SORGHUM ROOT ROT AND GRAIN MOLD PATHOGEN RESPONSES TO LEGUME ROTATION SYSTEMS

by

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DECLARATION

I, Masefudi Pinkie Mojaelo, 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 furthermore cede all copyright of this dissertation to the University of the Free State.

Masefudi Pinkie Mojapelo

Date: 30 January 2020

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PREFACE

This dissertation has four chapters with literature review included. The overall aim of this dissertation was to investigate how sorghum root rot and grain mold pathogens respond to legume rotation systems. This study looked at the cultural benefits obtained through rotation systems in promoting root and plant health and sustainable food security through the reduction of mycotoxin production.

The first chapter is the literature review, which introduces the main legume crops that were used to control root rot, grain mold and occurence of specific mycotoxins in sorghum. It detailed why there is a need to control these constraints affecting crop growth because of its reliance as a staple food in South Africa and many other developing countries. The introduction included the origin and distribution of sorghum, its production level globally and locally and its economic value. It further includes the constraints such as grain mold, root rot and mycotoxins and their cultural management strategies.

In Chapter 2 the impact of various crop rotation systems on the nutrient status of soil and the relationship between soil health and root rot severity in subsequent sorghum crops were established. Field trials were planted at Potchefstroom (ARC-Grain Crops, South Africa) during 2015/16, 2016/17 and 2017/18 respectively. The six main plots were established during the first season (2015/16) by either fallow or planting of sorghum, dry bean, soybean, cowpea and bambara. Plants were randomly collected from sorghum plots at Potchefstroom to quantify root colonization using ergosterol content and test for 12 root pathogens in root tissues using qPCR analyses. Soil samples were collected at the beginning of every season to test for soil nutrient elements ie. N (NO_3 and NH_4), P, K, Ca, Mg, Na, Fe, Cu, Zn and Mn and soil pH that contribute to improved soil and plant health.

In Chapter 3 colonization of *F. graminearum* Species Complex (*Fg*SC) on three sorghum cultivars and their response to grain mold pathogens under fallow, monoculture and legume rotation systems over a three season period (2015/16; 2016/17 and 2017/18) was determined. Ten plants were collected each season from a 10 m designated inner row per cultivar, per treatment for threshing. The threshed grains were visually rated for grain mold on a 1-5 scale. Total biofungal mass as a measure of colonization was quantified through ergosterol quantification, while *Fg*SC was quantified using qPCR analyses.

In Chapter 4 determination of whether legume based rotational systems assist in reducing deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) concentrations in sorghum grain and establishing whether the mycotoxin concentration in sorghum grain might be due to translocation of mycotoxins from the root system to sorghum grain was assessed. Ten plants were collected during each season from a 10 m designated inner row per cultivar, per treatment for threshing and assessment of root rot severity. The threshed grains were visually rated for grain mold on a 1-5 scale and roots were visually assessed on a scale of 0-4. Total biofungal mass as a measure of colonization was quantified through ergosterol quantification in grains and roots, while *Fg*SC was quantified using qPCR analyses. Mycotoxins were quantified using LC-MS/MS method. Relationships between *Fg*SC and mycotoxins were also determined.

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Chapter 1
Literature review

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is an essential economic crop, produced as both food and fodder (Ghani *et al.*, 2015). The crop, that serves as an important source of protein and calories in especially the African and Asian populations (Yousif and El Tinay, 2001), originated from the central parts of Africa, from where it later spread to Asia, America and Australia (Forbes *et al.*, 1986, Reddy *et al.*, 2010, Mesfin and Tileye, 2013). Sorghum is drought tolerant and is mostly planted in dry land areas with high temperature and low rainfall (Idris *et al.*, 2007, Ghani *et al.*, 2015). According to Awika and Rooney (2004), over 35% of all sorghum grown is estimated to be used for human consumption while the remaining 65% is used for alcohol production, animal feed and industrial products.

The constraints that affect sorghum quality and cause major losses in the sorghum industry include poor cultural practices, inadequate rainfall, weeds, insect pests and diseases caused by phytopathogenic fungi, which hinder its cultivation and result in low grain yield (Idris *et al.*, 2007, Al-Jedabi, 2009). Yield losses in sorghum annually have been estimated at 30% due to pests and diseases (Reddy and Zehr, 2004). Several species of soilborne fungi are known to cause root rot of sorghum, the most common being *Macrophomina phaseolina*, *Fusarium moniliforme (sensu lato)*, *Periconia circinata*, *Pythium* spp. and *Colletotrichum graminicola* (Reed *et al.*, 1983, Mughogho, 1984) with numerous other organisms being associated with this disease (van Rooyen, 2012). Root rot of sorghum remains a limiting factor in local sorghum production. Root rot of sorghum is often neglected because of the absence of obvious aerial symptoms (Tarr, 1962). Root rot results in low grain yield and root health is therefore important for quality and high yields as well as ensuring the market value of sorghum grain.

Grain mold and mycotoxins play a major role in the reduction of grain production and quality (Williams and Rao, 1981, Frederiksen, 1986, Ambekar *et al.*, 2011). Up to 100% yield loss can be expected in sorghum cultivars susceptible to grain mold (Williams and Rao, 1981). Grain mold is also referred to as head mold because of fungi that mold the grain as they mature on the head. Pathogens associated with grain mold of sorghum include *Fusarium graminearum* (*sensu lato*), *Fusarium proliferatum*, *Fusarium moniliforme* (*sensu lato*), *Fusarium semitectum*, *Curvularia lunata*, *Phoma sorghina* and *Alternaria* spp. (Forbes *et al.*, 1992). Weather conditions with moist soils and warm temperatures can influence infection, sporulation and dispersal of grain mold pathogens (Bandyopadhyay *et al.*, 1991).

Grain mold also contributes to the production of mycotoxins in most cereal crops, especially sorghum and wheat (*Triticum aestivum*). Hundreds of mycotoxins have been identified but the most important or common ones include aflatoxins, ochratoxins, fumonisins, zearalenone, trichothecenes and patulin (Udomkun *et al.*, 2016). Mycotoxins are harmful to both humans and animals. Over the ages, mycotoxins have been shown to exhibit four basic kinds of toxicoses towards humans and animals, namely acute, chronic, carcinogenic and teratogenic (Pitt *et al.*, 1994), leading to abnormalities in plant, animals and humans.

Alternative methods had to be sought to reduce root rot, grain mold and mycotoxins in sorghum crops. Crop rotation is an alternative cultural method that can be used to reduce inoculum in the soil. Sorghum can be rotated with legumes for 3–4 years in succession for sustainable crops in agriculture. Hardarson and Atkins (2003) reported that legumes are considered better alternatives in cropping systems with maize because of their ability to secure nitrogen economically and increase yield of maize (*Zea mays*), resulting in higher net profits of intercropping systems over monoculture. Cereal-legume intercropping is among the approaches that promote sorghum productivity (Sibhatu, 2015). Rhizobium bacteria absorb the free nitrogen from the air in the plant root tissue, in so doing increases soil nitrogen levels. The increased nitrogen levels aid in increasing the yield of the subsequent cereal crop that follows in the legume rotation system (Masindeni, 2006). The objective of this dissertation is to investigate how sorghum root rot and grain mold pathogens respond to legume rotation systems. Legumes are thought of as beneficial crops because they have the ability to increase natural soil fertility, able to adapt to drought stress, fix nitrogen and produce a reasonable crop when grown on poor soils. (Masindeni, 2006).

2. Crops used in the current study within the crop rotation system

2.1 Sorghum

Figure 1: Sorghum production in the field (Photo: P. Mojapelo).

2.1.1. Origin and distribution

Sorghum (*Sorghum bicolor* (L.) Moench) is the second largest staple food in East Africa after maize and is ranked among the five most important cereal crops in Africa (Idris *et al*., 2007). Sorghum is the major food crop grown in the western Hararghe in Ethiopia, accounting for 59.3% of the total cultivated area, followed by maize 32.8% and tef 4.15% (CSA, 2012). Ghani *et al.* (2015) states that sorghum is superior to maize as well as millet because of its ability to thrive in harsh environments with an ability to survive under drought, heat and salt stress conditions.

Sorghum is a member of the grass family *Graminea* (du Plessis, 2008). According to FAO (2009), sorghum is grown in a large belt in Africa spreading from the Atlantic Coast to Ethiopia and Somalia, bordering the Sahara in the north and the equatorial forest in the south. The production area extends through the drier parts of eastern and southern Africa, where rainfall is too low for the successful cultivation of maize. Commercial production of sorghum has shifted from the drier western production areas to the wetter eastern areas. This change was brought about by the identification and development of cultivars, which are more tolerant to lower temperatures (du Plessis, 2008). Since sorghum is a dietary staple food, about 500 million people especially from the rural areas are dependent on it, in more than 30 countries (Reddy *et al*., 2010).

2.1.2. Production level globally and in South Africa

Dicko *et al.* (2006) stated that during 2006, the global sorghum yield of more than 60 million tons was produced from 46 million ha of cultivated land. It is estimated that since 2006, approximately 70 million tons of grain have been produced internationally from 50 million ha of land, annually. USDA (2015) reported that in 2013/14 sorghum production was 60.46 million tons worldwide with 62.02 million in 2014/15. Reddy *et al.* (2010) attributes the increase of sorghum production to climate change and the ability of sorghum to survive drought stress. In South Africa, sorghum is produced in Limpopo, North West, Mpumalanga, Free State and Gauteng. Small-scale farmers grow sorghum on communal land. The total sorghum production by small-scale farmers is currently unknown as they consume their own product. Major commercial producing areas in South Africa include Free State, KwaZulu-Natal and Mpumalanga (Grain SA, 2017). The annual production yielded more in 2016/17 season compared to 2015/16 and 2017/18. A yield ($t.ha^{-1}$) of 3, 589 was obtained in 2016/17. Low yields obtained in 2015/16 and 2017/18 were attributed to drought caused by low rainfalls during these years (Grain SA, 2017).

2.2. Bambara groundnut

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) previously recognized as *Voandzeia subterranea* (L.) (Opoku, 2010), is a native leguminous crop that is cultivated throughout Africa (Vurayai *et al.*, 2011). It was the third essential grain legume crop after cowpea and groundnut (Doku and Karikari, 1970). This crop is better adapted in semi-arid areas of Africa where other leguminous crops cannot be grown (Doku and Karikari, 1971, Yamaguchi, 1983). In South Africa, bambara is cultivated in the Limpopo, KwaZulu-Natal, Mpumalanga, Eastern Cape and North West provinces by smallholder farmers (Masindeni, 2006). Coudert (1994) estimated that approximately 330 000 tons is produced annually of which half is produced on the African continent, with Nigeria being the major producing country.

