced1-2	*Curvularia trifolii*	0.00087	0.00000	**0.00043**
1/2Ced3-2	Dothideales	0.00323	0.00448	**0.00386**
1/2Ced10-1	Unidentified	0.00031	0.00066	**0.00049**
1/2Ced12-4	*Phoma macrostoma*	0.00764	0.00425	**0.00594**
1/2Ced33-4	*Curvularia trifolii*	0.00000	0.00000	**0.00000**
Ced Nie6-2	*Epicoccum nigrum*	0.00488	0.00904	**0.00696**
CedNie6-4	Dothideomycete	0.00801	0.00739	**0.00770**
CedNie20-4	*Curvularia trifolii*	0.00000	0.00000	**0.00000**
CedNie 21-1	*Fusarium oxysporum*	0.00532	0.00155	**0.00343**
Grey19-3	*Gibberella thapsina*	0.00325	0.00441	**0.00383**
Kl16-2	*Penicillium verruculosum*	0.00335	0.00399	**0.00367**
Kl17-1	*Gibberella thapsina*	0.00436	0.00671	**0.00554**
Kl20-1B	Unidentified	0.00399	0.00732	**0.00565**
Kl28-1	*Periconia macrospinosa*	0.00456	0.00779	**0.00617**

LSD ($P<0.05$): Root isolate = 0.0068; Cultivar = n/s; Root isolate x Cultivar interaction = n/s

Table 2.7 Continued

Fungi	Isolate	Ergosterol content (µg/g)		**Mean**
		PAN 8420	PAN 8706W	
Sen8-2	*Arthrinium phaeospermum*	0.00513	0.00819	**0.00666**
Sen14-1	*Colletotrichum capsici*	0.00206	0.00175	**0.00191**
Sen14-2	Unidentified	0.00469	0.00461	**0.00465**
Sen17-5	*Colletotrichum capsici*	0.00591	0.00709	**0.00650**
Sen25-1	*Fusarium solani*	0.00451	0.00289	**0.00370**
Sen27-4	*Fusarium oxysporum*	0.00424	0.00376	**0.00400**
Wint5-5	*Fusarium equiseti*	0.00114	0.00583	**0.00348**
Wint6-4	Unidentified	0.00428	0.00911	**0.00670**
Wint7-2	*Fusarium* sp.	0.00340	0.00564	**0.00452**
Wint7-4	Unidentified	0.00322	0.00616	**0.00469**
Wint8-4	*Phoma sorghina*	0.00617	0.00887	**0.00752**
Wint9-2	*Fusarium temperatum*	0.00351	0.00333	**0.00342**
Wint10-5	*Fusarium* sp.	0.00598	0.00881	**0.00740**
Wint21-1	*Fusarium temperatum*	0.00532	0.00155	**0.00343**
Mean		**0.00405**	**0.00541**	**0.00473**

LSD ($P<0.05$): Root isolate = 0.0068; Cultivar = n/s; Root isolate x Cultivar interaction = n/s

CHAPTER 3

EVALUATION OF SORGHUM CULTIVARS FOR ROOT ROT RESISTANCE

Abstract: Crop losses of sorghum as a result of pathogen or pest attack are estimated at 30% per year. Reducing these losses is essential to enhance food quality and quantity. Twenty-six sorghum cultivars were evaluated for root rot resistance in field trials at Cedara. All cultivars were susceptible to root rot which ranged from 29.23 - 45.60%. Cultivars PAN 8389, PAN 8229 and PAN 8625 were the most susceptible cultivars compared to PAN 8534 and PAN 8706W that were more resistant to the disease. Some cultivars compensated for the effects of root rot by having larger root volumes which ranged from 4.86 - 22.58 ml using water displacement, and thus ensured a larger effective root volume despite root rot being present. However, no relationship between plant growth and effective root volume was detected. Extracts from roots of 10 cultivars, representative of the range of root rot severities were used to detect inhibition of 13 root fungi *in vitro*. The highest inhibition was recorded with NS 5511 with a mean inhibition of 3.18%, relative to the control, while extracts of PAN 8657 with mean inhibition value of -3.24%, stimulated rather than inhibited the growth of test fungi. The total phenol content of roots of these cultivars was determined which ranged from 0.0220 mg/g in NS 5655 - 0.0973 mg/g in PAN 8648W. No significant relationship between total phenol content and root rot severity in the field was detected although a negative tendency was observed. PAN 8534 which was most resistant to root rot in the field (29.23%), had only moderate total phenol levels suggesting that resistance is conferred by other mechanisms.
Keywords: sorghum, root rot, resistance, root exudates, phenolic compounds

3.0 INTRODUCTION

Crop losses are estimated at 30% annually for sorghum as a result of pathogen and pest interference (Chandrashekar and Satyanarayana, 2006). The management of these attacks is of great importance to enhance food quality and the yield of sorghum produced.

Little attention has been given to root rot pathogens due to the absence of obvious symptoms in the aerial canopy (Tarr, 1962) and the difficulty of detection and quantification.

Root rot of sorghum is caused by a complex of soilborne fungi, including *Fusarium* spp., *Pythium* spp., *Macrophomina phaseolina*, *Colletotrichum graminicola* and *Periconia circinata* (Mughogho, 1984; Pande and Karunakar, 1992) although other pathogens such as *Rhizoctonia* spp., *Sclerotium rolfsii* and *Erwinia* spp. may also occur on roots (Tarr, 1962). The environment and host play a significant role in determining which pathogen causes the most damage by favouring a specific pathogen at a certain time (Mughogho and Pande, 1984). Certain pathogens, such as *Pythium* spp. are favoured by cold, wet soils and sorghum is more prone to root rot due to a slower germination rate, delayed emergence and a reduction in root growth (Forbes *et al*., 1986). *M. phaseolina*, on the other hand, is favoured by drier conditions and seasons with high soil temperatures (Tuinstra *et al.,* 2002).

Root diseases of sorghum can occur at any growth stage from planting to plant maturity. Symptoms caused by *Fusarium* spp. were described by Zummo (1984). Pathogens initially colonize the cortex tissues and subsequently move into the vascular bundles. This causes visible lesions on the roots which range from small scattered, red to purple discolorations which may coalesce to cover much of the root surface. As the disease progresses, lateral roots may sever from the main root system and roots generally, are devoid of root hairs. Although the extent of losses due root rots has not been fully established, the disease complex has been associated with reduced plant growth, poor grain fill and thus yield losses. Diseased roots reduce drought tolerance, reduce optimal use of soil moisture and nutrients and lodging may occur. Extrapolated from maize studies, root rot may cause losses from a few percent to in excess of 20 % (Fowler, 1980; Blanquet *et al.,* 1990; Channon and Farina, 1991).

Breeding for host plant resistance remains the most effective means of root rot control. McLaren (2002) found significant differences in the resistance of sorghum germplasm to

root rot although all entries were susceptible to the disease complex. Environment was a major factor in the reaction of germplasm to the disease complex and highly significant G x E interactions were recorded. Host physiology has also been reported to affect disease severity. Sugar and phenol levels in charcoal rot resistant sorghum genotypes have been reported to be 2 - 3 times higher than those in susceptible genotypes (Anahosur *et al.,* 1985; Patil *et al.,* 1985; Rao *et al.,* 1990). Anahosur & Naik (1985) found that total sugars and phenols in roots and first internode, were greater in a charcoal rot resistant genotype than in a susceptible genotype at physiological maturity. The quantity of the substances decreased in susceptible types from full flowering stage and they thus succumbed to *M. phaseolina*.

The aim of this study was to evaluate sorghum cultivars from the National Sorghum Cultivar Trials to determine the current level of resistance to root rot under field conditions during the 2008/09 growing season at Cedara, KwaZulu Natal. The effect of root extracts from test cultivars on selected test fungi was quantified to explain the observed resistance. Similarly, root total phenol content was quantified to determine a relationship with the response of cultivars to root pathogens.

3.1 MATERIALS AND METHODS

3.1.1 *Field trials*

Twenty-six sorghum cultivars submitted to the National Cultivar Trials were evaluated for root rot resistance. Cultivars were planted at Cedara, KwaZulu Natal during the 2008/2009 growing season, in a field where root rot had been detected in previous years. Cultivars were planted in single-row plots with a 1.2 m inter-row and 0.1 m intra-row spacing. Plots were replicated three times in a randomized block experimental design. Prior to planting the field was fertilized with 600 kg 2:3:2 (N: P: K (22)). Sorgomil gold (terbuthylazine, 497.2 g/l + S-metolachlor, 102.8 g/l) + Dual Gold (metolachlor, 915 g/l) were applied pre-emergence at rates of 3.7 + 0.6 l/ha for weed control. Subsequent

weeding was done by hand as required. Decis (deltamethrin, 25 g a.i./l) was applied for stalk borer and aphid control eight weeks after planting at a rate of 250 ml/ha using a knapsade spray.

Nine weeks after planting the plants were assessed for root rot severity and root volume. Five plants from each plot were carefully removed using a garden fork and spade, aiming to recover as much of the root system as possible. Roots were washed with running tap water to remove the soil adhering to the roots. Root rot severity was visually assessed using an estimate of the percentage visible root discolouration. Root volume was measured using water displacement. Root rot severity and root volume were used to calculate what was termed effective root volume (McLaren, 1999), i.e. E= [{(100-root rot severity)/100}*root volume] where E is effective root volume. At maturity, flag leaf height and total plant length were measured. Yield could not be measured due to excessive bird damage.

Data were analysed using NCSS (Hintze, 2001). Cultivar effects on root rot, root volume and effective root volume were analysed using Analysis of Variance and means separation was done using Fischer's LSD ($P<0.05$). Relationships between root variables and plant growth variables were analysed using regression analysis.

3.1.2 *Effect of cultivar root extracts on the growth of root fungi* **in vitro**

Ten cultivars selected from those planted in Cedara to represent the range from most resistance to most susceptible to root rot were planted in 3 ℓ plastic greenhouse pots containing a mixture of 60% loam soil and 40% peat. Eight pre-germinated seeds were planted and, upon emergence, thinned to six plants per pot. Plantings were replicated three times. Pots were maintained in the greenhouse at 25/22°C day/night temperature regime and watered daily with tap water. After 9 weeks plants were removed from the pots, roots were cut off and washed with tap water to remove all adhering soil. The roots

were frozen at -80°C to prevent the degradation of the root physical and biochemical structures until the roots were used.

The roots were ground into a fine powder with a pestle and mortar by adding liquid nitrogen and the root powders were weighed. For each g of root mass, 3 ml of sterile water was added and, after agitation on a vortex mixer (Vortex Genie 2, Scientific Industries), the solution was filtered through a 4.5 µl Millipore filter. Extracts (0.5 ml) were placed in a 7 mm in diameter well at the centre of Petri dishes containing malt extract agar (Biolab).

Thirteen test fungi, isolated and identified in Chapter 2 were tested against the sorghum extracts to determine their effect on fungal growth. Two test fungus disks 7 mm diameter from actively growing potato dextrose agar (PDA - BioLab) cultures, were placed on opposite sides near the margins of the Petri dishes with the respective extracts. Control treatments were prepared in the same manner but without root extract. Each treatment was replicated three times. Petri dishes were incubated for 5 days at 24°C under UV light and measured for colony growth using the radii in the direction of the extract well. Inhibition was determined as a percentage of the control. Data were analysed using NCSS (Hintze, 2001) and cultivar, isolate and Cultivar x Isolate interaction effects were quantified using Analysis of Variance and means were separated using Fischer's LSD.

3.1.3 *Total phenol content of sorghum roots*

A 0.5 g aliquot from each of the 30 sorghum root samples (three replications of each of the ten cultivars) were suspended in 6.25 ml of 75% aqueous acetone in 15 ml test tubes. The test tubes were vortex mixed for 2 hours on a Heidolph Multi Reax Shaker (Labotec) and the extracts were centrifuged at 3500 rpm for 6 minutes. One ml of the supernatant was added to a volumetric flask that contained 70 ml de-ionized water and 5 ml Folin-Ciocalteu phenol reagent (Waterman and Mole, 1994). A 15 ml aliquot of 20% sodium carbonate was added after one minute and the flask was filled to the 100 ml mark with

de-ionized water before a critical time of 8 minutes had passed. The solution was left at room temperature for two hours. A 2 ml aliquot of the solution was placed in polyester cuvettes and assayed for total phenol content at 760 nm (Khadambi, 2007) using a T60 UV VIS spectrophotometer. Data were analysed using Analysis of Variance (NCSS, Hintze, 2001) to determine cultivar effects on root total phenol content. The relationship between total phenol content and root rot severity recorded in the field trials was determined using regression analysis.

3.2 RESULTS

3.2.1 *Field trails*

Root rot severity ranged from 29.23 - 45.60% (Table 3.1) indicating that all cultivars tested were susceptible to root rot. Analysis of variance indicated that cultivars differed significantly ($P<0.05$) in their susceptibility to root rot. PAN 8389 (45.60%), PAN 8229 (45.42%) and PAN 8625 (44.82%) were the most susceptible cultivars compared to PAN 8534 (29.23%) and PAN 8706W (29.42%) that were more resistant to root rot. Similarly, analysis of variance indicated significant differences in root volume which ranged from 4.86 - 22.58 ml (Table 3.1). PAN 8420 (9.94 ml), NS5511 (8.18 ml), PAN 8706W (7.13 ml) and PAN 8625 (4.86 ml) had the lowest root volumes compared to PAN 8358 (22.58 ml), PAN 8816 (22.08 ml), PAN 8568 (21.67 ml), PAN 8474 (21.56 ml), PAN 8609 (21.33 ml) and PAN 8534 (20.42 ml) that had the highest root volumes.