Bambara groundnut possesses high crude protein content that ranges between 22 and 37% (Rowland, 1993). Doku (1995) reported that bambara groundnut has a relatively high carbohydrate content of 65% and protein content of 18 % which makes it a very good staple food which is less expensive than other protein sources. The crop plays an essential role in fighting malnutrition (Opoku, 2010). Other positive effects include the ability to increase natural soil fertility, produce a reasonable profit when grown on poor soils, adapt to drought stress and nitrogen fixation (Masindeni, 2006). It is therefore, a crop suited to low input cropping systems making it popular amongst farmers with limited resources.

2.3. Cowpea

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the underutilised native crops that has many advantages for both small-scale farmers and commercial farmers. It falls under the family *Fabacea* (Verdcourt, 1970). There has been ongoing debate as to its origin. Cowpea is believed to have originated from Africa, Asia or South America. Allen (1983) has however reported that cowpea was introduced from Africa to the Indian subcontinent over 3 000 years ago at the same time as sorghum and millet. The main cowpea producing areas in South Africa are Limpopo, Mpumalanga, North West and KwaZulu-Natal provinces.

Cowpea is cultivated for its grain (shelled green or dried), pods and/or leaves (which are consumed in fresh form as green vegetables and as dry haulms) and fodder (Singh *et al.*, 1997, Magloire, 2005). The grain, which is normally dried, is used commonly for human nutrition as a snack or meal, especially in Africa (Medupe, 2010). Singh *et al.* (1997) reported that 23-25% protein is contained in the cowpea and 50% starch. Magloire (2005) similarly reported the protein content in cowpea to range between 23-29% with the potential of 35% under favourable conditions. This grain legume crop is grown in tropics and subtropics regions due to the hot climate associated with these regions. Cowpea is the most important crop to the livelihood of poor people (Magloire, 2005, Medupe, 2010).

Just like bambara groundnut, cowpea, is drought tolerant. In high rainfall areas, it is subject to increased vegetative growth, resulting in higher disease incidence levels. (Singh *et al*., 1997, Watanabe *et al*., 1997).

Cowpea can be intercropped with cereals such as millet and sorghum (Magloire, 2005, Agbogidi *et al*., 2010). The legume-cereal association helps in sustaining crop rotation systems in semi-arid areas, due to the nitrogen fixing ability of cowpea. Cowpea can be used as a crop cover and its plant residues contribute to the crop being regarded as a soil improver (Singh *et al*., 1997, Magloire, 2005, Medupe, 2010). The Food and Agricultural Organisation (FAO) suspended reports on cowpea production due to challenges faced in getting reliable results of how much cowpea is produced per annum. In South Africa, only smallholder farmers cultivate cowpeas, therefore, no records with regard to the area under production and the quantities produced are available. Singh *et al.* (1997) however, mentioned that of the world's 8 million ha that is produced, Africa accounts for 6 million ha.

2.4 Dry bean and soybeans

Dry beans (*Phaseolus vulgaris* (L.) and soybeans (*Glycine max* (L.) Merr.) are the primary commercial legumes produced in South Africa. They contribute in enhancing profit, have agronomic value such as enhancing soil fertility, pest and disease limitations and produce high nutritional value. There is a higher demand for dry bean than is currently produced in South Africa which result in having to import the legumes from China (Grain SA, 2017). Dry beans have high dry protein content in seeds of 17–22%. Crop residues contribute to the overwintering of diseases and pests as they act as an inoculum source, however the advantage that soybeans have is that their residues are mostly leaves which decompose quickly, reducing the risk of increases in diseases (Sexton *et al*., 2014).

Extensive studies on high yields of cereals subsequent to soybeans have been reported in particular due to its ability to fix nitrogen (Robinson, 1966, Franzluebbers *et al*., 1995, Mwangi and Wanjekenche, 1997). Some crop residues have the ability to retain soil fetility and nutrient recycling (Sexton *et al*., 2009), however the opposite is normally experienced where wheat is cultivated after maize as a preceding crop as it is a host of *F. graminearum* that causes wheat head scab (Sexton *et al*., 2014). Soybean is normally used as a crop rotation legume as it has higher tolerable residue situations. Maize and soybean rotation gave a 10% to 22% yield benefit versus a continuous maize cropping in Minnesota and Nebraska (Reidell *et al*., 2009, Stanger and Lauer, 2008, Wilhelm and Wortmann, 2004). This was attributed to many factors including enhanced root growth (Nickel *et al*., 1995).

3. Root rot of grain sorghum

3.1. Incidence and impact of root rot on yield

Root rot is a significant problem in sorghum production (Mughogho, 1984, McLaren, 2002). Incidence of root rot is influenced by factors such as weather conditions, soil and locality (Agrios, 1997). However, a complex number of soilborne fungi are involved in the etiology of root rot with each differing in their ability to cause disease and each having their own distinct requirements for infection (Edmunds and Zummo, 1975). Colonization of roots by fungi affects plant stand and vigour that impact negatively on yield (Edmunds and Zummo, 1975). According to Flett (1996) root damage as a result of fungal infection, causes reductions in water and nutrient uptake and increased lodging due to stress.

Diseases caused by some pathogens occur at low levels that, individually do not always cause major concern but may accumulate resulting in net losses due to their prevalence. This causes a decline in plant quality and quantity of plant produce (Agrios, 1997). These infections often do not result in distinct aerial symptoms and the importance of the root rot complex has accordingly often been overlooked (Tarr, 1962). Yield losses are difficult to establish as root rot is hardly ever assessed unless there is a huge impact on the aerial growth. These losses are mostly attributed to environmental factors and poor soil conditions (Tarr, 1962, Edmunds and Zummo, 1975). In addition, symptoms on infected plants are often not recognized, as they are not evenly distributed throughout the field (Navarro *et al.*, 2008, Moussart *et al.*, 2013). The complexity of various pathogens involved as well as the lack of information on the topic makes researchers hesitant to venture into the field. Yield loss potential of root rot has been a challenge in sorghum production areas and has been extrapolated from other crops such as maize. Nel and Lamprecht (2011) demonstrated a yield decline of 1.81 t ha^{-1} for each 25% increase in maize root rot severity, which indicated that yield loss is suffered even at low levels of infection. McLaren (2002) suggested that yield losses in sorghum cultivars was the result of an integration of root rot severity and inherent root volume (termed effective root volume) and reported an approximate 2% reduction in head volume for every 1% reduction in effective root volume

3.2. Pathogens associated with root rot

The roots are affected by a spectrum of fungi found in the soil, which makes it difficult to link each disease to a specific causal agent. The most common root rot causal plant pathogens are documented to be *Fusarium* spp., *Pythium* spp., *Macrophomina phaseolina*, *Colletotrichum graminicola* and *Periconia circinata* (Mughogho, 1984). Other pathogens associated with the disease include *Pyrenochaeta terrestris*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Erwinia* spp. (Tarr, 1962).

Fusarium spp. such as *Fusarium equiseti* and *Fusarium oxysporum* are, considered the major incitant of root rot in the USA (Reed *et al*., 1983, Windels and Kommendahl, 1984). However, contrary to this, Horinouchi *et al. (*2008) reported *F. equiseti* as a plant growth-promoting fungus and suppressive organism against crown and root rot of some crops. *Exserohilum pedicellatum, F. oxysporum, M. phaseolina, Pythium* spp. and *R. solani* were reported as the major root pathogens associated with maize in South Africa (Hugo, 1995).

Hugo (1995) further classified *Phoma* spp., *Curvularia* spp., *Fusarium chlamydosporum* as root colonizers based on their isolation frequency from discoloured root tissue and "healthy" root tissue on maize plants. van Rooyen (2012) measured root rot severity using root discolouration criteria and ergosterol concentrations. In some instances, root discolouration severity was high but ergosterol quantification (which is an indication of the total fungal biomass), indicated low severity, questioning the reliability of the former criterion. It was shown that some fungi were able to colonise roots without producing visible symptoms when ergosterol was quantified in sorghum cultivars (van Rooyen, 2012). Many factors, including environmental and mechanical can be associated with root discolouration and poor development (Hugo, 1995) as well as production of phytoalexins caused by fungal infection of sorghum and stress. These phytoalexins are responsible for the pigmentations of infected tissues in sorghum. These pigmentations could also be due to host resistance response to infection by specific isolates.

3.3. Dissemination and symptoms associated with root rot

Much has been reported on the survival, source and form of root and stalk rot initial inoculum. Little has however been reported on pathogen dissemination (Mughogho, 1984). Waniska *et al*. (2002) stated that root rot fungi can be disseminated through wind, animals, agricultural equipment and persist as hyphae, spores or resting structures in the soil, as well as in plant debris. The life cycle stages of most root rot complex pathogens are very similar although some can differ greatly (Agrios, 1997). Idris *et al.* (2008) suggested that the germination of spores and resting structures are encouraged by root and seed exudates. Pathogens gain entry into the roots and seeds through natural root wounds or injuries caused by machinery, insects and/or other causes (Claflin, 2000).
Soilborne pathogens are thought to attack plants late in the season because of the distinctive symptoms associated with root rot such as lodging and stunting as well as premature senescence. In studies conducted by Hugo (1995) and Giorda *et al.* (1995) pathogens were detected in the soft tissues of sorghum and maize seedlings. Despite these early infections, aerial symptoms are rarely detected on infected plants until maturity stage (Tarr, 1962, Gossen, 2016). Root rot attacks the sorghum plant at any stage from seed germination to maturity under favourable weather conditions. Sorghum rootlets are more vulnerable than maize and they grow more slowly. Seed germination failure, stunted growth and mushy, soaked roots are symptoms caused by seedling disease pathogens such as *M. phaseolina, Pythium* spp. and *R. solani*. Symptoms on older plants infected

with *Fusarium* spp. first appear as small, circular to elongate, light red to dark purple lesions on roots, seeds, stalks and peduncles. The fungus spread from the roots throughout the whole plant resulting in premature death during grain development stage, reduced grain weight and lack of grain fill. Most of the affected roots do not have root hairs, thus reducing the plant's ability to tolerate drought stress.