Effective root volume (Table 3.1) indicated the extent to which root rot susceptibility negated the effect of root volume and *visa versa*. Thus PAN 8358 and PAN 8568 had root volumes of 22.58 ml and 21.67 ml respectively which were reduced to 13.13 ml and 12.18 ml respectively due to root rot, representing root efficiency reductions of 41.86% and 43.79 % respectively. In contrast, PAN 8534 had a root volume of 20.42 ml that was reduced to 14.45 ml representing a root efficiency reduction of 29.24 %, which is significantly less than the above cultivars but with a similar final root efficacy result.

Flag leaf height, as an indicator of plant growth, ranged from 64.57 - 111.73 cm (Table 3.1), indicating that cultivars differed significantly in growth. PAN 8568 (66.80 cm) and PAN 8474 (64.57 cm) had the shortest flag leaf height compared to the flag leaf height of NS 5511 (111.73 cm). Similarly, total plant length (Table 3.1) ranged from 100.07 (PAN 8474) - 147.23 cm (NS 5511) with significant ($P<0.05$) differences between cultivars. Regression analysis between root rot criteria (root rot severity, root volume and root efficiency index) and growth indicators (flag leaf height and total plant length) yielded no significant relationships.

3.2.2. *Effect of cultivar root extracts on the growth of root fungi* in vitro

The highest mean percentage inhibition of the selected root fungi with root exudates was recorded with cultivar NS 5511 with 3.18% (Table 3.2). In contrast, stimulation of growth was observed with cultivar PAN 8657, i.e. with -3.24% inhibition. Analyses of variance indicated significant ($P<0.05$) Cultivar x Isolate interactions and the highest interaction, with an inhibition of 24.74%, was recorded with PAN 8706W on test fungus Bet8-2 (unidentified). The cultivar that stimulated the highest test fungal growth, with an inhibition value of -21.33% was PAN 8625 on 1/2Bet36-2 (*Alternaria* sp.). Extracts of cultivar PAN 8657 had neither growth stimulation nor inhibition on test fungus CedNie27-1 (*F. oxysporum*). All the cultivars tested had inhibition effects on test fungi Sen12-1 (*F. oxysporum*) and Wint20-1 (*F. oxysporum*). Sen27-4 (*F. oxysporum*) was the only test fungus where all the culivars, apart from PAN 8568, stimulated its growth.

3.2.3 *Determining the total phenol content of sorghum roots*

The highest phenol content was observed in root samples from PAN 8648W (tan plant type) with a total phenol content of 0.0973 mg/g (Figure 3.1) compared to the lowest phenol content of 0.0220 mg/g of NS 5655 (red plant type). PAN 8706W (tan type plant)

also had a high phenol content of 0.0803 mg/g. PAN 8625 (red type plant) had the second highest phenol content of 0.0920 mg/g. Regression analysis between plant content and root rot severity in the field yielded a non-significant relationship although a tendency for reduced root rot was observed (Figure 3.2).

3.3 DISCUSSION

The expression of resistance of sorghum to root rot pathogens in a field experiment can be attributed to certain factors, including inherent host resistance, environment and the presence of antagonistic fungi. The field in which the sorghum cultivars were planted had previously been used for sorghum cultivation and it was assumed that diverse microbiota existed in this field that contained both pathogenic and antagonistic fungi that competed for living space and nutrients. The absence of significant differences ($P<0.05$) between the different replications suggests that the distribution of pathogens and the soil environment was sufficiently homogenous to provide reliable screening data.

Root rot (Table 3.1) occurred on all the cultivars tested and analysis of variance indicated a significant ($P<0.05$) effect of cultivar on root rot severity. Root rot severity ranged from 29.23 - 45.60% which is very similar to the range of 30.30 - 41.33% recorded by McLaren (2002) and indicates that little attention has been given to the development of root rot resistance in the new sorghum cultivars being released into the South African market over the intervening 5 year period.

A significant ($P<0.05$) effect of cultivar on root volume of sorghum was recorded. Root rot, as determined by water displacement, ranged from 4.86 - 22.58 ml which is considerably less than the range of 24.93 - 35.33 ml recorded by McLaren (2002), again supporting the notion of a lack of attention to root health in sorghum breeding programmes locally. This cultivar attribute is important in the ability of sorghum to compensate for the reduction in absorbent area of roots due to root rot. Thus, PAN 8706W which was more resistant to root rot with a root rot severity of 29.42%, but with a

root volume of 7.13 ml, had an effective root volume of 5.03 ml. PAN 8568 had an effective root volume of 12.18 ml despite root rot severity of 43.80% due to a root volume of 21.67 ml. McLaren (2000) recommended a dual criterion assessment for root rot assessment in sorghum cultivars. Thompson (1968) indicated a similar requirement in maize where, apart from the actual recorded root rot, root size and root clump mass were highly correlated with lodging incidence. Similarly, Nass and Zuber (1971) reported a relationship between root mass and volume with root pulling strength at maturity and this vertical pulling strength was used as an evaluation criterion for the selection of root rot resistant maize.

The absence of a significant relationship between plant growth assessment criteria and root rot or effective root volume in the current study is contrary to previous results (Hoffmaster, 1942; Sumner and Bell, 1982; McLaren, 2002) where stunting and poor head formation were recorded. In the current study plants were evaluated after nine weeks (*sensu* McLaren, 2002; 2004), the growth stage where, in previous studies, the highest correlations between growth and effective root volume were recorded. This was approximately two weeks prior to flowering. This time scale may have been premature and damage to plants in this interim period as a result of root rot may not have been accounted for. In future studies multiple assessment dates should be considered to re-assess root rot development in relation to plant growth.

In this study no relationship between the effect of root extracts (Table 3.2) on selected test fungi and observed root rot severity in the field was recorded. The closest relationship was R^2=0.35 between the effect of root extracts on Wint7-2 (*Fusarium* sp.) and observed root rot severity in the field. Tendencies, by inspection, were however observed. The highest colony growth of all the fungi tested against root extracts was with cultivar PAN 8657 where growth stimulation was observed. This cultivar also had a high root rot severity of 41.03% in the field.

However, PAN 8648W and PAN 8706W, both white-tan type sorghum cultivars stimulated and inhibited the growth of test fungi respectively but yielded field root rot

severities of 29.53% and 29.42%. This suggests that root biochemical content, as measured in the current study, does not play a major role in the expression of root rot resistance in the sorghum genotypes included in this study.

Furthermore, some of the fungi tested were inhibited and some were stimulated and this indicated that the effect root exudates had on the test fungi was, to some extent, dependent on the test fungus itself. Sen12-1 (*Fusarium oxysporum*) was the test fungus that was the most inhibited and CedNie7-2 (*F. oxysporum*) was the least inhibited indicating that intra-species diversity may also be an important factor. Previous studies have indicated a relationship between charcoal rot resistance in sorghum and sugar and phenol levels (Anahosur *et al.,* 1985; Patil *et al.,* 1985; Rao *et al.,* 1990), but this relationship between host biochemistry and disease resistances does not appear to apply to root rots and may only be relevant to the stalk tissues and the specific pathogen.

Chambers (1987) recorded differences in root rot resistance in maize cultivars but found no correlation between the reaction of cultivars to root rot and stalk rot resistance. The study therefore needs to be widened to identify other mechanisms of resistance. McLaren and Brönn (1987), in a field trial, found that resistant sorghum lines had higher levels of lignified exodermis cell layers compared to the susceptible lines. Cell wall thickness was also greater in resistant lines and these mechanisms may warrant further study. Similarly, Shertz & Rosenow (1977) in studies on resistance to charcoal rot, found large differences in the number of cells in sorghum stalks with lignified walls. Differences in the degree of lignification in the epidermis, sub-epidermis and vascular bundles were also recorded and lodging resistant lines generally had the most lignification.

All sorghum cultivars contain phenolic compounds, the only difference being the amount that is produced (Hahn et. al., 1984). Red sorghums types, especially those with a red pericarp, are generally thought of as more resistant to pathogen attacks, particularly to grain molds, due to a vast selection of metabolites, in particular considerable amounts of extractable phenols. White sorghums on the other hand have very low phenol content and are therefore expected to be more susceptible to pathogen attack (Awika and Rooney,

2004). Khadambi (2007) tested the antimicrobial activity of phenolic compounds from condensed tannin (red sorghum) and tannin-free (white sorghum) varieties on *Bacillus cereus, Escherichia coli* and *Listeria monocytogenes* at different concentrations and found that extracts from the condensed tannin variety had a higher significant inhibition on the bacterial isolates tested, compared to tannin-free sorghum extracts. It was concluded that the low levels of phenolic compounds found in tannin-free sorghum were unable to inhibit the bacterial isolates. This, however, was not apparent in root tissues observed in the current study. PAN 8648W and PAN 8706W, white-tan sorghum types, were in the top three cultivars with regard to root rot resistance and were among the cultivars that had the highest root phenol contents. This observation also indicates that root and head tissues may not be comparable as far as phenol and other physiological activities are concerned and that extrapolations are therefore not possible. This further calls for more detailed studies aimed specifically at root tissues.

Another consideration may be the extent to which differences in the ability of sorghum germplasm to react to infection occur. Hammerschmidt and Nicholson (1977), for example found that resistant and hypersensitive-resistant maize had different rates of changes in total phenols than susceptible lines subsequent to infection with *Colletotrichum graminicola.* This was mainly due to accumulation of phenols, probably flavones, in the surrounding tissues of the lesions. These compounds were also toxic to the fungus and this suggested that the rate of phenol accumulation was involved in the resistance of maize towards *C. graminicola*.

3.4 CONCLUSION

Plants seem defenseless, especially with their immobility, but they have a few inherent defense mechanisms not detectable by the naked eye and these defenses are triggered when plants are under attack by pathogenic fungi or other pests. Sorghum cultivars differed in their response to root rot pathogens although the level of resistance expressed was not sufficiently high to be a significant commercial value. All sorghum cultivars

tested contained biochemical compounds that either inhibited or stimulated the growth of test fungi which could play a role in the resistance or susceptibility of sorghum cultivars to pathogenic root fungi. A tendency for reduced sorghum root rot severity with increased total extractable phenols in roots was observed that indicated that higher phenol content resulted in higher resistance towards root rot. Other resistance mechanisms could also play a significant role in reducing the incidence of root rot, but this needs further investigation.

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Table 3.1 Root rot severity, root volume, effective root volume, flag leaf height and total plant length observed on 26 sorghum cultivars tested in the field at Cedara, Kwa-Zulu Natal during the 2008/09 growing season.

Cultivar	Root rot severity (%)	Root volume per plant (ml)	Effective root volume (ml)	Flag leaf height (cm)	Total pant length (cm)
PAN 8389	45.60	10.89	5.92	76.17	119.97
PAN 8229	45.42	13.44	7.34	92.13	130.50
PAN 8625	44.82	4.86	2.68	92.10	132.53
PAN 8568	43.80	21.67	12.18	66.80	103.13
PAN 8157	42.69	18.61	10.67	78.37	112.63
PAN 8358	41.87	22.58	13.13	82.40	116.97
PAN 8657	41.03	19.33	11.40	81.90	120.00
NS 5511	39.27	8.18	4.97	111.73	147.23
PAN 8420	38.48	9.94	6.12	79.27	118.70
PAN 8446	38.46	14.06	8.65	76.37	110.87
PAN 8564	37.17	19.42	12.20	104.13	140.63
PAN 8609	36.49	21.33	13.55	81.03	121.60
Banjo	36.03	14.53	9.29	70.67	106.87
Overflow	35.76	12.02	7.72	87.80	122.00
PAN 8553W	35.61	11.45	7.37	81.57	116.97
PAN 8474	35.60	21.56	13.88	64.57	100.07
PAN 8806	34.40	12.33	8.09	79.83	116.13
NS 5655	34.22	14.75	9.70	95.43	132.37
PAN 8816	33.67	22.08	14.65	82.33	117.67
PAN 8247	33.11	13.44	8.99	88.30	127.20
Mr.Buster	32.93	11.40	7.65	80.20	123.13
PAN 8353	32.62	12.17	8.20	83.03	121.50
PAN 8556	31.18	15.78	10.86	74.10	111.70
PAN 8648W	29.53	17.40	12.26	89.83	128.43
PAN 8706W	29.42	7.13	5.03	91.27	130.30
PAN 8534	29.23	20.42	14.45	75.23	110.17
LSD P<0.05	**5.77**	**5.2**	**2.8**	**3.45**	**4.05**

Table 3.2 Inhibition of fungi *in vitro* as a result of root extracts from 10 sorghum cultivars growing in the greenhouse and representing a range of root rot severities in the field.