3.4 Environmental influences on root rot and their manipulation in management strategies of sorghum root rot

There is a major misconception on the initiation of plant diseases because the interdependency of the factors is not always considered in their development (Edmunds and Zummo, 1975). Abiotic and biotic environments play a critical role in disease development, pathogen activity and stability of disease resistance (van Rooyen, 2012). A study showed the effect of genotype, environment and the G x E interaction on root rot development as 15.1%, 70.5% and 9.19% respectively indicating that environmental factors are a primary driving variable in root rot epidemiology (McLaren, 2002). The relationship between pathogen, host and environment is however interchangeable, as one cannot occur in the absence of the other (Edmunds and Zummo, 1975).

Sorghum is known for its ability to thrive through dry seasons and drought but stress factors such as plant population density and weeds, extreme temperatures, moisture and nutrients, drought and high insect populations may predispose the host to infection resulting in yield reductions (Mughogho, 1984, Sauer, 2012). Limiting stress factors causing root rot in sorghum is essential to avoid soil fertility imbalances and stunted plants through cultural practices (Claflin, 2000).

3.4.1 Soil environment

Temperature

Temperature plays an important role in the growth of sorghum from vegetative stage to grain filling stage and yield (van Rooyen, 2012). Sorghum is adapted to high temperature regions however, extreme temperatures lead to reduction in yield due to delayed flowering stage. Temperatures required for the optimal growth and yield range from 20–30ºC with a frost-free period of 120–140 days (du Plessis, 2008). The base temperature for germination is from 7 - 10ºC (du Plessis, 2008). Colonization by certain pathogenic fungi can be enhanced or delayed based on soil temperature.

Host-pathogen associations differ in their temperature needs. Some require cooler temperatures while others require higher temperature for infection to prevail depending on areas, seasons and years in which the crop was planted (Agrios, 1997). Diseases such as *M. phaseolina* and *Pythium* spp. have been reported to be more serious during and after high temperature periods. According to literature 40ºC was found to be the best temperature for growth of *M. phaseolina* on maize (Pareek,

1991). Similar results were observed for sorghum charcoal rot in Rajasthan (Arora and Pareek, 2013). *Fusarium* spp. and *Typhula*, which cause snow mold in cereals and turf grasses, prefer cool seasons or cold regions. Mughogho and Pande (1984) also reported that low soil temperatures favour infection by *Fusarium* spp. while high soil temperatures favour infection by *M. phaseolina* and *Pythium* spp.

Moisture

Sorghum production in South Africa is found on a wide range of soils, and under fluctuating rainfall conditions of approximately 400 mm in the drier western parts to 800 mm in the wetter eastern parts (du Plessis, 2008). Infectious diseases emerge when these conditions change over time. Physical and chemical constraints favour the growth of root systems into the deep layers of soil where water is easily accessible over a long period, although, over short periods, it is water movement through soil rather than root growth that allows uptake of sufficient quantities of water to prevent harmful desiccation (Mughogho, 1984). Water is a primary restriction that affects crop production in semi-arid regions (Govaerts *et al*., 2007).

Arora and Pareek (2013) reported maximum disease severity of *M. phaseolina* to be at 40% soil moisture level, whilst disease incidence decreased when soil moisture was at 100% capacity. Similar results were obtained where yield reduction was observed as a result of *M. phaseolina* in dry and high temperate fields (Mihail, 1989). According to Agrios (1997) *Fusarium solani* causing dry root rot of beans, *Fusarium roseum* causing seedling blights as well as *M. phaseolina* causing charcoal rot of sorghum and root rot of cotton prefer drier environments. *Pythium* affects roots, tubers and young seedlings in wet soils because the disease is proportional to the amount of soil moisture. It delays root growth and seed emergence (Forbes *et al*., 1986). As soil moisture level increases, the greater the chances of *Pythium* spreading and reducing the ability of a host to defend itself from pathogens as the host is deprived of oxygen in the waterlogged soils (Agrios, 1997).

3.4.2 Cultural practises

Crop rotation

Crop rotation is one of the oldest, most efficient cultural control strategies and has been a pillar of agricultural practice for ages (Bullock, 1992). It is a planned order of specific crops planted on the same field that ensures that the succeeding crop belongs to a different family than the previous (Brankatschk and Finkbeiner, 2015). Rotation of cereals and legumes is the preferred management strategy over sole cropping because of the ability to produce high yield (Baldock *et al*., 1981), its affordable production costs and less reliance on external inputs such as synthetic fertilizers and pesticides (Zegada-Lizarazu and Monti, 2011).

The succession of different crops assists with nutrient content produced in the soil and transfer of essential nutrients from one crop to the next. Alternating hosts results in the food source of the pathogen being taken out of cycle as different parasites and pathogens are linked to different kinds of crops (Brankatschk and Finkbeiner, 2015). A gap is therefore created to avoid crop-specific parasites feeding on the hosts. Some of the alternate hosts serve as cultivation breaks whilst others are suppressors of infectious agents. Crop rotation systems have positive benefits. These benefit include: the ability to reduce reliance on synthetic chemicals, decreasing soilborne disease incidence and pest abundance, prevent soil depletion, maintain soil fertility, lower erosion due to a longer period of land cover, improve populations of microorganisms and maintain long-term productivity and organic matter and to help control weeds (Brankatschk and Finkbeiner, 2015, Sibhatu, 2015, Orion *et al.*, 2016).

Shuaibu *et al.* (2015) discovered the significant effect of cowpea and soybean on sorghum's plant height, grain weight and grain yield when top-dressed with 60 kg.ha^{-1} as opposed to fallow. Fallow has always been the traditional method of soil fertility restoration however, with the increasing human population, monocropping has been the only source of food production leaving the soil exhausted and nutrient depleted (Gary *et al.*, 2003, Ncube *et al.*, 2007). In agreement with the above statement Ncube *et al.* (2007) found that sorghum grain yield increased after rotation with legume crops (bambara, cowpea, groundnut and pigeon pea) than when sorghum was planted after sorghum.

3.4.3 Biological control

Alternative methods that are environmentally friendly were sought after excessive use of chemicals for decades, which were a health hazard for animal and human life (Kennedy, 1998). Biological control methods aim at improving host's resistance by favouring microorganisms antagonistic to the pathogen (Agrios, 1997). Biological control using bacteria is thought to be the safest method in eradicating microorganisms causing harm to roots. There are some plant growth promoting rhizobacteria (PGPR) that have been selected as biocontrol agents as they contribute to a sustainable environment and the productivity of both agricultural systems and natural ecosystems (Lugtenberg *et al.*, 1991, Persello-Cartieaux *et al.*, 2003).

PGPR are known to colonise roots in the rhizosphere, suppressing microorganisms as well as soilborne pathogens (Rangajaran *et al.*, 2003, Barea *et al.*, 2005). There are two different mechanisms involved in the suppression of pathogens ie. direct and indirect mechanisms. The direct mechanisms include competition for colonisation or carbon and nitrogen sources as nutrients and signals, production of siderophores, phytohormones (Glick, 1995), inhibition by antibiotics and pathogenicity factors and parasitism. The indirect mechanisms include improvement of plant

nutrition, changes in the rhizosphere and activation of plant defence mechanisms leading to enhanced plant resistance.

The widely recognised biological control agents available on the market, include commercial solutions from both bacterial and fungal genera ie. *Bacillus* spp. (*B. cereus*, *B. subtilis*, *B. pumilis)* (Bai *et al*., 2002, Barea *et al*., 2005), *Pseudomonas* spp. (Amy *and* Germida*,* 2002), *Trichoderma* spp. (Harman *et al*., 2004), *Burkholderia* (Barea *et al*., 2005) and *Streptomyces* (Gopalakrishnan *et al*., 2013). *Trichoderma* suppresses plant pathogens by releasing lytic enzymes mainly chitinases, glucanases, and proteases and toxic compounds such as antibiotics, gliotoxin, gliovirin, and peptabiols.

The genus *Rhizobium* is the most well recognised group of growth plant promoters because of its association with plants and it has been commercialized with many practical applications in agriculture (Barea *et al*., 2005, Idris *et al*., 2007). The importance of the genus *Rhizobium* is their interaction with the legume roots forming nodules that fix nitrogen for plant growth improvement (Polenko *et al*.,1987, Barea *et al*., 2005). Due to complexity of microorganisms in the soil, suppression of a pathogen can be due to one or more mechanisms depending on the involved antagonist. It is thought that for effective suppression of pathogens a combination of mechanisms should be involved (Barea *et al*., 2005).

3.4.4 Chemical control

The use of chemicals in controlling root diseases has been practised for centuries and is still used because of the effective results in improving productivity in conventional agriculture (Idris *et al*., 2007). Such chemicals are, however, costly especially in developing countries and are potentially harmful to society by putting human and animal health at risk (Kennedy, 1998, Bowen and Rovira, 1999). Chemical control aims at curing the already present infection in plants or protecting the plant surfaces from the initial inoculum (Agrios, 1997). Chemicals are mainly used to increase the quality and yield of crops and to avoid crop losses as well as post-infections during storage (Abawi and Widmer, 2000).

Some chemicals are able to trigger defences in the plant (systemic acquired resistance) against harmful microorganisms. Other chemicals are phytotoxic and some such as broad-spectrum fungicides can create imbalances within the microbial community that result in unfavourable conditions that supress the activity of beneficial microorganisms as opposed to using pathogen specific fungicides (Idris *et al*., 2007, Al-Jedabi, 2009). Fungicides used for soil treatment to reduce nematodes as well as fungal and bacterial pathogens include metalaxyl, diazoben, pentachloronitro-benzene, captan and chloroneb (Agrios, 1997). Captan and chloroneb are mainly used as seed

treatment. There are different ways in which these fungicides are applied ie, as dusts, liquid drenches or granules to control damping off, seedling blights, crown and root rot (Agrios, 1997).

According to Vatchev and Maneva (2012) a reduction in plant mortality of 11.1% - 84.81% and 23.8% - 77.1% was recorded when the roots of cucumber plants infected with *Fusarium oxysporum* were drenched with a combination of fungicides. Fungicides included in the study were thiophanate methyl, propamocarb hydrochloride and benomyl. It has been reported that a combination of three fungicides reduced the inhibitory effect on the disease as opposed to one or two treatments. In contrast, Idris *et al*. (2007) used benomyl in reducing root rot of sorghum in Ethiopia but did not succeed because of the phytotoxicity towards sorghum (Benhamou, 1992). Benomyl is a systemic fungicide (Agrios, 1997). Idris *et al*. (2008) reported that *Pythium ultimum* can be controlled by metalaxyl on sorghum but it was not the case when metalaxyl was applied in Ethiopia's Alemaya areas with cooler and wetter soils and control of *P. ultimum* root rot was unsuccessful.