Cultivar	Percentage Inhibition (mm)						
	Bet8-2 (unidentified)	Bet26-4 (*Acremonium strictum*)	1/2Bet36-2 (*Alternaria* sp.)	Bet28-1B (*Alternaria alternata*)	Bet13-2 (*F. oxysporum*)	CedNie7-2 (*F. oxysporum*)	CedNie27-1 (*F. oxysporum*)
NS 5511	9.55	-0.12	14.09	8.20	-5.20	-4.96	4.31
NS 5655	1.67	-2.51	15.18	4.55	-3.29	0.88	2.90
PAN 8420	5.95	0.86	8.19	-4.34	1.26	-8.78	-2.43
PAN 8657	-11.57	-5.33	-9.58	-12.16	-2.05	-2.53	0.00
PAN 8534	-11.39	2.42	14.83	-4.45	-0.84	-2.71	-2.89
PAN 8806	7.01	-0.82	-9.62	6.08	-1.05	1.09	-0.64
PAN 8625	2.38	0.25	-21.33	6.94	-3.01	-6.73	6.59
PAN 8648W	-6.63	0.39	-10.16	-6.14	-6.58	-5.56	3.93
PAN 8706W	24.74	5.62	-20.98	4.11	-7.52	-4.23	-0.78
PAN 8568	2.15	-0.33	9.29	5.94	2.92	-2.47	1.69
Mean	**2.39**	**0.04**	**-1.01**	**0.87**	**-2.54**	**-3.60**	**1.27**

LSD ($P<0.05$): Cultivar = 0.08; Isolate=0.13; Cultivar x Isolate interaction=1.08;

Table 3.2 Continued

Cultivar	Percentage Inhibition (%)						
	Sen12-1 (*F. oxysporum*)	Sen27-4 (*F. oxysporum*)	Wint10-5 (*Fusarium* sp.)	Wint23-1 (*F. oxysporum*)	Wint20-1 (*F. oxysporum*)	Wint7-2 (*Fusarium* sp.)	**Mean**
NS 5511	11.44	-0.40	0.19	-2.49	5.13	1.57	**3.18**
NS 5655	11.90	-3.17	1.83	2.95	5.60	1.67	**3.09**
PAN 8420	6.29	-1.11	9.57	1.92	1.24	-0.51	**1.39**
PAN 8657	3.14	-2.96	6.24	-5.63	0.87	-0.53	**-3.24**
PAN 8534	16.64	-6.67	-3.06	-2.48	0.18	-0.67	**-0.08**
PAN 8806	11.55	-7.13	3.79	-1.24	3.46	1.29	**1.06**
PAN 8625	6.73	-7.47	6.64	-0.03	0.51	-1.12	**-0.74**
PAN 8648W	9.41	-4.49	-1.36	0.66	0.55	6.41	**-1.51**
PAN 8706W	5.55	-3.02	2.84	-2.19	3.30	7.36	**1.14**
PAN 8568	11.86	1.43	-5.49	-0.55	3.34	1.79	**2.43**
Mean	**9.45**	**-3.50**	**2.12**	**-0.91**	**2.42**	**1.73**	**0.67**

LSD (P<0.05): Cultivar = 0.08; Isolate=0.13; Cultivar x Isolate interaction=1.08;

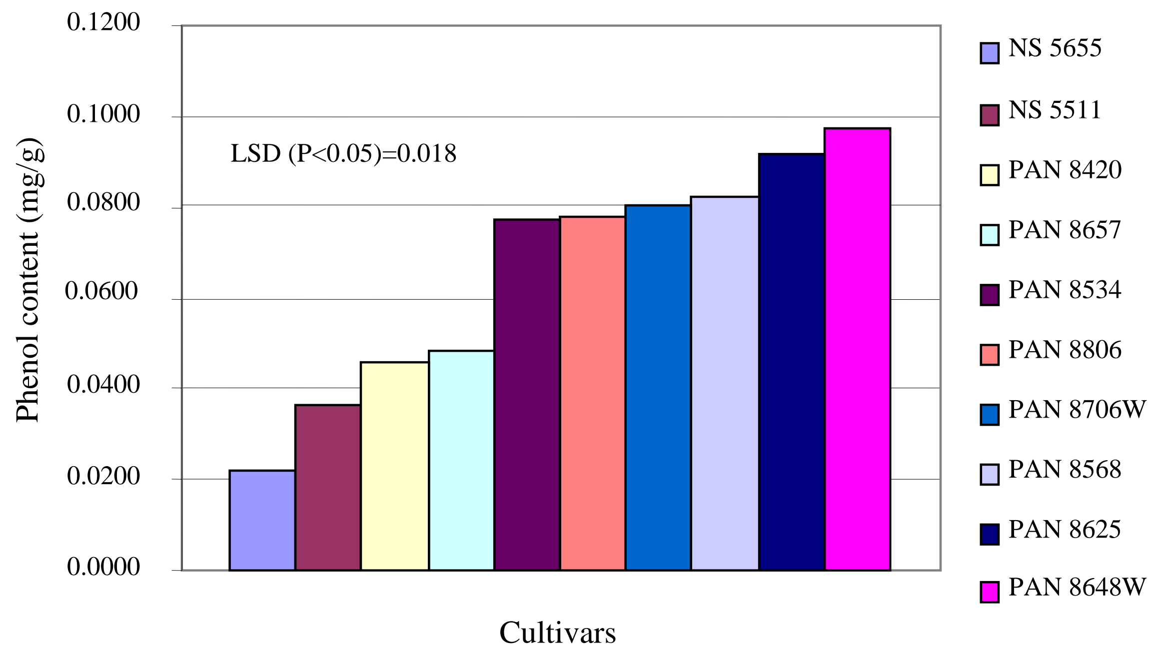

Figure 3.1 Total phenol content of root extracts from 10 sorghum cultivars representing a range of root rot severities in the field grown in the greenhouse

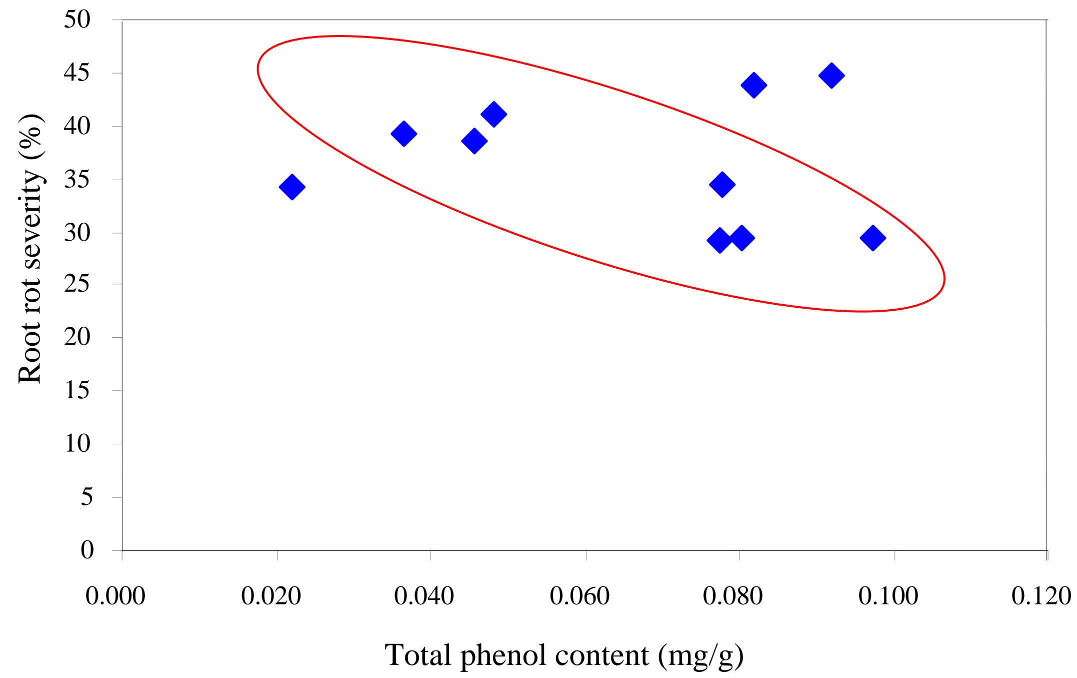

Figure 3.2 Relationship between root rot severity recorded in 10 selected sorghum cultivars in a field trial at Cedara and total phenol content of roots

CHAPTER 4

EVALUATION OF BIOLOGICAL CONTROL AGENTS FOR THE SUPPRESSION OF SORGHUM ROOT ROT PATHOGENS

Abstract: Root rot diseases of sorghum are caused by a complex of soilborne fungi, including a number of *Fusarium* spp. Root rot control has not received much attention due to symptoms not being obvious in the aerial canopy. In this study, biological control was considered as a control measure. Isolates of *Trichoderma* spp. from sorghum roots were tested against a number of fungi isolated from sorghum roots. In dual culture, mean inhibition of growth of test fungi ranged from 66.84% with isolate CedNie8-1 (*Trichoderma* sp.) to 79.71% with isolate Bet18-1 (*Trichoderma* sp.). Inhibition of test fungal growth, as a result of either the production of volatile or non-volatile substances, was measured. Isolate Sen2-2 (*T. harzianum*) resulted in the highest inhibition of 21.48% compared to Kl30-1 (*Trichoderma* sp.) that showed only 4.70% inhibition due to volatile substances. Kl23-1 (*T. virens*) had the highest inhibition of 39.30% compared to Wint4-2 (*T. harzianum*) of 6.79% due to non-volatile substances. Root colonization of the *Trichoderma* spp. on four sorghum cultivars was measured *in vitro*. CedNie10-3 (*T. harzianum*) provided the highest mean colony count of 34222 cfu (colony forming units) compared to Sen16-2 (*T. harzianum*) only 4780 cfu's indicating differences in the colonization ability of isolates. A significant ($P<0.05$) Cultivar x Isolate interaction was also recorded. In the greenhouse, using naturally infested soil, Ced3-1 (*T. hamatum*) resulted in the lowest root rot percentage of 56.15% compared to Kl23-1 (*T. virens*) with 61.66% which was higher than the control, suggesting that isolate Kl23-1 (*T. virens*) was responsible for some of the symptoms observed. Overall, *Trichoderma* spp. treatments were not effective in the control of sorghum root rot. Rhizosphere colonization was highest with Ced3-1 (*T. hamatum*) with 7745 cfu/g soil compared with 3627 cfu/g by Kl23-1 (*T. virens*) and competition for food and space was suggested as a mode of action.
Keywords: sorghum, root rot, biological control, *Trichoderma* spp.,

4.0 INTRODUCTION

Pest and pathogen attacks can lead to annual yield losses of 30% in sorghum (Chandrashekar and Satyanarayana, 2006). The management of diseases and pests are therefore important to enhance food quality and the yield of sorghum. Root rot pathogens are overlooked due to symptoms on roots not being obvious in the aerial canopy (Tarr, 1962) and the ability of sorghum to compensate to some extent for root loss due to root pathogens. Root rot of sorghum is caused by soilborne fungi, which include *Macrophomina phaseolina, Colletotrichum graminicola, Periconia circinata*, *Fusarium* spp. and *Pythium* spp. (Mughogho, 1984). The disease is usually the result of a complex of these and other opportunistic fungi and colonization of the host tissue is dependent on environmental conditions that predispose the host to infection and enhance the infection process of a specific pathogen at a specific time (Mughogho and Pande, 1984).

The first International Symposium on soilborne plant pathogens was held at Berkeley in 1963, and subsequently, there has been a growing awareness of the threat associated with the use of harmful fungicides to control plant diseases in agriculture. This, as well as the cost of developing a new fungicide (approximately $30,000,000 in 1990) and pathogen resistance towards fungicides have led to increased efforts to control plant diseases without chemical application (Chet, 1990). This gave rise to the development of biological control. Biological control can be considered an alternate or supplementary method of reducing the use of fungicides (Compant *et al*., 2005). Biological control can be defined as "the action of parasites, predators and pathogens in maintaining another organism's density at a lower average than would occur in their absence" (DeBach, 1964).

Fungi are a very diverse group of organisms, with about 230,000 species dispersed in each ecosystem. Only a few of these species display biocontrol properties. The possibility of using antagonistic microorganisms to control pathogenic fungi has received increasing attention (Chet, 1990). *Trichoderma* species are usually considered soilborne organisms associated with the roots of plants (Bailey *et al.,* 2008) and have been

particularly effective as biological control agents against pathogens such as *Rhizoctonia solani* (Chet, 1990).

Mechanisms involved in the inhibition of pathogens by biocontrol agents (BCA's) include competition (Compant *et al*., 2005; Leipoldt, 2007), antibiosis (Dennis and Webster, 1971a; Dubey *et al.*, 2007; Leipoldt, 2007), hyper-parasitism (Leipoldt, 2007), rhizosphere/root colonization (Leipoldt, 2007), nutrient mineralization (Leipoldt, 2007) and systemic acquired resistance (Leipoldt, 2007). Antibiosis is probably the most important mechanism. Studies have shown that antagonistic strains of *Trichoderma* spp. and *Fusarium* spp. have the ability to produce volatile and non-volatile antimicrobial metabolites that aid in their biocontrol activity (Dennis and Webster, 1971a; Dennis and Webster, 1971b; Mathivanan *et al.,* 2008).

The minimum amount of *Trichoderma* spp. needed for effective biological control is estimated at 1 x 10^6 cfu (colony forming units)/g of soil and methods of introducing *Trichoderma* spp. into the field include the distribution of *Trichoderma* spp. on the surface of infested soil, placing *Trichoderma* spp. into the planting furrow, loading *Trichoderma* spp. into the rhizosphere (soil surrounding roots) before seedlings are planted and coating of seeds with *Trichoderma* spp. spores using an adhesive. The last three methods are more cost effective compared to the surface distribution method (Chet, 1990). *T. harzianum* isolate T-22 is marketed for the control of different diseases in many plant crops and applied as either a soil amendment or seed treatment. In the treatment of foliar disease of apple, grape or other fruit crops, it can be applied directly to leaves (Bailey *et al.,* 2008).