4. Grain mold of sorghum and mycotoxins

Figure 2: Symptoms of grain mold on sorghum caused by *Fusarium* spp. (Choi *et al*., 2013).

Grain mold causes yield loss and affects the market value of sorghum grains (Ambekar *et al*., 2011). Grain mold of sorghum is common in areas with high humidity and moderate temperature (Williams and Rao, 1981). Many complex pathogenic and opportunistic fungi from different genera are associated with grain mold of sorghum (Menkir *et al*., 1996). This complex of fungi includes various *Fusarium* spp. such as *Fusarium thapsinum* Klittich, Leslie, Nelson, and Manasas, *Fusarium semitectum* Berk. and Ravenel, *Fusarium proliferatum* (Matsushima) Nirenberg, and *Fusarium andiyazi* Marasas, Raheeder, Lamprecht, Zeller and Leslie, *Curvularia lunata* (Wakk.) Boedijn, *Alternaria alternata* (Fr.) Keissler, and *Curvularia sublineola*, (Williams and Rao, 1981, Pitt *et al*.,

1994; Das *et al*., 2012). *P. sorghina* has also been reported to cause grain mold (Forbes *et al*., 1992, Pitt *et al*., 1994).

Even though various fungi in the soil are capable of causing grain mold of sorghum, each has a specific requirement for infection in terms of climatic conditions (Lahouar *et al*., 2015, Cuevas *et al*., 2016). Predominant species differ among geographic locations and across years (Denis and Girard, 1980). Grain mold occurs at any time between anthesis, harvest, storage and transport (Menkir *et al*., 1996). Due to the complex of fungi found in the soil, inoculum is ever present depending on the climatic patterns at flowering and grain filling stages of the crop (Tarr, 1962). White grain pericarp sorghum cultivars are the most susceptible compared to the brown and red grain pericarp cultivars (Ambekar *et al*., 2011).

Biotic factors, such as insects, can also play an essential role in facilitating infection of grain by grain mold pathogens (Ratnadass *et al*., 2003). Physical damage of the grain appears as softened and chalky endosperm, discoloured pericarp, decreased grain size and density, occurrence of mycotoxins and different composition of phenolic compounds (Salifu, 1981, Williams and Rao, 1981). Grain mold also result in losses in seed mass, grain density, seed germination, storage quality, food and feed processing quality and market value (Ambekar *et al*., 2011). The visible symptoms in the field appear as pink, white, orange and black discolouration of the grain surface (depending on the pathogen) (William and Rao, 1981, Bandyopadhyay *et al*., 2000).

Grain mold pathogens may also produce mycotoxins, which are a health hazard to humans and animals, limiting the use of grain sorghum as food, and feed (Castor and Frederiksen, 1980, Thiel *et al*., 1992, Chu and Li, 1994). Mycotoxins are secondary metabolites produced by fungi that induce a toxic response when introduced in low concentrations to higher vertebrates and other animals (Bennett, 1987). The name was derived in the 1960's from the Greek word "mykes" meaning mold, and "toxicum" meaning poison (Mavhunga, 2013). Ismaiel and Papenbrock (2015) further explained that mycotoxin was first used in 1960 to describe the contamination of peanuts in animal feed by toxins and the loss of turkeys in England. Several filamentous fungi that include *Acremonium*, *Alternaria*, *Aspergillus*, *Claviceps*, *Fusarium* and *Phomopsis* spp. cause mycotoxin contamination (Barrett, 2000, Śvábová and Lebeda, 2005, Zain, 2011, del Palacio *et al*., 2016).

More than 400 mycotoxins have been documented, with the most recognised being aflatoxins, ochratoxins, fumonisins, zearalenone, trichocenes and patulin (Udomkun *et al*., 2016). Toxigenic fungi that produce mycotoxins are divided into two groups namely "field" and "storage" fungi. Field fungi (e.g., *Cladosporium*, *Fusarium* and *Alternaria* spp.) are referred to as those that invade and produce toxins before harvest whilst "storage" fungi (e.g., *Aspergillus* and *Penicillium* spp.) produce toxins in the storage room after harvest (Miller, 1995). Production and accumulation of mycotoxins in the soil is mostly due to the factors that favour phytopathogenic growth resulting in cell death of

the crop's vascular tissue (Wagacha and Muthomi, 2008). These factors include moisture, water activity, substrate temperature, aeration and substrate availability (Mavhunga, 2013).

4.1 Mycotoxins produced by *Fusarium* spp. and their effect on humans and animals

4.1.1. Trichothecenes: Deoxynivalenol and nivalenol.

Deoxynivalenol (DON) is the most common, but less toxic of the trichothecene mycotoxins that occur worldwide (Miller, 1995, Langseth *et al*., 1999, Bennett and Klich, 2003). *F. graminearum* (*sensu lato*) strains in most cases fail to hydroxylate the C-4 position and accumulate DON rather than Nivalenol (NIV) (Desjardins, 2006). The toxin is water-soluble that may be translocated in the phloem of stalks and ears, devoid of *F. graminearum* (*sensu lato*) (Mavhunga, 2013). Covarelli *et al*. (2012) reported that symptoms on wheat seedlings inoculated with *Fusarium culmorum* were observed up to the third node whilst DON was present in all stem segments and heads. DON was more concentrated in tissues beyond those colonized by fungus, translocating to the head where it accumulated mainly in the rachis with significant quantities in the grain.

Lancova *et al*. (2008) reported that DON was found in hyphae of fungal colonised grains, of which the concentrations correlated highly with ergosterol concentrations. This mycotoxin is mainly found in contaminated wheat, maize (Bennett and Klich, 2003, Audenaert *et al*., 2013) barley, rye, sunflower seeds and mixed feeds (Bennett and Klich, 2003). Fungal species closely associated with production of DON include *F. graminearum (sensu lato), F. culmorum* and *Fusarium crookwellense* as well as other related species based entirely on the geographic origin of the isolate (Miller *et al*., 1991). Distribution of these pathogens in the grain depend solely on temperature as some proliferate in cooler temperatures whilst others prefer warm temperate regions (Miller, 1995). Under favourable conditions, agronomic practices play an important role in the impact of this mycotoxin (Miller, 1995).

DON targets domestic pigs (Miller, 1995). Poultry and cattle are more tolerant to DON in their diets than pigs (Prelusky *et al*., 1994). Cattle are able to degrade the secondary metabolites in the rumen (Miller, 1995). Although, poultry are tolerant to DON, it does affect egg production and quality. Effect from ingestion of feed contaminated with DON on domesticated pigs and cattle results in feed refusal (Prelusky *et al*., 1994). Human beings are most susceptible to DON. Carcinogenicity of DON and NIV is of no special concern but their co-occurrence with aflatoxin may synergize the carcinogenicity of aflatoxins (Ueno *et al*., 1992). It is commonly referred to as vomitoxin based on the symptoms that can be observed in humans that have consumed DON infected foods (Bennett and Klich, 2003).

NIV causing fungi include *F. graminearum* (*sensu lato*), *F. culmorum*, *Fusarium cerealis* and *Fusarium poae* (Desjardins and Proctor, 2011). Sorghum in South Africa is solely colonized by the NIV producers *Fusarium meridionale, Fusarium acaciae-mearnsii* and *Fusarium cortaderiae*

(Mavhunga, 2013). NIV is the trichothecene biosynthesis product and DON is regarded as the pathway intermediate (Desjardins, 2006). Trichothecenes have a wide host range including sorghum, rye, barley, wheat and oats. NIV is found in lower concentrations than DON in host crops, however it is thought to be more virulent (Pestka, 2007). NIV chemotype produces NIV and 4–ANIV, which were reported in Africa and Asia (Desjardin, 2006). However, Boutigny *et al.* (2012) reported that NIV producing *F. graminearum* (*sensu lato)*. are infrequently detected in South African wheat and maize. Its effect on chickens include reduced feed consumption and liver weight (Hedman *et al.*,1995). Inhibition of protein, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis in humans and livestock causes cell necrosis and toxicosis to lymphoid and intestinal organs.

4.1.2. Zearalenone

Zearalenone (ZEA) is one of the three most frequently distributed mycotoxins contaminating agricultural products globally (Elbashir and Ali, 2014, Beukes *et al.*, 2016). Its discovery was in African and Asian grain imported into Japan. The tested grain contained concentration of 7260 µg.kg^{-1} (Aoyama *et al.*, 2001). *Fusarium graminearum* Species Complex (*Fg*SC) is one of the primary producers of ZEA. Fungi causing ZEA include *F. graminearum* (*sensu lato)*, *F. cerealis*, *F. culmorum, F. equiseti and F. semitectum* (Martins and Martins, 2000). ZEA has a wide range of hosts including sorghum, maize, barley, oats and contaminated bread made of wheat (Mavhunga, 2013).

It is a nonsteroidal resorcyclic acid lactone and is transformed into derivatives α and β zearalenol, which trigger its toxicity (Mavhunga, 2013). Swine are reportedly the most sensitive to ZEA and poultry are the least affected. ZEA is referred to as an oestrogenic toxin resulting in infertility of animals and pubertal changes in children (JECFA, 2000). The pre-pubertal conditions experienced in female swine included vulva reddening and swelling which is caused by the excessive release of oestrogen. Human cervical and breast cancer have been reported (Ahamed *et al.*, 2001). Livestock fed contaminated cereals can be used as an indication to record the extent of contaminated feed, duration of exposure, persistence of the animal and species difference in terms of metabolism (Magan and Olsen, 2004). Effects such as interference with conception, ovulation, implantation, foetal development and viability of the newborns have been reported with concentrations ranging from 50–100 parts per million (ppm). The regulated lowest maximum allowable limit of ZEA is 100 µg.kg^{-1}. The tolerable daily intake limit of ZEA infected products is set to 0.25 µg.kg^{-1} body weight (Marroquin-Cardona *et al.,* 2014).

4.1.3. Fumonisins

Fumonisins are influential phytotoxins that cause electrolyte loss and interfere with the formation of complex sphingolipids (Abbas *et al.*, 1993, Dutton, 1996). They were discovered in 1988 after an outbreak of Elem, a well-known disease in horses (Gelderblom *et al.*, 1988). Fumonisins have also

been associated with human oesophageal cancer in parts of southern Africa (Transkei) (Marasa et al., 1988). There are three types of fumonisins i.e. B_1, B_2 and B_3. B_3 is the most common as well as toxic and occurs at highest concentrations (Miller, 1995, Bennett and Klich, 2003). Fumonisins are commonly detected in maize but have also been reported in grains of sorghum and pearl millet (Vismer *et al.*, 2015). Maize crops grown in cold areas are more likely to escape the fumonisins causing pathogens (Miller, 1995) compared to warmer areas which favour the development of *F. verticillioides* which is the most prevalent ear disease causal organism of maize (Udomkun *et al.,* 2016). Janse van Rensburg *et al.* (2015) reported that fumonisisn levels on local field sorghum are low.