The most important factors that determine the success of a potential biocontrol agent (BCA) is the ability to effectively colonize the plant root and survive in the soil in the presence of growing roots and indigenous microorganisms over an extended time period (Compant *et al*., 2005). A major concern in the use of biocontrol agents in suppressing pathogens is the non-target effects this may have on the environment, non-target organisms and biogeochemical cycles (Winding *et al.,* 2004). Many biocontrol agents

not only suppress a narrow group of target pathogens, but can also inhibit other organisms in the soil and on different crops (Winding *et al.,* 2004). More research is needed to determine the impact BCA's can have on the environment.

The purpose of this study was to quantify the effect of *Trichoderma* spp., isolated from the roots and rhizosphere of commercially grown sorghum, on root rot fungi, determine the modes of action that inflict the inhibiting effect and to determine whether the biological control agent can survive on the root surface and in the soil surrounding the root.

4.1 MATERIALS AND METHODS

4.1.1 *Isolation of Biological control agents (BCA's)*

Nineteen isolates of *Trichoderma* spp. (Table 4.1) were identified from fungi initially isolated from sorghum roots (Chapter 2) and maintained on Petri-dish plates containing potato dextrose agar (PDA; BioLab). These *Trichoderma* spp. were identified using sequencing as fully discussed in Chapter 2. Six isolates appear to be unmapped species and could not be fully identified (Table 4.1).

4.1.2 *Dual Cultures*

The potential of BCA's in the inhibition of the growth of root rot pathogens was tested using 19 *Trichoderma* spp. and 15 fungi isolated from sorghum roots (Table 4.1). The latter were selected to represent the range of isolates from sorghum roots (Chapter 2). Petri-dishes with PDA (BioLab) were inoculated with a 7 mm disk of the respective BCA using an ethanol-and-heat sterilized cork borer and placed 5 mm from the edge of the Petri-dish. Similarly, a 7 mm disk of the test fungus was placed in the centre of the Petri dish. Controls had agar disks without BCA's. Each treatment was replicated four times.

The Dual Cultures were grown under UV light at 25°C. The radial growths of the test fungi were measured after four days and inhibition was determined as a percentage of the control (Dubey *et al.,* 2007).

4.1.3 ***Substances produced by*** **Trichoderma** ***species.***

4.1.3.1 *Volatile substances*

Tests were conducted to determine whether the inhibition recorded in the Dual Culture experiment was a result of the production of volatile substances. Petri-dishes containing 5% malt extract agar (MEA; BioLab) were each inoculated with a single 7 mm centrally placed agar disk cut from each of 11 *Trichoderma* spp. cultures (Table 4.3) grown on PDA and selected from those displaying inhibition in Dual Culture. The cultures were placed near a UV light for one week, after which the lid of each dish was discarded and replaced by the bottom of a Petri dish containing 5% MEA inoculated with a 7 mm disk of each of the 15 test fungi. The plates were sealed with parafilm. The control plates were not inoculated with *Trichoderma* spp. but the lids were replaced in the same way. Treatments were replicated four times. After four days near UV light at 25°C the diameter of colonies of the test fungus were measured and compared with the control plates. Inhibition was determined as a percentage of the control (Dennis and Webster, 1971b).

4.1.3.2 *Non-volatile substances*

To determine whether non-volatile substances produced by *Trichoderma* spp. play a role in the inhibition of root pathogens, a piece of heat-sterilized cellophane (dialyses membrane) was placed onto Petri-dishes containing 5% malt extract agar. Petri-dishes were left overnight on a laminar flow bench to ensure the evaporation of excess moisture. Disks, 7 mm in diameter from 11 *Trichoderma* spp. cultures (Table 4.5), growing on

PDA, were placed in the centre of the respective Petri-dishes and the Petri-dishes were placed near UV light at 25°C for 48 hours. The cellophane with the *Trichoderma* spp. was removed and the 15 test fungi were placed in the centre of the respective Petri dishes, where the *Trichoderma* spp. isolate had been positioned. Treatments were replicated four times. After 48 hours incubation at 25°C near UV light, the colony diameters of the test fungi were measured. Inhibition was determined as a percentage of the control (Dennis and Webster, 1971a).

4.1.4 *Root colonization* in vitro

Ten 7 mm disks of each of the 19 *Trichoderma* spp. actively growing on PDA (BioLab) were placed in Erlenmeyer flasks containing 100 ml nutrient broth. Flasks were placed on a rotary shaker for five days at 25°C for inoculum production. Sorghum seeds from four cultivars (Table 4.7) were surface sterilized in 70% ethanol for five minutes followed by one minute in 1% sodium hypochlorite, and rinsed three times with sterile tap water. Fifteen seeds were placed on Petri-dishes containing water agar (WA; BioLab). One milliliter of each inoculum was added to the seeds on the WA and incubated overnight to ensure the adhesion of the *Trichoderma* spp. to the seeds. The control seeds were inoculated with nutrient broth without *Trichoderma* spp. The seeds were transferred onto fresh WA and incubated near UV light at 25°C for another five days to allow root development. The seed and newly developed roots were placed in sterile water, shaken vigorously for one minute and serially diluted to 10^{-3}. One milliliter of the aliquot was placed on half strength malt extract agar (BioLab) and incubated to determine colony counts (Idris *et al.,* 2007).

4.1.5 *Greenhouse evaluation of* Trichoderma *spp. suppression of sorghum root rot*

Sorghum seeds of PAN 8706W and PAN 8420 were surface sterilized five times in hot water at a temperature of 60°C for approximately five minutes and pre-germinated on

WA (BioLab) for 24 hours at 25°C. Six germinated seeds were planted into one liter greenhouse pots containing soil from a sorghum plot collected at the ARC-Grain Crops Institute in Potchefstroom and naturally infested with root rot pathogens. Conidial suspensions of three *Trichoderma* spp. prepared from five 7 mm PDA agar plugs shaken up in 10 ml sterile water and left overnight near UV light, were added to the seeds in the soil at a rate of 0.25 ml solution per seed per pot (Dubey *et al.,* 2007). Treatments were replicated three times. A control in which no seed treatment was applied, was included. Six weeks after planting, each sorghum plant was carefully removed from the pots and washed with running tap water. Sorghum root rot was recorded as the percentage visible root discolouration. Root (crown to the tip of the longest root) and shoot length (crown tot the tip of the longest leaf) was also measured.

4.1.6 *Rhizosphere colonization*

To determine rhizosphere colonization, rhizosphere soil from each of the pots used in the greenhouse experiment was collected and one g of soil was placed into glass vials containing 10 ml sterile water. Serial dilutions to 10^{-3} were made and 1 ml was plated onto Petri-dishes containing half strength malt extract agar. The plates were incubated near UV light at 25°C and colony counts were done after 1 - 2 days. Treatments were replicated three times (Idris, *et al.,* 2007).

Treatment effects of all the studies were analyzed using NCSS (Hintze, 2001) by means of analyses of variance and means separation was done using Fischer's LSD ($P<0.05$).

4.2 RESULTS

4.2.1 *Dual Cultures*

Analysis of variance indicated significant ($P<0.05$) differences in the inhibition of test fungi by *Trichoderma* spp. as well as a significant *Trichoderma* spp. x Test fungus interaction. Inhibition was observed with all 19 *Trichoderma* spp. against all 15 test fungi (Table 4.1). Bet18-1 (*Trichoderma* spp.) displayed the highest mean inhibition of 79.71% tested against all the test fungi with CedNie 8-1 (*Trichoderma* spp.) having the lowest mean inhibition of 66.84%. The highest inhibition percentage of 92.96% was recorded with Sen16-2 (*T. harzianum*) on test fungus Wint6-4 (unidentified), with the least inhibition of 30.56% with Ced3-1 (*T. hamatum*) on PBet39-1 (unidentified). Wint6-4 (unidentified) was the most inhibited test fungus with a mean percentage inhibition of 87.22% and PBet39-1 (unidentified) the least with a mean of 57.76%.

When the actual physical growth of the test fungi was compared (Table 4.2), analysis of variance indicated significant ($P<0.05$) differences and respective interaction effects. CedNie8-1 (*Trichoderma* spp.) had the least effect on the growth of the test fungi with a mean radial growth of 16.54 mm and Wint21-2 (*T. harzianum*) had the most effect on the growth of the fungi tested with a mean radial growth of 10.36 mm. The test fungus with the lowest growth was 1/2Bet36-2 (*Alternaria* sp.) with a radial growth of 9.34 mm and the test fungus with the highest growth was Wint10-5 (*Fusarium* sp.) with a radial growth of 19.70 mm when all the treatments were considered. The lowest test fungal growth was with Sen2-5 (*T. harzianum*) which restricted the growth of test fungus PBet39-1 (unidentified) the most with a mean radial growth of 3.96 mm while Sen2-2 (*T. harzianum*) resulted in the least restriction of test fungus Wint10-5 (*Fusarium* sp.) with a mean radial growth of 26.55 mm.

4.2.2 *Substances produced by* **Trichoderma** *species*

4.2.2.1 *Volatile substances*

Analysis of variance indicated significant (P<0.05) differences in the inhibition of test fungi by *Trichoderma* spp. as a result of volatile substances as well as *Trichoderma* spp. x Test fungus interactions. Kl30-1 (*Trichoderma* sp.) resulted in the lowest percentage inhibition of 4.70% and Sen2-2 (*T. harzianum*) the highest with 21.48% (Table 4.3). This corresponded with the radial growths of *Trichoderma* spp. where Kl30-1 (*Trichoderma* sp.) had the highest mean radial growth of 39.47 mm compared to the mean radial growth of 32.01% for Sen2-2 (*T. harzianum*) (Table 4.4). CedNie7-2 (*F. oxysporum*) was the test fungus best inhibited by all the *Trichoderma* spp. tested with a mean inhibition percentage of 37.41% while the lowest inhibition with Wint10-5 (*Fusarium* sp.) of -29.90%, indicated a stimulation in the growth of the test fungus. This could be a result of some of the metabolites that triggered rapid test fungal growth and this growth stimulation can be seen with all, except three of the *Trichoderma* spp. tested i.e. 1/2Bet 13-1 (*Trichoderma* sp.), Sen2-2 (*T. harzianum*) and Sen16-2 (*T. harzianum*) which inhibited all the test fungi without stimulating any growth.

4.2.2.2 *Non-volatile substances*

Analysis of variance indicated significant (P<0.05) differences in the inhibition of test fungi by *Trichoderma* spp. as a result of non-volatile substances as well as interactions between the two entities. *Trichoderma* spp. isolate Wint4-2 (*T. harzianum*) resulted in the least overall inhibition of 6.79%, compared with Kl23-1 (*T. virens*) which provided the highest mean inhibition of 39.30% (Table 4.5). Bet8-2 (unidentified) was the test fungus most inhibited by a mean of 35.81% and 1/2Bet36-2 (*Alternaria* sp.) was the least inhibited with a mean inhibition of 7.75%. There were some *Trichoderma* spp. that stimulated test fungus growth (Table 4.6), but this was much less compared with that produced by volatile substances. For example with volatile substances, Wint10-5

(*Fusarium* sp.) experienced growth stimulation with eight of the eleven *Trichoderma* spp. used, but with non-volatile substances none of the *Trichoderma* spp. stimulated any growth of Wint10-5 (*Fusarium* sp.), which suggests that growth stimulation as a result of metabolite production is also dependant on the susceptibility of that test fungus to a particular metabolite.

4.2.3 *Root colonization* in vitro

Analysis of variance indicated significant ($P<0.05$) cultivar, *Trichoderma* spp. and Cultivar x *Trichoderma* spp. interactions in the colonization of roots *in vitro* by *Trichoderma* spp. CedNie10-3 (*T. harzianum*) was the most efficient colonizer of roots with a mean of 34222.50 colony forming units (cfu), compared to Sen16-2 (*T. harzianum*) with a mean colonization of 4780.00 cfu (Table 4.7). Some *Trichoderma* spp. treatments resulted in incompatible interactions, notably Sen16-2 (*T. harzianum*) that only colonized one cultivar, namely PAN 8389 with 19120.00 cfu.

4.2.4 *Greenhouse evaluation of* Trichoderma *spp. suppression of sorghum root rot*

Although there were no significant differences between the *Trichoderma* spp. treatments on root rot severity (Table 4.8), there was a significant difference between cultivars and their susceptibility to root rot. PAN 8706W had the lowest mean percentage root rot of 55.56%, compared with PAN 8389 with a mean root rot severity of 60.92%. A tendency occurred where the percentage root rot was lower (56.15%) in pots treated with Ced3-1 (*T. hamatum*) and Ced3-2 (58.28%), compared with Kl23-1 (*T. virens*) with a mean root rot percentage of 61.66% compared to the control with a percentage root rot of 60.58%. The highest root rot percentage was recorded in PAN 8625 treated with Kl23-1 (66.75%) and the lowest root rot percentage in PAN 8625 treated with Ced3-1 (52.11%). It is only with PAN 8389 that the control treatment had the highest percentage root rot compared to the *Trichoderma* spp. treatments. With all the other cultivars, some of the *Trichoderma*

spp. caused more root rot than the control and it is suggested that *Trichoderma* spp. are capable of producing some of the symptoms recorded on the roots.