F. verticillioides and *F. proliferatum* are common pathogens thought to produce fumonisins especially in maize (Miller, 1995, Miller, 2008). Udomkun *et al.* (2016) mentioned that *Aspergillus niger* also plays a role in fumonisins production. *F. moniliforme* (*sensu lato)* systemically occur in leaves, stems, roots and kernels of maize even in healthy crops because of the mutual relationship they have, in which the pathogen supplies the crop with fusaric acid that is beneficial to plant growth (Miller, 2008).

Lew *et al.* (1991) reported that European maize borer increased *F. moniliforme* (*sensu lato).* and fumonisin concentrations in a maize field. *F. verticillioides* is ubiquitous in almost all maize samples. As not all strains produce toxins, the presence of fungus does not imply the presence of mycotoxin (Plumplee and Galey, 1994). This pathogen thrives in warmer temperatures above 28^{0} C and accumulates more in stressed plants (Miller, 2008). Fumonisins cause pulmonary edema and hydrothorax in swine (Harrison *et al.*, 1990), leukoencephalomalacia (hole in the head syndrome) in equines (Marasas *et al.*, 1988) and rabbits (Bucci, *et al.*, 1996), pulmonary edema and hydrothorax in swine, hepatotoxic and carcinogenic effects (Miller, 1995) and apoptosis in the liver of rats (Pozzi *et al.*, 2000). In humans, there is a probable link with esophageal cancer (Marasas *et al.*, 1988).

4.1.4. Moniliformin

The discovery of moniliformin came about after screening for toxic products on a North American isolate of *F. moniliforme* Sheldon (*Fusarium verticillioides* [Sacc.] Nirenberg) cultured on a maize medium (Cole *et al.*, 1973). After its discovery, two more Northern American isolates (Burmeister *et al.*, 1980) and one South African maize isolate of *F. moniliforme*, which produced moniliformin, were identified (Ismaiel and Papenbrock, 2015). A broad number of pathogens are associated with moniliformin production with *F. proliferatum* and *Fusarium subglutinans* being the most common (Carmen *et al.*, 2004). Rabie *et al.* (1982) conducted a study in moniliformin production and toxicity of different *Fusarium* spp. in southern Africa and discovered four new *Fusarium* spp. that were associated with moniliformin production viz. *Fusarium acuminatum*, *Fusarium concolor*, *F. equiseti*,

and *F. semitectum*. Moniliformin occurs as a water-soluble sodium or potassium salt (Steyn *et al*., 1978).

Moniliformin was first discovered in Transkei-South African in 1982 on maize (Thiel *et al*., 1982). Most mycotoxins produced are commonly associated with maize although, Rabie *et al*. (1982) reported that *Fusarium fusarioides* found in millet, sorghum, peanuts, dried fish, and soil are also capable of producing moniliformin. Moniliformin has an inhibitory effect mainly on leaf development rather than on roots of wheat seedlings (Wakulinski, 1989). It has a phytotoxic effect on plant systems as well as animals including ducklings, rats, mice and mink. Moniliformin is poorly understood as few studies have been conducted as it is regarded as a non-carcinogenic toxin. It is neglected because it occurs in small doses, however during processing its stability is not known whereby its extent in relation to consumer exposure is uncertain (Carmen *et al*., 2004).

5. Root rot and grain mould detection methods

5.1. Visual scoring

Visual scoring of root rot and grain mold can be used to estimate severity (degree of colonization of a uniform sample indicated by signs or discolouration), incidence (proportion of root or grain affected), or damage (reduction in root or grain size) (Forbes *et al*., 1992). The visual method used to assess root rot and grain mold depends entirely on estimates of root or grain discolouration either on a rating scale or a percentage scale (Forbes *et al*., 1986). Pathogens can be detected through plating out techniques where roots and grains are cultured on growth media that favour growth of specific pathogens, after which samples are identified based on their morphological characteristics and quantified (Leslie and Summerell, 2006). However, the method is thought to be unreliable because it excludes fastidious organisms, or slow growing pathogens (Gossen, 2016). One study showed that identification of microorganisms should not only be dependent on the morphological characteristics. O'brien and Thirumalachar (1969) discovered that charcoal rot had two causal agents thus, *M. phaseolina* and *Botryodiplodia solani-tuberosi* both of which are alike at their mycelial stage but differed in their pcynidiospores.

Visual rating has been the common means of quantifying grain mold to date (Forbes *et al*., 1992). It is mostly unreliable because the results are based on the "rater" with which their accuracy will always differ from one rater to the other (Madden *et al*., 2007). Other factors interfering with visual rating accuracy include the frequency, timing and sampling size. A scale of 1–5 is used in scoring grain mold, where 1 = no deterioration, 2 = 10% of grain surface deteriorated, 3 = moderate deterioration with 11–25% of the grain surface deteriorated, 4 = considerable deterioration with 26–50% of the grain surface deteriorated and 5 = extensive deterioration with more than 50% of the grain surface deteriorated (Audilakshmi *et al*., 2007). Grain mold visual rating offers only the qualitative data not

quantitative and correlation between level of damage and corresponding level of disease is of outmost importance (Forbes *et al.*, 1986).

5.2. Molecular analysis

Molecular techniques are thought to be the most reliable methods of detecting and quantifying the extent of tissue colonization by pathogens, as they can be both qualitative and quantitative (Coetzee, 2015). Such techniques include polymerase chain reaction (PCR), quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA). PCR techniques are rapid and sensitive (Henson and French, 1993) but are thought to be time consuming and not quantitative compared to qPCR (Muppa, 2009). qPCR is time efficient, specific and quantitative. It is called real time because during amplification one is able to monitor the cycles as the machine runs. It quantifies the total fungal biomass present in a sample with specific primers (Muppa, 2009). Optimisation of qPCR reaction is aimed at amplifying the target DNA (Mavhunga, 2013, Schoeman, 2016).

The target DNA of any studied pathogen should be of high quality, pure and not at too high or too low a concentration. DNA concentration with A260/280 of ~1.8 is accepted as “pure” for DNA (Schoeman, 2016). Too high a DNA concentration results in a combination of primers and dNTPs binding to the DNA with no amplification whilst too low a DNA concentration with too high primers and dNTPs cause primer dimers (false positives) (Burggraf and Olgemöller, 2004).

There are three basic steps involved in the qPCR technique viz. denaturation, annealing and elongation. The thermal cycling includes the denaturing of DNA strands at 94ºC for 10 min and primers bind at specific regions of the target DNA that require amplification between 40-65°C (annealing) and the primer extension to provide a second strand of DNA to be synthesized (Coetzee, 2015). The elongation method requires the use of thermostable DNA polymerase and deoxyribonucleoside triphosphates (dNTPs). Repetition of these steps for 40 cycles at 94ºC for 30 sec; 60ºC for 30 sec and 72ºC for 30 sec, 95ºC for 30 sec and 40ºC for 30 sec (Schoeman, 2016). This allows for accurate amplification of the multiplication of the specific DNA to an amount, which allows the DNA to be detected and quantified (Henson and French, 1993).

The detection and quantification of the target DNA can be conducted through the Hydrolysis assay approach as it is primer specific, uses fluorescent probes and gives accurate amount of fungal biomass (Muppa, 2009). Three types of dyes and probes used in the Taqman qPCR approach include Taqman^R probes, fluorescent resonance energy transfer (FRET) probes and molecular beacons (Schaad and Frederick, 2002). The probes carry a florigenic reporter dye and a quencher dye. When the probe is intact, the reporter dye fluorescent emission is absorbed by the quencher dye (Mavhunga, 2013). The probe is then digested by Taq DNA Polymerase, which activates the

reporter dye fluorescence. The fluorescence intensity increases exponentially with repeated cycle (Hogg *et al.*, 2007)

A cycle threshold (Ct) is calculated every time a target gene makes contact with a probe or dye during amplification (Coetzee, 2015). Use of qPCR is of paramount importance because it offers a more accurate assessment of fungal biomass in roots and grains, compared to microbiological methods. Successfully implemented real-time PCR can be useful for assessing the impacts of many environment and management practices.

5.3. Ergosterol concentration

Ergosterol is a predominant sterol mostly found in cell membranes of filamentous fungi. It is present in smaller quantities in higher plants (Pasanen *et al.*, 1999). Disease severity should be established through both visual scoring as well as through quantification of the actual fungal colonization of the affected plant part (Mpofu, 2009). Ergosterol concentration measurement is used to estimate the total (viable and nonviable) fungal biomass which considers all fungal growth events e.g. in soil, aquatic systems or indoor environments (Pasanen *et al.*, 1999) but neglects the survival of yeast cells (Pasanen *et al.*, 1999). It is the preferred fungal biomass measure because of its sensitivity as opposed to methods such as chitin that were used to detect the mycelium quantity in maize and wheat (Donald and Mirocha 1977). The ergosterol concentration procedure is used to distinguish levels of grain mold resistance in grain crops (Jambunathan *et al.*, 1991). This measurement determines the internal damage that mold or rot exerts on the grain/roots by colonizing it and the process cannot be seen with an unaided eye (Mpofu, 2009). Therefore, a combination of assessment severity of different fungi (visually or on agar) in a grain/root sample in conjunction with ergosterol measurement indicates the identity of the fungi and their quantity.