The highest mean shoot length (Table 4.9) of 24.91 cm was recorded with Ced3-2 (*T. hamatum*) and the lowest of 22.38 cm with Ced3-1 (*T. hamatum*) although the differences were not significant. On cultivar PAN 8625 the differences in the ability of the two isolates within the same species to influence plant growth is evident in length differences of 2.53 cm. This indicates some diversity between strains of the same species.

On cultivar PAN 8706W (white grain) the shoot length (Table 4.9) of the control was higher than in the treated pots and on cultivar NS 5511 (red grain) the situation was reversed with the control having the lowest shoot length compared to the treated pots. This demonstrates the different effects of treatments on shoot growth, by either stimulating or inhibiting growth, therefore illustrating a *T* x C interaction. Kl23-1 (*T. virens*) resulted in the highest overall shoot length of 32.39 cm.

4.2.5 *Rhizosphere colonization*

Analysis of variance indicated significant ($P<0.05$) differences in the *Trichoderma* spp. treatments and cultivars tested, although there was no significant *Trichoderma* spp. x Cultivar interaction. Soil from pots with PAN 8625 had the highest mean counts of 9326.67 cfu/g of soil and cultivar NS5511 the lowest with 3743.33 cfu (Table 4.10). *Trichoderma* spp. isolates Ced3-1 (*T. hamatum*) and Ced3-2 (*T. hamatum*) had almost twice the colony counts of isolate Kl23-1 (*T. virens*).

Trichoderma hamatum isolates Ced3-1 and Ced3-2 not only showed significant root colonization *in vitro* in the previous study, but also colonized the rhizosphere of the sorghum plants effectively, whereas isolate Kl23-1 (*T. virens*) had a lower mean colony count. The highest rhizosphere colonization was recorded with Ced 3-1 (*T. hamatum*) on cultivar PAN 8625 with a colony count of 15740.00 cfu and the poorest colonization with Kl23-1 (*T. virens*) on PAN 8625 of 2680.00 cfu. On cultivar PAN 8389 the highest

colony count was found with Ced3-1 (*T. hamatum*) of 5580.00 cfu, with the colony counts of the other isolates being similar to one other. On cultivar PAN 8706W the roles are changed, with Ced3-2 (*T. hamatum*) having the highest colony counts of 11170.00 cfu, compared to the others. On cultivar NS 5511 all three isolates portrayed approximately the same colonization ability.

4.3 DISCUSSION

Numerous *Trichoderma* spp. have been used as antagonists against plant pathogens, in particular *T. virens*, *T. harzianum*, *T. viride* and *T. hamatum* (Mathivanan *et al.,* 2008). In the present dual culture study all the *Trichoderma* spp. isolated from sorghum roots inhibited the growth of the test fungi. These included *T. virens*, *T. harzianum*, *T. hamatum* and *T. spirale.* According to Dubey *et al.* (2007), cultures of *T. viride* are the highest fungal growth inhibitors compared with other *Trichoderma* spp. against chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris,* followed closely by *T. harzianum* in dual culture experiments. Dual culture studies performed by Coskuntuna and Özer (2008) showed that *T. harzianum* inhibited the growth of *Fusarium oxysporum* f. sp. *cepae*, the causal organisms of basal rot on onion, by up to 73.3%. *T. harzianum* and *T. viride* inhibited the growth of *F. oxysporum*, the cause of root rot of sorghum, by 57.7% and 49.8% respectively, with the control completely overgrown in dual cultures (Al-Jedabi, 2009). Dual culture studies conducted by Dubey *et al.* (2007) indicated that the observed hyphal interactions on plates may be due to hyphae of the *Trichoderma* spp. being able to coil around pathogen hyphae and kill them, penetrate them directly or just overgrow them. *Trichoderma* spp. have the ability to grow rapidly, giving them an important advantage against plant pathogenic fungi in the search for nutrients and space (Barbosa *et al*., 2001).

Trichoderma spp. also have the ability to produce metabolites such as a variety of volatile and non-volatile substances which have different effects on different target fungi. These effects were recorded in the current study where 67% and 73% of all the *Trichoderma*

spp. tested reduced the growth of the test fungi by the production of volatile or non-volatile substances respectively. All the *Trichoderma* spp. tested showed some degree of inhibition when the mean response of test fungi were taken into account with respect to both volatile and non-volatile substances. Differences in the inhibition effect with different isolates of the same species were also observed where five isolates of *Trichoderma harzianum* displayed differences in their inhibition of all the test fungi. This could be as a result of a different range of chemical compounds being produced or metabolites from the same complexities, but in different proportions (Dennis and Webster, 1971b). In a study conducted by Barbosa *et al*. (2001), *Trichoderma* spp. produced compounds such as trichodermin, trichodermol, harzianum A and harzianolide that were mostly responsible for the inhibition of *Cladosporum herbarum*.

The effect of volatile and non-volatile substances is also dependent on the test fungus. This effect was observed with Wint10-5 (*Fusarium* sp.) where volatile substances stimulated growth of this fungus, compared to non-volatiles where the growth was inhibited. Dubey *et al.* (2007) found that volatile substances produced by *T. viride* inhibited the growth of *F. oxysporum* f. sp. *ciceris,* followed by *T. virens* and *T. harzianum*, while non-volatile substances produced by *T. harzianum* caused maximum growth inhibition of the pathogen. Dennis and Webster (1971b) in studies of volatile substances produced by *Trichoderma* spp., found that mycelial growth inhibition or stimulation could be explained by the variability in the growth potential of the pathogen tested.

Barbosa *et al*. (2001) showed that when *Cladosporium herbarum* was grown on a Petri-dish previously inoculated with *Trichoderma* spp., antagonism was seen and non-volatile substances were primarily responsible for the inhibition of growth and sporulation. *T. polysporum* isolate was more effective than *T. harzianum* and *T. viride*. *T. polysporum* was also the only isolate that inhibited some fungal growth by volatile substances, indicating dual modes of action.

Some substances can initiate different growth patterns in test fungi. Dennis and Webster (1971b) indicated differences in growth pattern of cultures of *Pythium ultimum* grown in the presence or absence of volatile metabolites produced by *T. viride*. In the presence of volatile metabolites the test fungus displayed stunted growth with the hyphae being more branched than normal.

Bailey *et al.* (2008) suggested that isolates of *T. harzianum*, *T. hamatum* and *T. asperellum* were the most consistent colonizers of cacao seedling roots with moderate to severe discolouration of roots with inoculation on water agar plates. *T. ovalisporum* isolates and an unidentified *Trichoderma* spp. were less capable of colonizing the roots with moderate discolouration of the roots. *T. stromaticum* and *T. spirale* were the poorest colonizers with the lowest root discolouration. This variation supports the data from the current study where the highest overall colonization ability was recorded with *T. harzianum* with 34222.50 cfu compared with Grey9-6 (*T. spirale*), which displayed poor colonization with 14390.00 cfu. *In vitro* studies conducted by Al-Jedabi (2009) indicated that *Trichoderma harzianum* had a higher colonization on sorghum roots compared to *Trichoderma viride* and this advantage of *Trichoderma harzianum* contributed to its ability to inhibit the growth of *Fusarium oxysporum* that causes sorghum root and crown rot.

Although no significant differences in root rot severity were observed with the *Trichoderma* spp. tested in the current study, a tendency occurred where small differences in root rot were recorded between *T. virens* and both isolates of *T. hamatum* compared with the control. The root rot severity on *T. virens* was more than on the control, which suggested that *T. virens* could be responsible for causing some of the symptoms itself. In trials conducted by Al-Jedabi (2009) sorghum seeds treated with *T. harzianum* and *T. viride* and planted in *F. oxysporum* infested pots, showed only a small percentage root rot of 20%, compared to the control with 100% root rot and it is suggested that the effective colonization of the sorghum roots is responsible for the suppressive effect. Therefore the tendency for less root rot recorded with Ced3-1 (*T.*

hamatum) and Ced3-2 (*T. hamatum*) could be a result of effective colonization of the sorghum roots.

It is not clear how *Trichoderma* spp. cause discolouration of the roots after colonization. Howell *et al*., (2000) recorded an increase in the synthesis of terpenoid compounds in cotton roots that was linked to the isolates' ability to protect cotton seedlings against *Rhizoctonia solani.* It is suggested that this could have induced root discolouration in the current study. Recent molecular interaction studies conducted by Bailey *et al.* (2006) on the relationship between *Trichoderma* spp. isolates and cacao seedlings verified an alteration of gene expression during colonization by *Trichoderma* spp. This discolouration of the roots could be a response of the cacao seedlings to indicate recognition of the presence of the specific *Trichoderma* spp. A similar response may be a reason for the observed lesions on the sorghum roots in the pots treated with *Trichoderma* spp. in the current greenhouse study.

The higher shoot lengths in pots treated with *Trichoderma* spp. isolates may be due to the ability of *Trichoderma* spp. to act as plant growth promoters (Rojo *et al*., 2007). These authors observed an increase in peanut yield in trials treated with *Trichoderma* spp. The increase in plant growth that contributes to a higher yield is assumed to be due to a defense response triggered in the host plant subsequent to inoculation with *Trichoderma* spp. in the early stages of root development and by the biocontrol of major and minor plant pathogens. Growth and health of *Pinus radiata* seedlings was enhanced by the addition of *T. hamatum* in a commercial nursery. The shoot heights were increased by 16%, root weight increased by 31% and the mortality rate decreased by 29% (Hohmann *et al*., 2011).

The difference in soil colonization in the rhizosphere of the cultivars may either be attributed to the production of metabolites by the sorghum cultivars, such as phenolic compounds or tannins, the release of nutrients, enzymes, phenolic compounds and sugars in the form of root exudates and the interaction of other fungi found in the naturally infested soil from Potchefstroom. This indicated that the mode of action for *T. hamatum*

isolates Ced3-1 and Ced3-2 was likely to be the competition for space and food, as the amount of colony forming units was almost twice that of Kl23-1 (*T. virens*) for both these *Trichoderma* spp.

Studies performed by Bailey *et al.* (2008) indicated that the *Trichoderma* spp. isolates used against diseases of cacao seedlings not only colonized the plant tissues surrounding the point of inoculation, but also colonized distant tissues, including root tips, roots, stems, plumules and leaves and when the cacao seedlings were examined, *Trichoderma* spp. were isolated from the xylem. And the only way this could have happened was for the immature bark (relatively thick tissue) to be penetrated by the *Trichoderma* spp., therefore portraying the colonizing ability of *Trichoderma* spp.

Studies conducted by Hohmann *et al*. (2011) showed the difference in colonizing abilities between two *Trichoderma* spp., one being a poor colonizer and the other being a strong rhizosphere colonizer and the effect these had on plant health. *T. atroviride* had no significant effect on plant performance, while the dominant colonizer, *T. hamatum*, not only enhanced plant growth of *Pinus radiata* seedlings, but also reduced mortality of seedlings by 29%. This supports the findings of the rhizosphere colonization study where *T. hamatum* isolates dominated the *T. virens* isolate.

4.4 CONCLUSION

Chemical control is not always effective in controlling soilborne pathogens and because of its negative impact on the environment, for instance, loss of non-target beneficial organisms, groundwater pollution, and the development of resistant pathogens (Dubey *et al.,* 2007), other control methods are needed. For these reasons biological control is preferred as pathogens are managed by the natural suppressiveness of the soil where the disease is found. In our study *Trichoderma* spp. were chosen as a biological control agent and proved effective when tested *in vitro*. However, greenhouse results were less

satisfactory, as no significant differences were observed between *Trichoderma* spp. treatments and root rot severity. Further research is needed and will continue.

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Table 4.1 Inhibition as a result of direct interaction between *Trichoderma* spp. and test fungi on dual culture plates.