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Chapter 2

Changes in macro/micro elements associated with legume/sorghum-based rotation systems and their relationship with root rot

Abstract

Maintenance of soil fertility is essential if sorghum production, nutrient value and market value are to be sustained. Most soils in South Africa have deteriorated due to the significant challenges brought about by unfavourable environmental conditions, monocropping, diseases and agrochemicals that have led to unsustainable agriculture. The effect of crop rotation systems on the nutrient status of soil and the relationship between soil health and root rot severity in subsequent sorghum crops were evaluated from 2015 to 2018. Plants were randomly collected from sorghum plots at Potchefstroom to quantify root colonization using visual ratings and ergosterol content and to test for 10 root pathogens in root tissues using qPCR analyses. Soil samples were also collected at the beginning of every season to test for soil nutrient elements ie. N (NO_3 and NH_4), P, K, Ca, Mg, Na, Fe, Cu, Zn and Mn and soil pH that contribute to improved soil and plant health. Yields of three sorghum cultivars, PAN8706W, PAN8816 and NS5511 were compared, with the latter yielding the highest in both seasons (2.367 and 2.35 t.ha^{-1}) despite its high visual root rot severity. Significant increases in P and K were observed in 2017/18 season under bambara, cowpea and monoculture rotation systems. Higher ergosterol levels, ranging between 124.5–1049.2 were recorded in a drybean/sorghum rotation system. Root rot severity index was significantly higher in NS5511 (2016/17 = 177.5 and 2017/18 = 358.5) and lower in PAN8706W (2016/17 = 0.0; 2017/18 = 152.5). The prominent fungal pathogens *Fusarium chlamydosporum*, *Fusarium oxysporum* and *Phoma* spp. were significantly affected by crop rotation systems with a decline in the successive seasons showing the effect that crop rotation has on soil borne pathogen populations. Regression relationships suggested that only a few macro- and micronutrient had an influence on root rot severity of sorghum. Certain ranges of nutrient concentrations occurred at which the root rot severities were lowest indicating that any concentration lower or higher than the range thresholds would result in increased root rot severity. Yield and root rot were not significantly affected by legume-based rotations with sorghum. The overall results indicate that the benefits of legume rotation systems on root rot of sorghum were limited although tendencies in increased availability of nutrients and disease suppression were evident in the various legume rotation systems.

2.1 Introduction

Nutrient deficiency is often experienced in African soils due to their nature and prevailing environmental conditions resulting in low fertility status (Padwick, 1983, Bado *et al.*, 2011). Factors associated with low fertility status include low infiltration rate, compaction, acidity and organic carbon stocks (Mnkeni and Mkile, 2006) that generally affect soil density, porosity, water infiltration and root growth in crops, thereby reducing crop yield (Tavares Filho *et al.*, 2001). According to Primavesi (1990) crop production on biologically inactive and physically deteriorated soils, result in crops responding less to applied chemical inputs. South African soils have in the past, been regarded as low in organic matter content (du Toit and du Preez, 1995), but the use of different crop rotation systems could help in improving soil fertility and nutrient absorption efficiency, thus increasing yield through nutrient cycling (Moreti *et al.*, 2007).

A degree of root rot is omnipresent in every plant, but the tell-tale aerial symptoms do not always manifest when severities are low (White, 1999). The absence of visible aerial symptoms often result in root rot not being considered a cause of yield loss (Tarr, 1962), and such losses are generally attributed to other factors, such as climatic conditions or general poor performance of the cultivar. Quantification of yield losses associated with root rot remains a challenge due to the difficulty in obtaining healthy controls (White, 1999). Nel and Lamprecht (2011) were able to demonstrate a yield decline of 1.81 $t.ha^{-1}$ for each 25% increase in maize root rot severity, which indicated that yield loss is suffered even at low levels of infection. A number of soil fungi have been associated with root rot, including *Macrophomina phaseolina*, *Fusarium moniliforme (sensu lato)*, *Periconia circinata*, *Pythium* spp. and *Colletotrichum graminicola* (Reed *et al.*, 1983, Pande and Karunakar, 1992). van Rooyen (2012) in isolation, sequencing and pathogenicity studies indicated *Fusarium solani*, *Fusarium temperatum*, *Alternaria* spp., *Phoma macrostoma*, *Phoma sorghina*, *Acremonium strictum*, *Curvularia trifolii* and *Colletotrichum capsici* in sorghum roots.

Agronomic and economic success of crop rotation systems are dependent on the choice of crops (Malik, 2010), high dry matter production, yield and a capability to recycle nutrients and improve soil health (Malik, 2010, Oliveira *et al.*, 2011). Legumes are traditionally ideal crops to be rotated with cereals as they improve soil fertility (Bagayoko *et al.*, 2000) through their nitrogen fixation ability (Bationo and Ntare, 2000). Groundnut/sorghum and cowpea/sorghum rotations increased soil mineral N by 36% and 52%, respectively in Burkina Faso (Bado *et al.,* 2011). Nutrients play a vital role on a plant's yield and may change the host defence mechanism as well as its tolerance to diseases (Dordas, 2008). Mineral nutrition has the ability to interfere with resistance mechanisms *viz.* formation of mechanical barriers and synthesis of natural defence compounds such as phytoalexins, antioxidants and flavonoids (Schumann *et al.*, 2010). Plant pathogens utilise their hosts as a food source. The nutrient status of tissues utilised by the pathogen can affect plant development and the eventual disease severity observed, either negatively or positively (Schumann *et al.*, 2010).

Most soils are nutrient deficient because of the lack of nutrient input in developing areas (Bado *et al.*, 2011). Schumann *et al.* (2010) reported a study on a N deficient plant population using an ammonium-based fertilizer and nitrate-based fertilizer. The latter resulted in a decline incidence of diseases such as *Fusarium* and *Phytophthora* root rots, while the ammonium-fertilizer increased the incidence of the two disease. Carley and King (1968) similarly reported an increase in pea root rot when N was applied in the form of ammonium as opposed to nitrate. Weinke (1962) found root rot of bean caused by *Fusarium solani* to be severe when ammonium-N as opposed to nitrate or urea-N was applied.

The aim of this study was to establish the impact of various crop rotation systems on the nutrient status of the soil and the relationship between soil health and root rot severity in subsequent sorghum crops.

2.2 Materials and methods

2.2.1 Field trial

Field trials were planted at Potchefstroom (ARC-Grain Crops (26°43'43.16"S - 27°04'47.71"E), South Africa) during 2015/16, 2016/17 and 2017/18 with the objective of simulating sorghum-legume rotation systems over a three-year period. The six main plots were established during the first season (2015/16) by either fallow or planting of sorghum, dry bean, soybean, cowpea and bambara (Table 1). Plots were 18 m in length with a 0.9 m inter-row spacing to accommodate 12 rows. In 2016/17, main plots were split into two sections of six rows each. One section of six rows was re-planted with the respective crop from the previous season e.g. dry bean, while the second section was planted to three diverse sorghum cultivars i.e. PAN8816 (low tannin red), PAN8706W (tannin, white) and NS5511 (high tannin brown). This created a legume/sorghum, fallow/sorghum, sorghum monoculture as well as a repeated season of the initial cropping system. Due to space limitations, only two rows were planted per sorghum cultivar. During the 2017/18 season the whole plot (i.e. each of the two sub-sections) was planted with the sorghum cultivars resulting in a two rows per cultivar in each of the double initial system/sorghum treatment and initial treatment/double sorghum treatment. Trial layout was a split plot (crop rotation system as main plot, and sorghum cultivar as sub-plot factor) with three replicates. The trial was executed as a no-till trial, with no fertilizer applications for the entire duration of the trial. Dimethenamid (75 $g.l^{-1}$) was applied directly after planting at a rate of 2000 $ml.ha^{-1}$ for pre-emergence grass control, followed by Basagran® (Bendioxide - thiadiazine 480 $g.l^{-1}$) post-emergence as required at 1.5 $l.ha^{-1}$ for broadleaf weeds. Hand weeding was also done when required. Decis® (deltamethrin, 25 $g.l^{-1}$) was applied at 250 $ml.ha^{-1}$ for pest control, including stalk borer and aphids.

2.2.2 Establishing soil nutrient status of various legume based crop rotation systems

Soil was collected at the beginning of each planting season (October) using a 90 cm stainless steel soil auger. Soil samples were collected at depth of 15-30 cm. Two soil samples per plot were randomly taken, mixed and a representative sample was sent for analyses. During October 2016/17, the samples were taken from the whole plot in order to establish the soil nutrient status as a result of the initial crop system (planted during 2015/16) and to compare the effect on sorghum root rot severity and associated root rot pathogens during the 2016/17 season. During October 2017/18, the samples were only taken on the sub-plots where sorghum was planted in the preceding season (due to cost implications). This allowed the nutrient status as a result of crop rotation with a legume followed by sorghum and the effect on sorghum root rot and associated soil borne pathogens in the third year of the rotation system (e.g. cowpea/sorghum/sorghum) to be quantified. The analyses were conducted at ARC-Industrial Crops (IC), Rustenburg, South Africa. Elements tested for included macronutrients N (NO_3 and NH_4), P, K, Ca, Mg, and Na, micronutrients Fe, Cu, Zn and Mn and soil pH.

2.2.3 Field sampling and yield calculation

Ten randomly selected sorghum plants per cultivar per plot were sampled every season at soft dough stage to establish root rot severity. The root samples were also used to identify and quantify fungi that colonized the sorghum roots. Samples were taken from a part of the inner row of each cultivar allocated for destructive sampling and did not form part of the row area allocated for yield calculation. At physiological maturity, plants from a 10 m designated inner row of each cultivar were harvested by hand. Harvested panicles were threshed and grain weight was obtained. Grain moisture was determined with a Twist Grain pro moisture meter (Draminski Elektronics, London, U.K). Yield ($t.ha^{-1}$) was calculated at 12.5% moisture using the following equations:

$$Grain\ yield\ (t.ha^{-1}) = \frac{grain\ mass\ (kg)}{1000}\ x\ Adjusted\ grain\ moisture\ x\ Area\ factor \quad [1]$$

where:

$$Adjusted\ grain\ moisture\ (\%) = ((100 - grain\ moisture\ \%))/((100 - 12.5)) \quad [2]$$

and

$$Area\ factor\ (m^2) = \frac{10{,}000}{(row\ length\ x\ row\ width)} \quad [3]$$

2.2.4 Visual root rot severity rating and sample processing

Root samples were washed under running tap water to remove soil and left to dry for 24 hours to avoid dampness prior to visual rating. Roots were visually assessed for root rot severity based on the percentage of root discolouration. Root rot incidence was quantified as the percentage of the plants sampled per plot that demonstrated some degree of rot (visual discolouration). A root disease index (RDI) was used to record disease severity of each plant sampled based on an adjusted scale of 0–4 where, 0 = no symptoms, 1 = 1–24% rot, 2 = 25–49% rot, 3 = 50–75% rot and 4 = 75–100% rot (Soonthornpoct *et al.*, 2000). Disease severity in the sampled population was accordingly calculated as the product of disease incidence x RDI (Soonthornpoct *et al.*, 2000). After rating, the entire root system of every plant was cut into 2 mm pieces. Root material of the ten sampled plants per cultivar per plot were pooled and thoroughly mixed. A 6 g subsample was taken and ground in liquid nitrogen using a pestle and a mortar. Approximately 1 g of the powdered material was added to 1 ml of DNA Extraction Buffer (DEB) in 2 ml Eppendorf tubes, which were maintained at -80°C until further use. Five g of the powdered sample was used for ergosterol quantification. Samples were stored in brown paper bags in a cold room at 4°C.