Trichoderma spp.		Test fungus inhibition (%)						
Isolate	Identification	1/2Bet36-2 (*Alternaria* sp.)	PBet39-1 (Unidentified)	Bet8-2 (Unidentified)	Bet13-2 (*F. oxysporum*)	Bet26-4 (*Acremonium strictum*)	Bet28-1B (*Alternaria alternata*)	CedNie7-2 (*F. oxysporum*)
1/2Bet13-1	*Trichoderma* sp.	64.96	61.78	65.17	74.83	60.61	72.71	66.51
Bet18-1	*Trichoderma* sp.	77.29	73.80	73.73	90.57	84.74	79.07	70.93
Ced3-1	*Trichoderma hamatum*	63.18	30.56	68.40	74.24	54.86	68.66	65.53
Ced3-2	*Trichoderma hamatum*	64.28	39.99	66.25	72.63	61.07	74.78	67.00
CedNie8-1	*Trichoderma* sp.	54.23	43.18	65.61	66.48	60.54	69.81	63.96
CedNie10-3	*Trichoderma harzianum*	63.67	59.81	69.26	75.82	63.54	72.86	75.04
Grey9-6	*Trichoderma spirale*	70.57	39.88	76.51	79.26	62.91	80.62	77.51
Kl4-2	*Trichoderma* sp.	66.79	57.27	72.80	82.13	68.45	75.38	78.64
Kl8-3	*Trichoderma* sp.	63.67	59.90	76.26	78.01	70.32	77.97	72.30
Kl23-1	*Trichoderma virens*	59.83	67.88	80.56	75.24	78.53	83.20	75.16
Kl30-1	*Trichoderma* sp.	64.03	80.99	65.00	77.97	52.84	71.22	73.18
Sen2-2	*Trichoderma harzianum*	67.45	63.47	66.29	77.60	65.67	71.36	78.25
Sen2-5	*Trichoderma harzianum*	56.62	81.66	66.37	74.68	64.86	79.11	78.69
Sen16-2	*Trichoderma harzianum*	68.05	40.13	82.27	78.68	63.65	81.02	79.17
Sen16-4	*Trichoderma harzianum*	70.36	53.96	78.96	83.45	66.89	72.59	80.52
Wint4-2	*Trichoderma harzianum*	62.81	65.27	74.60	80.14	62.83	78.89	79.94
Wint16-1	*Trichoderma harzianum*	63.29	56.38	73.15	81.22	70.31	77.81	73.55
Wint21-2	*Trichoderma harzianum*	72.28	58.26	79.09	85.96	74.41	75.29	78.01
Wint22-2	*Trichoderma harzianum*	67.52	63.23	80.41	80.08	79.38	77.80	71.17
Mean		**65.31**	**57.76**	**72.67**	**78.37**	**66.65**	**75.80**	**73.95**

LSD ($P<0.05$) *Trichoderma* spp.= 0.19; Test fungi = 0.15; *Trichoderma* spp x Test fungi=2.85;

Table 4.1 Continued

Isolate	Test fungus inhibition (%)								**Mean**
	CedNie27-1 (*F. oxysporum*)	Sen12-1 (*F. oxysporum*)	Sen27-4 (*F. oxysporum*)	Wint6-4 (Unidentified)	Wint7-2 (*Fusarium* sp.)	Wint10-5 (*Fusarium* sp.)	Wint20-1 (*F. oxysporum*)	Wint23-1 (*F. oxysporum*)	
1/2Bet13-1	74.44	72.49	76.17	87.09	77.45	70.91	73.85	77.14	**71.74**
Bet18-1	87.65	70.38	80.41	92.01	77.92	61.47	92.13	83.54	**79.71**
Ced3-1	66.49	65.77	72.65	80.33	79.87	70.87	73.58	72.57	**67.17**
Ced3-2	70.95	68.18	73.88	78.48	77.30	62.42	73.13	67.06	**67.83**
CedNie8-1	66.76	66.07	72.65	83.99	82.38	64.59	70.57	71.82	**66.84**
CedNie10-3	65.49	72.35	78.32	84.93	74.94	63.67	76.50	73.81	**71.33**
Grey9-6	77.37	73.12	74.54	90.13	77.49	67.70	79.23	71.94	**73.25**
Kl4-2	79.05	76.12	77.49	89.19	78.33	70.15	85.00	79.19	**75.73**
Kl8-3	75.41	80.15	75.75	86.43	77.81	73.92	77.78	70.32	**74.40**
Kl23-1	75.73	78.80	73.99	92.34	82.03	76.82	81.53	76.75	**77.23**
Kl30-1	67.00	73.06	73.49	80.67	83.17	66.93	68.54	73.62	**71.45**
Sen2-2	82.86	74.16	75.17	82.81	78.10	52.76	82.24	77.64	**73.06**
Sen2-5	76.55	87.86	75.86	89.12	73.62	55.69	85.90	76.16	**74.85**
Sen16-2	81.02	76.75	77.62	92.96	84.79	79.60	86.96	79.01	**76.78**
Sen16-4	81.34	75.21	75.97	90.77	88.08	73.41	83.16	81.78	**77.10**
Wint4-2	81.63	75.04	76.12	84.68	75.74	55.06	82.70	80.32	**74.38**
Wint16-1	76.41	77.11	79.59	90.16	80.97	79.94	77.41	77.70	**75.67**
Wint21-2	78.12	85.31	70.47	92.29	90.09	78.94	80.65	79.73	**78.59**
Wint22-2	78.91	72.61	75.33	88.75	83.38	76.65	77.21	77.13	**76.64**
Mean	**75.96**	**74.77**	**75.55**	**87.22**	**80.18**	**68.50**	**79.37**	**76.17**	**73.88**

LSD (P<0.05) *Trichoderma* spp.= 0.19; Test fungi = 0.15; *Trichoderma* spp x Test fungi=2.85;

Table 4.2 Growth of test fungi on dual culture plates with *Trichoderma* spp.

Trichoderma spp.		Test fungus growth (mm)						
		1/2Bet36-2 (*Alternaria* sp.)	PBet39-1 (Unidentified)	Bet8-2 (Unidentified)	Bet13-2 (*F. oxysporum*)	Bet26-4 (*Acremonium strictum*)	Bet28-1B (*Alternaria alternata*)	CedNie7-2 (*F. oxysporum*)
Isolate	Identification							
1/2Bet13-1	*Trichoderma* sp.	8.62	8.30	16.81	14.77	15.00	14.14	20.51
Bet18-1	*Trichoderma* sp.	5.60	5.63	12.72	5.51	5.80	10.84	17.78
Ced3-1	*Trichoderma hamatum*	9.07	14.98	15.30	15.09	17.11	16.23	21.07
Ced3-2	*Trichoderma hamatum*	8.79	12.96	16.32	16.03	14.74	13.06	20.19
CedNie8-1	*Trichoderma* sp.	11.19	14.19	16.73	19.63	14.96	15.64	22.08
CedNie10-3	*Trichoderma harzianum*	8.95	8.67	14.85	14.15	13.82	14.05	15.25
Grey9-6	*Trichoderma spirale*	7.24	12.99	11.37	12.16	14.07	10.04	13.77
Kl4-2	*Trichoderma* sp.	8.17	9.23	13.14	10.46	11.99	12.75	13.06
Kl8-3	*Trichoderma* sp.	8.94	8.66	11.52	12.92	11.28	11.41	16.93
Kl23-1	*Trichoderma virens*	9.90	6.93	9.42	14.50	8.16	8.70	15.21
Kl30-1	*Trichoderma* sp.	8.85	4.11	16.89	12.90	17.91	14.91	16.40
Sen2-2	*Trichoderma harzianum*	8.02	7.87	16.31	13.09	13.09	14.83	13.31
Sen2-5	*Trichoderma harzianum*	10.67	3.96	16.27	14.83	13.32	10.81	13.04
Sen16-2	*Trichoderma harzianum*	7.88	12.95	8.61	12.49	13.80	9.83	12.73
Sen16-4	*Trichoderma harzianum*	7.30	9.93	10.18	9.64	12.56	14.20	11.93
Wint4-2	*Trichoderma harzianum*	9.16	7.49	12.33	11.65	14.11	10.93	12.27
Wint16-1	*Trichoderma harzianum*	9.04	9.41	12.96	11.00	11.29	11.49	16.17
Wint21-2	*Trichoderma harzianum*	6.80	9.02	10.11	8.21	9.74	12.79	13.44
Wint22-2	*Trichoderma harzianum*	8.00	7.94	9.50	11.68	7.83	11.50	17.62
Control		24.66	21.62	48.52	58.69	38.16	51.80	61.20
Mean		**9.34**	**9.84**	**14.99**	**14.97**	**13.94**	**14.50**	**18.20**

LSD ($P<0.05$): *Trichoderma* spp.= 0.08; Test fungi = 0.06; *Trichoderma* spp x Test fungi=1.20;

Table 4.2 Continued

Isolate	Test fungus growth (mm)								**Mean**
	CedNie27-1 (*F. oxysporum*)	Sen12-1 (*F. oxysporum*)	Sen27-4 (*F. oxysporum*)	Wint6-4 (Unidentified)	Wint7-2 (*Fusarium* sp.)	Wint10-5 (*Fusarium* sp.)	Wint20-1 (*F. oxysporum*)	Wint23-1 (*F. oxysporum*)	
1/2Bet13-1	16.04	16.48	9.10	9.13	16.03	16.34	18.16	15.40	**14.32**
Bet18-1	7.76	17.68	7.47	5.71	15.73	21.72	5.91	11.08	**10.46**
Ced3-1	21.06	20.42	10.45	13.38	14.35	16.50	18.34	18.46	**16.12**
Ced3-2	18.26	18.90	9.98	14.55	16.12	20.79	18.65	22.18	**16.10**
CedNie8-1	20.88	20.23	10.44	10.39	12.46	20.00	20.36	18.96	**16.54**
CedNie10-3	21.67	16.52	8.28	10.45	17.89	20.37	16.38	17.64	**14.60**
Grey9-6	14.22	16.05	9.72	6.07	15.99	18.18	14.55	18.87	**13.02**
Kl4-2	13.16	14.30	8.59	6.69	15.43	16.89	10.68	14.01	**11.90**
Kl8-3	15.45	11.92	9.26	8.28	15.79	14.73	15.53	19.98	**12.84**
Kl23-1	15.25	12.66	9.95	7.04	12.80	13.05	13.01	15.65	**11.48**
Kl30-1	20.73	16.07	10.10	13.17	11.99	18.72	21.71	17.76	**14.81**
Sen2-2	10.77	15.40	9.48	11.82	15.57	26.55	12.54	15.04	**13.58**
Sen2-5	14.74	7.28	9.22	7.42	18.79	24.85	10.08	16.04	**12.76**
Sen16-2	11.93	13.93	8.54	4.90	10.80	11.30	9.37	14.12	**10.88**
Sen16-4	11.73	14.79	9.18	6.45	8.44	15.06	11.92	12.28	**11.04**
Wint4-2	11.55	14.90	9.12	10.05	17.26	25.36	12.23	13.25	**12.78**
Wint16-1	14.82	13.62	7.78	6.98	13.55	11.30	15.76	15.01	**12.01**
Wint21-2	13.74	8.75	11.30	5.29	7.06	11.86	13.60	13.63	**10.36**
Wint22-2	13.26	16.28	9.42	7.98	11.80	13.09	15.90	15.40	**11.81**
Control	62.84	60.03	38.22	68.08	71.23	57.40	67.65	67.35	**53.16**
Mean	**17.49**	**17.31**	**10.78**	**11.69**	**16.95**	**19.70**	**17.12**	**18.61**	**15.03**

LSD ($P<0.05$): *Trichoderma* spp.= 0.08; Test fungi = 0.06; *Trichoderma* spp x Test fungi=1.20;

Table 4.3 Inhibition of test fungi on 5% malt extract agar plates during bioassay for volatile substances produced by *Trichoderma* spp.

Trichoderma spp.		Test fungus inhibition (%)						
Isolate	Identification	1/2Bet36-2 (*Alternaria* sp.)	PBet39-1 (Unidentified)	Bet8-2 (Unidentified)	Bet13-2 (*F. oxysporum*)	Bet26-4 (*Acremonium strictum*)	Bet28-1B (*Alternaria alternata*)	CedNie7-2 (*F. oxysporum*)
1/2Bet13-1	*Trichoderma* sp.	21.66	9.99	22.77	7.29	6.99	19.41	41.99
Bet18-1	*Trichoderma* sp.	28.01	19.17	24.01	13.00	7.10	27.56	37.31
Grey9-6	*Trichoderma spirale*	23.61	5.74	27.60	-1.16	14.93	9.46	45.33
Kl4-2	*Trichoderma* sp.	27.16	16.31	27.84	22.63	12.07	20.16	41.34
Kl23-1	*Trichoderma virens*	21.86	11.58	26.42	4.67	15.07	15.25	33.21
Kl30-1	*Trichoderma* sp.	13.19	2.42	26.41	0.18	1.44	7.58	41.82
Sen2-2	*Trichoderma harzianum*	19.23	25.88	17.95	10.34	20.21	28.29	30.54
Sen2-5	*Trichoderma harzianum*	17.80	9.34	25.69	22.98	7.43	14.39	32.07
Sen16-2	*Trichoderma harzianum*	19.62	8.74	1.88	1.46	9.07	20.36	36.47
Wint4-2	*Trichoderma harzianum*	27.72	25.12	32.78	2.36	6.49	13.34	33.36
Wint21-2	*Trichoderma harzianum*	24.83	10.50	21.25	4.07	7.37	17.28	38.07
Mean		**22.24**	**13.16**	**23.15**	**7.98**	**9.83**	**17.55**	**37.41**

LSD ($P<0.05$): *Trichoderma* spp.= 0.58; Test fungi = 0.79; *Trichoderma* spp x Test fungi=8.69;

Table 4.3 Continued

Isolate	Test fungus inhibition (%)								Mean
	CedNie27-1 (*F. oxysporum*)	Sen12-1 (*F. oxysporum*)	Sen27-4 (*F. oxysporum*)	Wint6-4 (Unidentified)	Wint7-2 (*Fusarium* sp.)	Wint10-5 (*Fusarium* sp.)	Wint20-1 (*F. oxysporum*)	Wint23-1 (*F. oxysporum*)	
1/2Bet13-1	6.50	7.70	5.13	6.86	17.28	13.37	27.72	9.67	**14.96**
Bet18-1	24.36	5.09	37.80	9.57	26.01	-34.16	15.50	14.32	**16.98**
Grey9-6	10.52	1.60	8.32	4.31	12.75	-49.16	16.80	0.56	**8.75**
Kl4-2	25.73	9.87	18.87	10.99	29.23	-17.97	20.34	12.90	**18.50**
Kl23-1	15.40	14.08	20.78	20.56	11.73	-32.81	33.99	11.32	**14.87**
Kl30-1	13.45	-0.79	26.82	-0.19	6.16	-78.15	9.15	0.94	**4.70**
Sen2-2	23.67	54.90	20.13	8.43	15.38	6.50	27.13	13.64	**21.48**
Sen2-5	6.86	17.76	10.10	9.47	25.97	-63.74	27.71	15.98	**11.99**
Sen16-2	20.51	25.86	35.85	39.19	36.81	2.50	17.62	29.34	**20.35**
Wint4-2	12.88	35.66	25.22	12.35	43.54	-22.72	24.08	16.86	**19.27**
Wint21-2	9.05	16.00	11.45	10.90	16.64	-52.55	22.07	8.81	**11.05**
Mean	**15.36**	**17.07**	**20.04**	**12.04**	**21.95**	**-29.90**	**22.01**	**12.21**	**14.81**

LSD (P<0.05): *Trichoderma* spp.= 0.58; Test fungi = 0.79; *Trichoderma* spp x Test fungi=8.69;

Table 4.4 Growth of test fungi on 5% malt extract agar plates during bioassay for volatile substances produced by *Trichoderma* spp.

Trichoderma spp.		Test fungus growth (mm)						
Isolate	Identification	1/2Bet36-2 (*Alternaria* sp.)	PBet39-1 (Unidentified)	Bet8-2 (Unidentified)	Bet13-2 (*F. oxysporum*)	Bet26-4 (*Acremonium strictum*)	Bet28-1B (*Alternaria alternata*)	CedNie7-2 (*F. oxysporum*)
1/2Bet13-1	*Trichoderma* sp.	16.92	36.60	23.63	36.85	38.62	26.79	41.99
Bet18-1	*Trichoderma* sp.	15.64	32.90	23.24	34.66	38.55	24.02	37.31
Grey9-6	*Trichoderma spirale*	16.52	38.34	22.12	40.21	35.32	30.13	45.33
Kl4-2	*Trichoderma* sp.	15.75	34.03	22.04	30.70	36.48	26.71	41.34
Kl23-1	*Trichoderma virens*	16.95	35.96	22.44	37.94	35.26	28.21	33.21
Kl30-1	*Trichoderma* sp.	18.80	39.69	22.50	39.73	40.91	30.86	41.82
Sen2-2	*Trichoderma harzianum*	17.50	30.15	25.17	35.63	33.12	23.69	30.54
Sen2-5	*Trichoderma harzianum*	17.79	36.87	22.70	30.56	38.42	28.32	32.07
Sen16-2	*Trichoderma harzianum*	17.42	37.08	30.04	39.19	37.71	26.44	36.47
Wint4-2	*Trichoderma harzianum*	15.61	30.47	20.55	38.79	38.81	28.88	33.36
Wint21-2	*Trichoderma harzianum*	16.26	36.35	24.07	38.10	38.41	27.42	38.07
Control		21.70	40.70	30.57	39.76	41.52	33.40	42.86
Mean		**17.24**	**35.76**	**24.09**	**36.84**	**37.76**	**27.90**	**37.86**

LSD ($P<0.05$): *Trichoderma* spp.= 0.21; Test fungi = 0.26; *Trichoderma* spp x Test fungi=3.15;

Table 4.4 Continued

Isolate	Test fungus growth (mm)								Mean
	CedNie27-1 (*F. oxysporum*)	Sen12-1 (*F. oxysporum*)	Sen27-4 (*F. oxysporum*)	Wint6-4 (Unidentified)	Wint7-2 (*Fusarium* sp.)	Wint10-5 (*Fusarium* sp.)	Wint20-1 (*F. oxysporum*)	Wint23-1 (*F. oxysporum*)	
Bet18-1	42.60	42.54	46.10	43.00	50.42	23.39	33.78	38.90	**36.14**
1/2Bet13-1	34.51	43.70	30.06	41.75	44.86	36.19	39.31	36.80	**34.23**
Grey9-6	40.83	45.29	44.55	44.20	52.76	40.26	38.36	42.78	**38.47**
Kl4-2	33.95	41.48	39.41	41.10	43.49	31.82	37.33	37.49	**34.21**
Kl23-1	38.56	39.56	38.53	36.71	53.66	35.85	31.12	38.07	**34.80**
Kl30-1	39.40	46.42	35.71	46.26	56.81	48.09	42.51	42.62	**39.47**
Sen2-2	34.79	20.72	38.70	42.28	51.56	25.26	33.99	37.14	**32.01**
Sen2-5	42.46	37.82	43.67	41.80	44.29	44.15	33.94	36.19	**35.40**
Sen16-2	36.18	34.16	30.93	28.08	37.57	26.32	38.60	30.28	**32.43**
Wint4-2	39.72	29.53	36.22	40.47	34.54	33.07	35.58	35.76	**32.76**
Wint21-2	41.42	38.64	43.02	41.14	50.74	41.12	36.32	39.24	**36.69**
Control	45.61	46.11	48.59	46.18	61.12	26.97	47.12	43.10	**41.02**
Mean	**39.17**	**38.83**	**39.62**	**41.08**	**48.48**	**34.37**	**37.33**	**38.20**	**35.64**

LSD ($P<0.05$): *Trichoderma* spp.= 0.21; Test fungi = 0.26; *Trichoderma* spp x Test fungi=3.15;

Table 4.5 Inhibition of test fungi on 5% malt extract agar plates during bioassay for non-volatile substances produced by *Trichoderma* spp.

Trichoderma spp.		Test fungus inhibition (%)						
Isolate	Identification	1/2Bet36-2 (*Alternaria* sp.)	PBet39-1 (Unidentified)	Bet8-2 (Unidentified)	Bet13-2 (*F. oxysporum*)	Bet26-4 (*Acremonium strictum*)	Bet28-1B (*Alternaria alternata*)	CedNie7-2 (*F. oxysporum*)
1/2Bet13-1	*Trichoderma* sp.	18.55	8.04	43.37	10.17	22.23	35.76	13.60
Bet18-1	*Trichoderma* sp.	25.61	13.81	46.50	21.75	16.70	42.77	61.73
Grey9-6	*Trichoderma spirale*	5.56	2.08	40.21	16.30	30.85	36.99	5.66
Kl4-2	*Trichoderma* sp.	6.56	7.62	30.36	15.48	7.38	13.03	19.16
Kl23-1	*Trichoderma virens*	24.41	34.56	43.73	38.20	27.34	43.89	33.50
Kl30-1	*Trichoderma* sp.	2.92	2.82	53.29	4.52	17.72	48.61	10.34
Sen2-2	*Trichoderma harzianum*	-9.00	1.85	23.09	-0.09	6.24	10.26	9.40
Sen2-5	*Trichoderma harzianum*	6.03	2.30	35.14	-2.14	1.60	8.38	7.13
Sen16-2	*Trichoderma harzianum*	4.31	0.82	40.82	6.12	16.51	-5.59	6.18
Wint4-2	*Trichoderma harzianum*	-8.23	5.11	18.01	0.57	5.15	1.15	22.73
Wint21-2	*Trichoderma harzianum*	8.52	10.95	19.34	9.70	8.99	-4.63	11.43
Mean		**7.75**	**8.18**	**35.81**	**10.96**	**14.61**	**20.97**	**18.26**

LSD ($P<0.05$): *Trichoderma* spp.= 0.47; Test fungi = 0.64; *Trichoderma* spp x Test fungi=7.04;

Table 4.5 Continued

Isolate	Test fungus inhibition (%)								**Mean**
	CedNie27-1 (*F. oxysporum*)	Sen12-1 (*F. oxysporum*)	Sen27-4 (*F. oxysporum*)	Wint6-4 (Unidentified)	Wint7-2 (*Fusarium* sp.)	Wint10-5 (*Fusarium* sp.)	Wint20-1 (*F. oxysporum*)	Wint23-1 (*F. oxysporum*)	
1/2Bet13-1	13.34	12.95	45.15	39.69	29.05	16.76	22.11	14.22	**23.00**
Bet18-1	26.84	69.26	36.98	24.38	35.57	56.25	33.88	18.91	**35.40**
Grey9-6	19.80	13.43	41.88	3.99	15.13	14.68	14.33	19.02	**18.66**
Kl4-2	26.20	12.88	8.54	-0.01	17.49	14.26	22.49	24.33	**15.05**
Kl23-1	46.64	29.16	35.24	68.21	27.39	51.91	50.61	34.68	**39.30**
Kl30-1	18.54	12.80	39.80	46.25	31.67	5.64	12.88	14.79	**21.51**
Sen2-2	17.34	7.04	11.30	-1.13	12.76	15.53	18.89	13.00	**9.10**
Sen2-5	9.63	0.60	26.80	-6.60	5.77	10.71	10.01	10.61	**8.40**
Sen16-2	12.75	10.37	21.38	-2.27	12.34	10.70	18.14	9.87	**10.83**
Wint4-2	26.56	9.09	4.82	-6.61	2.94	7.82	5.95	6.78	**6.79**
Wint21-2	9.62	17.20	26.60	0.36	11.02	8.35	11.28	11.79	**10.70**
Mean	**20.66**	**17.71**	**27.14**	**15.11**	**18.28**	**19.33**	**20.05**	**16.18**	**18.07**

LSD ($P<0.05$): *Trichoderma* spp.= 0.47; Test fungi = 0.64; *Trichoderma* spp x Test fungi=7.04;

Table 4.6 The growth of test fungi on 5% malt extract agar plates during bioassay for non-volatile substances produced by *Trichoderma* spp.

Trichoderma spp.		Test fungus growth (mm)						
Isolate	Identification	1/2Bet36-2 (*Alternaria* sp.)	PBet39-1 (Unidentified)	Bet8-2 (Unidentified)	Bet13-2 (*F. oxysporum*)	Bet26-4 (*Acremonium strictum*)	Bet28-1B (*Alternaria alternata*)	CedNie7-2 (*F. oxysporum*)
1/2Bet13-1	*Trichoderma* sp.	11.29	18.90	14.06	20.30	19.26	12.85	22.30
Bet18-1	*Trichoderma* sp.	9.99	17.73	13.19	17.67	20.64	11.45	9.90
Grey9-6	*Trichoderma spirale*	13.13	20.09	14.85	18.87	17.08	12.57	24.35
Kl4-2	*Trichoderma* sp.	12.88	18.94	17.29	19.11	22.94	17.44	20.87
Kl23-1	*Trichoderma virens*	10.45	13.49	13.91	13.99	17.98	11.28	17.14
Kl30-1	*Trichoderma* sp.	13.44	19.94	11.50	21.55	20.35	10.23	23.19
Sen2-2	*Trichoderma harzianum*	14.99	20.18	18.94	22.64	23.19	18.00	23.46
Sen2-5	*Trichoderma harzianum*	12.93	20.05	16.10	23.05	24.35	18.32	24.05
Sen16-2	*Trichoderma harzianum*	13.15	20.36	14.62	21.23	20.69	21.15	24.23
Wint4-2	*Trichoderma harzianum*	14.88	19.45	20.59	22.42	23.49	19.69	19.89
Wint21-2	*Trichoderma harzianum*	12.68	18.31	19.94	20.39	22.52	20.90	22.89
Control		13.84	20.59	24.92	22.62	24.76	20.08	25.86
Mean		**12.80**	**19.00**	**16.66**	**20.32**	**21.44**	**16.16**	**21.51**

LSD (P<0.05): *Trichoderma* spp.= 0.10; Test fungi = 0.13; *Trichoderma* spp x Test fungi=1.50;

Table 4.6 Continued

Isolate	Test fungus growth (mm)								Mean
	CedNie27-1 (*F. oxysporum*)	Sen12-1 (*F. oxysporum*)	Sen27-4 (*F. oxysporum*)	Wint6-4 (Unidentified)	Wint7-2 (*Fusarium* sp.)	Wint10-5 (*Fusarium* sp.)	Wint20-1 (*F. oxysporum*)	Wint23-1 (*F. oxysporum*)	
1/2Bet13-1	22.38	23.73	16.83	16.37	22.63	23.47	19.65	21.89	**19.06**
Bet18-1	18.91	8.38	19.28	20.50	20.57	12.33	16.67	20.63	**15.85**
Grey9-6	20.81	23.58	18.03	26.07	27.06	24.02	21.65	20.66	**20.19**
Kl4-2	19.10	23.74	28.12	27.10	26.32	24.04	19.56	19.28	**21.12**
Kl23-1	13.80	19.31	19.94	8.61	23.13	13.59	12.48	16.79	**15.06**
Kl30-1	21.05	23.76	18.55	14.56	21.80	26.56	21.98	21.70	**19.34**
Sen2-2	21.40	25.24	27.24	27.44	27.82	23.81	20.51	22.23	**22.47**
Sen2-5	23.37	27.16	22.43	28.96	30.06	25.11	22.76	22.82	**22.77**
Sen16-2	22.61	24.45	24.19	27.86	27.97	25.16	20.72	22.94	**22.09**
Wint4-2	19.07	24.71	29.29	28.94	30.97	26.01	23.76	23.76	**23.13**
Wint21-2	23.42	22.55	22.55	27.07	28.38	25.76	22.37	22.52	**22.15**
Control	25.94	27.29	30.76	27.18	31.92	28.18	25.28	25.57	**24.99**
Mean	**20.99**	**22.82**	**23.10**	**23.39**	**26.55**	**23.17**	**20.61**	**21.73**	**20.68**

LSD (P<0.05): *Trichoderma* spp.= 0.10; Test fungi = 0.13; *Trichoderma* spp x Test fungi=1.50;

Table 4.7 Root colonization of four sorghum cultivars by *Trichoderma* spp. isolates *in vitro*.