2.2.5 Fungal target DNA quantification

2.2.5.1 Fungal reference isolates for qPCR analysis

Eight fungal isolates (*Curvularia eragrostidis*, *Exserohilum pedicellatum*, *Fusarium chlamydosporum*, *Fusarium equiseti*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Phoma* spp., and *Rhizoctonia solani)* were purchased from the National Collection at the Agricultural Research Council–Plant Protection Research Institute (PPRI), Pretoria, South Africa, for specificity tests, standard curves and positive controls. In addition, a *Fusarium verticillioides* isolate (MRC 826) was provided by PROMEC-MRC and a *Fusarium graminearum (sensu lato)* isolate was provided by Stellenbosch University, Plant Pathology Department, Stellenbosch, South Africa. These 10 pathogens are known as common root colonizers of sorghum (Hugo, 1995). The isolates were plated onto full-strength potato dextrose agar (PDA) and incubated for 7 days at room temperature (25⁰C) on a laminar flow bench prior to DNA Extraction.

2.2.5.2 DNA extraction solutions

1M Tris–HCl (pH 8) was prepared by adding 60.5 g Tris to 400 ml H_2O. The pH was adjusted to 8 using a pH meter and the solution was autoclaved at 120°C for 20 min.

NaAc was prepared by adding 40.81 g of NaAc to 100 ml of H_2O. pH was adjusted to 5.5 with acetic acid.

DNA extraction buffer (DEB) was prepared and consisted of 40 ml of 1M TRIS HCl, 30 ml of NaCl (5.844 g in 100 ml H_2O and autoclaved) 10 ml 0.5 M EDTA (pH 8) (18.61 g of EDTA in 80 ml H_2O + 2 g of NaOH pellets; NaOH pellets were added until the solution was clear), and 10 ml SDS (10 g in 100 ml H_2O; the solution was heated to 68 ^{0}C and stirred until dissolved)

2.2.5.3 DNA extraction

DNA extraction was carried out using the modified CTAB (Cetyl trimethylammonium bromide) method (Möller *et al.*, 1992) for both the reference fungi as well as the powdered root samples kept at -80^{0}C. Mycelium from each of the 10 reference fungi was scraped from the culture plates into 2 ml Eppendorf tubes and 1 ml of DEB pre-warmed to 65°C was added. Mycelium was crushed for 1 min using a glass rod. The samples were frozen in liquid nitrogen and subsequently placed in boiling water for five min. A 600 µl chloroform:IAA mixture (25 phenol:24 chloroform:1 isoamylalcohol v/v) was added to the tubes. These were mixed five times by inversion. The samples were centrifuged at 14000x g for 15 min at 4°C to spin down cell debris. The aqueous upper layer was added to new 2 ml Eppendorf tubes with 200 µl of CTAB and 400 µl chloroform:IAA and these were mixed by inversion. Samples were centrifuged at 14000x g for 15 min at 4°C. The new upper layer was added to 2 ml Eppendorf tubes containing 60 µl of 3M NaAc and 800 µl of 100% ice-cold ethanol and these were mixed by inverting the tubes five times. Samples were centrifuged at 14000x g for 10 min at 4^{0}C. The supernatant was discarded and a pellet was observed at the bottom of the tube. The pellet was dissolved in 500 µl 70% ethanol to wash the DNA and centrifuged at 14000x g for five min at 4^{0}C. All liquid was removed and the tubes were drained and placed upside down to air-dry for one hour at room temperature in a laminar flow bench. The pellets were re-suspended in 50 µl Low TE buffer. Two µl RNase A was added to remove RNA contamination and tubes were incubated at room temperature overnight. DNA concentration was quantified using a Nanodrop 2000c spectrophotometer (Nanodrop, Wilmington, DE, USA, and Thermoscientific). All DNA samples were diluted to a concentration of 20 ng with molecular grade water. Extracted and diluted DNA was frozen at -80°C until qPCR analyses. DNA purity was evaluated at the absorbance ratio A260/280.

2.2.5.4 qPCR analyses

qPCR analyses was carried out using the threshed root samples of sorghum. The purpose of the assay was to use species specific primers to identify and quantify 10 fungal isolates (Table 2) in the root samples. Two methods of qPCR were performed; SYBR green and Hydrolysis assay (Taqman® probe). A volume of 23 µl reaction mix consisting of 10 µl iTaq™ Universal SBYR Green Supermix (Bio-Rad, Hercules, USA), 11.75 µl PCR H_2O and 0.625 µl of both forward and reverse primers (10 µM) was added to 2 µl of DNA sample to make a total volume of 25 µl. The thermal cycling was as follows: denaturation at 94^{0}C for 10 min, 40 cycles of 94^{0}C for 30 sec; 60^{0}C for 30 sec and 72^{0}C for

30 sec, 95ºC for 30 sec and 40ºC for 30 sec. A melt curve was between 60ºC and 95ºC with an increment of 1ºC for 10 sec each step.

Hydrolysis assay (Taqman® probe) consisted of a total volume of 20 µl with 1.6 µl of DNA sample and 18.4 µl of the reaction mix, which included: 10 µl iQ™ Supermix, 6.8 µl H_2O, 0.6 µl of both the forward and reverse primers (10 µM), 0.4 µl (10 µM) of the probe. The thermal cycling included denaturation at 95ºC for 10 min followed by 40 cycles of 95ºC for 15 sec and annealing at 60ºC for 1 min.

The SYBR Green method was applied to all the reference fungi excluding *F. equiseti* and *F. verticillioides,* which were quantified using the hydrolysis assay (Taqman® probe). Six standards were used as a fungal reference to construct a standard curve. The concentrations of the standards were 20 000 pg, 5000 pg, 1250 pg, 312.5 pg, 78.125 pg and 19.5 pg. A negative control was included where DNA samples were not added in comparison with the positive control where known concentrations of the 10 fungal samples were used. qPCR assays were carried out on a C1000™ Thermal cycler (Bio-Rad, Hercules, CA, USA) and a 96-well Hard-Shell® PCR Plates (Bio-Rad Laboratories, Hercules, CA, USA). The PCR reactions were performed in triplicate for each standard curve sample.

2.2.6 Ergosterol quantification

Ergosterol was extracted from roots of the three sorghum cultivars in the various rotation systems over the respective seasons to determine total fungal biomass. Roots were ground into fine powder using liquid nitrogen. Ergosterol was quantified using the modified method of Jambunathan *et al.* (1991). Five g powder of each sample was added to 25 ml methanol (extraction grade). A magnetic stirrer was used to mix the extracts in a 100 ml beaker for 30 minutes. After the mixture was allowed to settle, 12.5 ml of the clear upper extract was added to a 50 ml test tube with a screw cap, containing 1.5 g potassium hydroxide (KOH). The mixture was stirred in a vortex mixer (Vortex Genie 2, Scientific Industries) to dissolve the KOH. Five ml of n-hexane was added to the mixture and tubes were incubated for 30 min in a water bath at 75ºC. The mixture was allowed to cool to room temperature and test tubes were covered with foil to avoid ergosterol degradation. Ergosterol is light sensitive and degrades when exposed to UV light (Robine *et al.*, 2005). Distilled water (2.5 ml) was added, the suspension was mixed in a vortex mixer and the hyperthermal reaction was allowed to cool to room temperature. The upper hexane layer was transferred to a glass tube. Five ml 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 tube. This step was repeated. The hexane extract was evaporated until dry in the glass test tube in a water bath at 75ºC. The residue was re-suspended in 2.5 ml methanol (HPLC grade) and filtered through a 0.45 µl Pall Acrodisc syringe filter. The filtrate was analysed using a Perkin Elmer high performance liquid chromatograph (HPLC) with a SIL-20A

auto sampler. The extracts were loaded onto a reverse phase column (Phenomenex, C18 125 A 10 µm particle size, 150 mm x 4.6 mm) at 50°C. The mobile phase consisted of methanol:water (96:4) at a flow rate of 1.2 $ml.min^{-1}$. Standard ergosterol (Sigma) was used to develop a standard curve in the range of 7500 to 29 $\mu g.g^{-1}$. Standards were prepared by mixing 0.2 g Ergosterol stock solution with 4 ml methanol (HPLC grade). Standards were prepared through serial dilution. Ergosterol was determined from the peak area at 282 nm with a retention time of approximately 7 min.

2.2.7 Data analysis

Genstat 18th Edition was used to conduct analysis of variance (ANOVA) on all variables measured and mean separation was done using Fischer's protected test LSD (P=0.05). During 2016/17 data was analysed as a split plot experimental design with rotation sequence as main plot and cultivar as sub-plot factors. During 2017/18 data was analysed as a split-split plot experimental design, with the crop planted in year 1 (2015/16) as the main plot (referred to as 'year 1'), rotation sequence of two consecutive seasons of sorghum as opposed to one season of sorghum included in the rotation system as the sub-plot factor (referred to as 'number of seasons of sorghum') and cultivar as the sub-sub plot factor. Relationships between variables were analysed using regression analyses including simple, non-linear and multivariate regression methodologies in Excel and Genstat.

2.3 Results

2.3.1 Sorghum yield

ANOVA indicated that yield ($t.ha^{-1}$) was not significantly affected by the crop rotation systems applied during 2016/17 or 2017/18 (Table 3). Cultivar differences were recorded during both seasons, with NS5511 yielding the highest in both seasons (2.367 and 2.35 $t.ha^{-1}$ respectively). During 2016/17, PAN8816 yielded 2.22 $t.ha^{-1}$ which did not differ significantly from the yield of NS5511, while in the 2017/18 season, PAN8706W yielded 2.12 $t.ha^{-1}$ which did not differ significantly from the yield of NS5511. Although not significant, yield in the second sorghum crop following soybean tended to be lower than the similar sequence following bambara in cultivar NS5511. The inverse was recorded with PAN8706W while little variation between systems was recorded where a single crop of sorghum followed the rotation systems, irrespective of prior single or double legume system. There was little variation as well in PAN8816 under both systems (legume/legume/sorghum and sorghum/sorghum/legume) with the first sorghum crop following dry bean yielding higher than the rotation system where legumes were planted in two consecutive years.