Trichoderma spp.		Cultivar root colonization (cfu/ml)				
Isolate	Identification	PAN 8625	PAN 8389	PAN 8706W	NS 5511	**Mean**
1/2Bet13-1	*Trichoderma* sp.	0.00	17430.00	29360.00	10.00	**11700.00**
Bet18-1	*Trichoderma* sp.	9030.00	27440.00	27790.00	24080.00	**22085.00**
Ced3-1	*Trichoderma hamatum*	32350.00	27790.00	32280.00	25460.00	**29470.00**
Ced3-2	*Trichoderma hamatum*	37530.00	27850.00	39880.00	22150.00	**31852.50**
CedNie8-1	*Trichoderma* sp.	28820.00	38780.00	35660.00	32470.00	**33932.50**
CedNie10-3	*Trichoderma harzianum*	33810.00	32160.00	38510.00	32410.00	**34222.50**
Grey9-6	*Trichoderma spirale*	20.00	27060.00	1110.00	29370.00	**14390.00**
Kl4-2	*Trichoderma* sp.	32120.00	4750.00	43190.00	36750.00	**29202.50**
Kl8-3	*Trichoderma* sp.	27350.00	31970.00	25930.00	30000.00	**28812.50**
Kl23-1	*Trichoderma virens*	0.00	12060.00	3340.00	4680.00	**5020.00**
Kl30-1	*Trichoderma* sp.	900.00	21780.00	39180.00	25150.00	**21752.50**
Sen2-2	*Trichoderma harzianum*	32090.00	24820.00	29540.00	0.00	**21612.50**
Sen2-5	*Trichoderma harzianum*	25260.00	43700.00	25480.00	35030.00	**32367.50**
Sen16-2	*Trichoderma harzianum*	0.00	19120.00	0.00	0.00	**4780.00**
Sen16-4	*Trichoderma harzianum*	30420.00	17270.00	26670.00	15040.00	**22350.00**
Wint4-2	*Trichoderma harzianum*	23520.00	0.00	14340.00	15770.00	**13407.50**
Wint16-1	*Trichoderma harzianum*	36720.00	6220.00	0.00	1880.00	**11205.00**
Wint21-2	*Trichoderma harzianum*	29760.00	31160.00	41830.00	26360.00	**32277.50**
Wint22-2	*Trichoderma harzianum*	24010.00	0.00	10250.00	4090.00	**9587.50**
Mean		**21247.89**	**21650.53**	**24438.95**	**18984.21**	21580.39

LSD (P<0.05): *Trichoderma* spp.= 369.27 Cultivar=n/s; *Trichoderma* spp x Cultivar=1477.09;

Table 4.8 Effect of *Trichoderma* spp. on the severity of root rot on four sorghum cultivars grown in naturally infested soil in the greenhouse.

Trichoderma spp.		Cultivar root rot (%)				
Isolate	Identification	PAN 8625	PAN 8389	PAN 8706W	NS 5511	**Mean**
Ced3-1	*Trichoderma hamatum*	52.11	57.98	55.43	59.09	**56.15**
Ced3-2	*Trichoderma hamatum*	58.75	60.00	53.28	61.08	**58.28**
Kl23-1	*Trichoderma virens*	66.75	61.53	60.37	58.00	**61.66**
Control		65.33	64.17	53.17	59.67	**60.58**
Mean		**60.74**	**60.92**	**55.56**	**59.46**	**59.17**

LSD (P<0.05): *Trichoderma* spp.= n/s; Cultivar=1.18; *Trichoderma* spp x Cultivar=n/s;

Table 4.9 Effect of *Trichoderma* spp. on the shoot length of four sorghum cultivars.

Trichoderma spp.		Cultivar shoot length (cm)				
Isolate	Identification	PAN 8625	PAN 8389	PAN 8706W	NS 5511	**Mean**
Ced3-1	*Trichoderma hamatum*	18.51	26.76	19.00	25.27	**22.38**
Ced3-2	*Trichoderma hamatum*	28.38	25.37	19.30	26.58	**24.91**
Kl23-1	*Trichoderma virens*	22.53	24.45	16.83	32.39	**24.05**
Control		24.18	22.13	23.40	24.81	**23.63**
Mean		**23.40**	**24.68**	**19.64**	**27.26**	**23.74**

LSD ($P<0.05$): *Trichoderma* spp.= ns; Cultivar=2.64; *Trichoderma* spp x Cultivar=n/s;

Table 4.10 Total colony forming units (cfu) of three *Trichoderma* spp. in the rhizosphere of four sorghum cultivars planted in naturally infested soil in the greenhouse.

Trichoderma spp.		Cultivar rhizosphere colonization (cfu/g)				
Isolate	Identification	PAN 8625	PAN 8389	PAN 8706W	NS 5511	**Mean**
Ced3-1	*Trichoderma hamatum*	15740.00	5580.00	5620.00	4040.00	**7745.00**
Ced3-2	*Trichoderma hamatum*	9560.00	3350.00	11170.00	3450.00	**6882.50**
Kl23-1	*Trichoderma virens*	2680.00	3440.00	4650.00	3740.00	**3627.50**
Mean		**9326.67**	**4123.33**	**7146.67**	**3743.33**	**6085.00**

LSD (P<0.05): *Trichoderma* spp.= 111.54; Cultivar=148.72; *Trichoderma* spp x Cultivar=n/s;

SUMMARY

Crop losses are estimated at 30% for sorghum annually as a result of invasion of pathogens or pests. Root rot is usually the result of a complex of soilborne fungi such as *Fusarium* spp., *Pythium* spp., *Macrophomina phaseolina*, *Colletotrichum graminicola* and *Periconia circinata* and other fungi. Colonization of host plant tissues are dependant on the environment that favours a specific pathogen at a particular time and host predisposition.

Fungi were isolated and tested for pathogenicity on two sorghum cultivars. Root discolouration as a measure of root infection indicated a range of host responses, from severe root rot to some isolates that suppressed root rot and stimulated host growth. Isolate Bet9-3 (unidentified) resulted in the highest percentage root rot on both PAN 8420 and PAN 8706W. In most cases an Isolate x Cultivar interaction was recorded indicating that cultivars differ in their responses to pathogens although overall, PAN 8706W was more resistant to root rot than PAN 8420. All the isolates were identified by means of sequencing. A number of isolates that proved capable of causing severe root damage could not be identified suggesting that species, not previously identified, may be associated with root rots locally. Similarly, a number of species were isolated that have not previously been recorded on sorghum. Ergosterol concentrations in sorghum roots were used as a measure of root colonization by the test isolates. Isolate Bet26-4 (*Acremonium strictum*) resulted in the highest ergosterol level but with a moderate root rot discolouration. This disparity between the two evaluation criteria has resulted in the reliability of the evaluation criteria generally used to evaluate root rot, being questioned.

Root rot severity, flag leaf length, total plant length, root volume and effective root volume of 26 sorghum cultivars from the National Cultivar Trials, planted in a naturally infested field trial at Cedara, were measured. All the cultivars were susceptible to root rot with PAN 8389 being the most susceptible (45.60%) and PAN 8534 the most resistant (29.23%). No significant relationship between root rot, root volume or effective root volume and plant growth parameters was recorded. A study using root extracts from the

different cultivars showed only limited suppression of test fungal growth. Similarly, extractable phenol content from roots showed a tendency with reduced root rot severity in the field but this relationship was not significant. Some of the highest phenol content was observed in roots of PAN 8706W and PAN 8648W (white tan cultivars) and these cultivars had the second and third lowest root rot severities respectively. The high phenol content in roots of these cultivars is contrary to the low phenol contents normally reported in white sorghums and further studies of root physiology in relation to root rot resistance, as well as other mechanisms of root rot resistance, are warranted.

The negative effects and risks associated with the use of agricultural chemicals have led to the search and discovery of new methods of disease control in plants. The potential of *Trichoderma* spp. associated with field sorghum as biological control agents was considered. *Trichoderma* spp. isolated from sorghum roots were tested for their effect on the growth of root sorghum isolates. *In vitro* inhibition of these isolates of up to 80 % was recorded due to modes of action including direct colonization and the production of volatile and non-volatile substances by *Trichoderma* spp. *Trichoderma* spp. differed in their ability to colonize sorghum seed, rhizospheres and roots. In a greenhouse study, in soil infested with sorghum root pathogens, Ced3-1 (*T. hamatum*) resulted in the lowest root rot severity while Kl23-1 (*T. virens*) had the least suppressive effect on disease development. Although *Trichoderma* spp. differed in their efficacy to suppress root rot, none was sufficiently so to be of significant commercial value.

OPSOMMING

Gewasverliese as ‘n direkte gevolg van patogene of peste word jaarliks op ongeveer 30% vir sorghum beraam. Wortelvrot is gewoonlik die gevolg van ‘n kompleks van grondgedraagde fungi soos *Fusarium* spp., *Pythium* spp., *Macrophomina phaseolina*, *Colletotrichum graminicola* en *Periconia circinata* en verskeie ander fungi. Die kolonisering van gasheerweefsel is afhanklik van die omgewing wat ‘n spesifieke patogeen bevorder op ‘n bepaalde tydstip, asook die vatbaarheid van die gasheer.

Fungi is geïsoleer en getoets vir patogenesiteit op twee sorghum kultivars. Wortel verkleuring as ‘n aanduiding van wortel infeksie, het ‘n reeks gasheer reaksies aangedui, van strawwe wortelvrot tot die onderdrukking van wortelvrot en gestimuleerde plantegroei. Isolaat Bet 9-3 (onbekend) het die hoogste persentasie wortelvrot veroorsaak op beide PAN 8420 en PAN 8706W. In die meeste gevalle is ‘n Isolaat x Kultivar interaksie waargeneem wat aandui dat kultivars verskil in hulle reaksies teenoor patogene, alhoewel PAN 8706W in die algemeen meer weerstandbiedend was. Al die isolate is geïdentifiseer deur DNS nukleotied volgorde bepaling. Verskeie isolate wat instaat was om strawwe wortelvrot te veroorsaak, kon nie suksesvol geïdentifiseer word nie, wat aandui dat plaaslike, onbekende spesies dalk ‘n rol speel by wortelvrot. So ook is spesies geïdentifiseer wat nog nie vantevore op sorghum gerapporteer is nie. Vir bevestigingsdoeleindes is ergosterol vlakke in sorghum wortels gemeet om die koloniseringspotensiaal van die isolate te bepaal. Isolaat Bet 26-4 (*Acremonium strictum*) het hoë ergosterol vlakke getoon, alhoewel net ‘n gemiddelde wortelvrotstrafheid deur hierdie isolaat veroorsaak is. Hierdie waarnemings het daartoe gelei dat die betroubaarheid van huidige evaluasie tegnieke bevraagteken word.

Die strafheid van wortelvrot, vlagblaar lengte, totale plant lengte en wortel volume van 26 sorghum kultivars van die Nasionale Kultivar Proewe, in ‘n veldproef in Cedara wat natuurlik met sorghum patogene besmet is, is gemeet. Al die kultivars was vatbaar vir wortelvrot met kultivar PAN 8389 die vatbaarste (45.60%) in vergelyking met PAN 8534 wat die meeste weerstand gebied het (29.23%). Geen betekenisvolle verhouding tussen

wortelvrot, wortel volume en groei parameters was aangeteken nie. Slegs 'n beperkte groei inhibisie is ondervind in 'n studie waarin wortel ekstrakte teenoor fungi groei getoets is. Die vlak van geëkstraheerde fenool vanaf wortels het 'n tendens met verminderde wortelvrot strafheid getoon, maar die verhouding was nie betekenisvol nie. Sommige van die hoogste fenool vlakke is in wortels van PAN 8706W en PAN 8648W (beide wit kultivars) aangetref en hiedie kultivars het ook die tweede en derde laagste wortelvrot strafheid getoon. Die hoë fenool vlakke in hierdie kultivar wortels stem nie ooreen met die algemene lae verwagte hoeveelhede wat gewoonlik in wit sorghum aangetref word nie. Verdere studies is dus nodig om wortel fisiologie in verhouding met weerstand, sowel as ander weerstandsmeganismes, te ondersoek.

Die negatiewe effekte en risikos wat met die gebruik van landbou chemikalieë gepaard gaan, het gelei tot die soektog na nuwe metodes om plantsiektes te beheer. Die potensiaal van *Trichoderma* spp. as biologiese beheer agent is oorweeg. *Trichoderma* spp. wat geïsoleer is vanaf sorghum wortels is getoets teenoor swam isolate vanaf sorghum wortels. Tot 80% inhibisie van patogeen isolate is aangeteken, deur of direkte kontak of deur die produksie van vlugtige of nie-vlugtige stowwe wat produseer word deur *Trichoderma* spp. *Trichoderma* spp. het veskil ten opsigte van hul potensiaal om die sorghum saad, rhizosfeer en wortels te koloniseer. In die kweekhuis het Ced3-1 (*T. hamatum*) die meeste wortelvrot onderdrukking getoon teenoor Kl23-1 (*T. virens*) wat die minste inhibisie getoon het. Alhoewel *Trichoderma* spp. verkil het in hul meganismes om wortelvrot te onderdruk, was dit nie suksesvol genoeg vir kommersiële gebruik nie.