2.3.2 Soil nutrient status

ANOVA indicated that neither cultivar nor the interaction between cultivar and the crop planted during the first season of the study (2015/16) had a significant effect on the macro- and micronutrients, as evaluated in the beginning of the 2016/17 season (Tables 4 and 5). Analysis of macro- and micronutrients at the beginning of the 2017/18 plant season, however, indicated that both P and K were significantly affected by the crop rotation system applied during 2017/18, irrespective of the cultivar (Tables 4 and 5). P occurred at significantly (P=0.05) higher levels where CP/SG/SG rotation was applied, while the monoculture sorghum rotational systems (SG/SG/SG) yielded the highest K concentrations followed by BA/SG/SG and CP/SG/SG. A notable decline in these two elements was however, observed in the subsequent season as opposed to the preceding seasons. The rotation system had no significant effects on Ca, Mg, Na, and NH_4 (although small increases in these nutrient levels were observed), soil pH and NO_3.

2.3.3 Visual root rot severity rating

Higher root rot severity was generally observed during 2017/18 compared to the preceding season (Table 6). Cultivars differed significantly with respect to root rot severity across seasons. NS5511 yielded a significantly greater root rot severity in both 2016/17 (root rot severity score = 177.5) and 2017/18 (root rot severity score = 358.5), while PAN8706W resulted in the lowest root rot severity in both seasons (2016/17 = 0.0; 2017/18 = 152.5) (Table 6). Root rot severity was, however, not significantly affected by the crop rotation system or the interaction between the crop rotation system applied and the cultivar planted in both the 2016/17 and 2017/18 seasons (Table 6). Although not significant there was a distinctly lower root rot severity with soybean compared with cowpea in the 2017/18 season (Table 6). PAN8706W is a white tan plant type where visual symptoms are not as evident as in the reds, which give an anthocyanin response. The zero values are an anomaly (Table 6) as indicated by ergosterol (Table 7) where colonization was equivalent to the other cultivars.

2.3.4 Ergosterol quantification

Ergosterol was not significantly affected by rotation systems during 2016/17 (Table 7). In contrast with the expectation, higher levels of ergosterol were detected in some legume rotations as opposed to monoculture sorghum. An increase in ergosterol level was detected in 2017/18 season in comparison to the 2016/17 season. All the rotational systems applied did not differ significantly from each other except PAN8816 under the dry bean rotation system which yielded higher ergosterol levels (1049.2 $\mu g.g^{-1}$) (results not included).

2.3.5 Fungal pathogen response

Tables 10 to 19 indicate fungal DNA concentrations of the ten fungi included in the current study, associated with the crop rotation systems. ANOVA indicated that significant differences in cultivars were only obtained for *F. chlamydosporum* and in rotation system x cultivar for *F. oxysporum* and *Phoma* spp. during 2016/17 (Table 8). qPCR analyses indicated that *F. chlamydosporum* was only significantly affected by cultivar (Table 12) with PAN8706W having significantly greater levels of thispathogen than the remaining two cultivars which did not differ significantly. Although not significant, low levels of *F. chlamydosporum* were detected under fallow in comparison to cowpea rotation systems (Table 12). However, contrary to this, an inverse in PAN8816 was observed. *Fusarium oxysporum* and *Phoma* spp. were significantly affected by an interaction between crop rotation system applied and cultivar planted during 2016/17. *Fusarium oxysporum* occurred at significantly higher levels in bambara/sorghum rotation system in PAN8816 (Table 15; 2016/17) compared to all other treatment combinations (Table 15). Higher levels of *F. oxysporum* were detected in both PAN8706W and NS5511 under bambara rotation systems even though not significant in 2017/18 (Table 15). *Phoma* spp. occurred at significantly higher levels in the roots of PAN8706W in the cowpea/sorghum crop rotation system followed by bambara (Table 18).

2.3.6 Relationships between macro- and micronutrients with root rot severity

Non-linear regression analyses indicated that the polynomial regression model fitted the data the best in the majority of cases and suitable fits could be obtained ($R^2 > 0.6$). The relationships suggest that certain ranges of nutrient concentrations occurred at which the root rot severities were lowest and that any concentration lower or higher than the range thresholds would result in increased root rot severity (Table 20). Few relationships were obtained suggesting that not many macro- and micronutrients could actually have an influence on root rot severity of sorghum. Figure 3 provides the regression curves for 2016/17 (A-C) and 2017/18 (D-E) respectively. The polynomial regression model fitted for K, Mn and root rot severity of NS5511 and PAN8816, in 2016/17 suggested that the lowest root rot severities for both cultivars were obtained at K concentrations of ±170 $mg.kg^{-1}$ and Mn concentrations of ±78 $mg.kg^{-1}$. At concentrations lower or higher than this, root rot severity increased. During the same season, a NO_3 level of ± 5.9 $mg.kg^{-1}$ resulted in the lowest root rot severity in PAN8816. During the following season (2017/18), K concentration of ± 100 $mg.kg^{-1}$ however yielded the lowest root rot severity in NS55511 and PAN8816 (Figure 3D) and Mn concentrations of ±30 $mg.kg^{-1}$ yielded the lowest root rot. PAN8706W yielded the lowest root rot severity at P concentration of ±15 $mg.kg^{-1}$ (Figure 3F). From the data presented here it is evident that the concentrations at which the lowest root rot was observed differed between seasons. It must also be noted that PAN8706W, which constantly yielded the lowest root rot of the three cultivars, was not affected in the same manner by changes in macro-and micronutrient conservations in the soil compared to the more root rot susceptible cultivars NS5511 and PAN8816.

Figures 4 to 13 indicate the relationships between macro- and micronutrient ranges and the target DNA levels in the 10 fungal pathogens in the soil from the rotations systems included in the study, with Table 20 showing the nutrient concentrations that allowed for low pathogen levels in the soil. Polynomial regression between Ca and *F. verticillioides* on NS5511 and PAN8706W in 2016/17 and 2017/18 differed in their DNA concentration ranges (±1425-1490 and ±1525-1580) (Figure 4; Table 20) suggesting that each cultivar has its own nutrient level requirements which differ across years and seasons. PAN8706W showed variation between Ca and *Curvularia eragrostidis* and *Exserohilum pedicellatum* from the same season (Figure 4; Table 20). Cu ($mg.kg^{-1}$) concentrations occurred in lower traces in the soil as evident in (Figures 5A and 5B) however, pathogen reactions differed in their responses as a reduction in *F. chlamydosporum* was observed with low Cu concentrations in PAN8816 and an increase of *E. pedicellatum* in PAN8706W. The nutrient concentration range did not differ amongst the two cultivars (PAN8816 and PAN8706W) in both seasons. Regression analysis indicated that the relationship between *F. equiseti, M. phaseolina* and Mg significantly decreased the population density of the two pathogens with target DNA concentrations ranging from 645-740 in PAN8706W (Figure 7A and 7B). K was observed as a beneficial nutrient in the 2017/18 season as it controlled a variety of pathogens in PAN8706W and *F. oxysporum* in PAN8816 (Table 20).

2.4 Discussion

The benefits of legume rotation systems on root rot of sorghum, as indicated in the current study were limited although tendencies in increased availability of nutrients and disease suppression were evident in the various legume rotation systems. These responses were not unexpected as root rot etiology of sorghum is extremely complex (Tarr, 1962) and it has proven difficult finding specific management strategies for the disease because of the diversity in the fungal community in the soil. Visual estimation criteria and plating techniques have been used in the past to identify and quantify the presence of soil borne pathogens, however these methods are thought to be unreliable as they are not sufficiently specific. Ergosterol does not distinguish between beneficial organisms and pathogens hence qPCR has been selected as the best method because of its quantitative ability and specificity (Nicolaisen *et al.*, 2009).

The prominent fungal pathogens detected during the 2016/17 season i.e. *F. chlamydosporum*, *F. oxysporum* and *Phoma* spp. were similar to those reported by Beyers 2017 unpublished (North-West University, Potchefstroom campus) with maize. Studies conducted in the USA, found *F. oxysporum* to be the most prominent root rot causal pathogen in the *Fusarium* genus (Reed *et al.*, 1983, Windels and Kommendahl, 1984). Hugo (1995) added that *F. oxysporum* was a major root rot incitant in South African maize. McLaren (1987) also reported *F. oxysporum* as a seedling pathogen of

sorghum. *Fusarium chlamydosporum* and *Phoma* spp. were classified as root colonisers based on their isolation frequency from maize root tissues (Hugo, 1995). Fungal pathogens are reportedly detected in the late stages of plant growth due to their ability to cause lodging and senescence (Dodd, 1980). Hugo (1995) however, detected root pathogens in the juvenile stages of plant growth and colonisers in the later growth stages as opposed to the findings of Dodd (1980). This current study has detected both root pathogens and colonisers in the soft dough stages of the sorghum growth. The results contradict both Dodd (1980) and Hugo (1995). This could be attributed to different host plants, locality and environmental interactions causing variation in disease severity (McLaren, 2002). Root colonisers are reported to be unable to inhabit actively growing tissues (Hugo, 1995) which was not the case in the current study as *F. chlamydosporum and Phoma* spp. were detected at the milk growth stages. This highlights the importance of knowing the survival mechanisms of certain pathogens, their ability to thrive and infect plants and at what different growth stages.

Phoma spp. was detected in higher levels in PAN8706W under the cowpea/sorghum rotation system as was *F. chlamydosporum*. These results are in contrast with those of root rot severity as PAN8706W was the least, visually affected cultivar. PAN8706W could thus potentially be a susceptible cultivar to root pathogens. Root rot symptoms are not as distinct on white tan plants because of the absence of an anthocyanin reaction, hence detection of pathogens in the absence of discoloured root tissues. Fallow systems resulted in lower *F. chlamydosporum* than cowpea rotation systems in PAN8706W which could be attributed to the ability of fallow retaining soil moisture and breaking crop pest and disease cycles while an inverse in PAN8816 could be due to the cultivar being less tolerant to soilborne pathogenic root fungi. Although not included in the current study, Bado *et al.* (2011) in Burkina Faso reported an increase in nematode activity on sorghum roots in cowpea/sorghum rotation systems as opposed to a groundnut system, which may have provided entry wounds for these pathogens although this did not have an effect on yield. This could mean that cowpea might be a host of unknown pathogens resulting in diseases that affect the PAN8706W cultivar. High occurrence of *Phoma and F. oxysporum* were detected in PAN8816 under bambara rotation systems. Bambara has been reported to be a pest and disease free crop unless attacked by weevil during storage (Gibbon and Pain, 1985, Doku, 1995, Tanimu and Aliyu, 1995). It has however been reported to be affected by *Fusarium* wilt caused by *F. oxysporum* (Brink *et al.*, 2006). This could indicate that bambara is susceptible to occurrence of *F. oxysporum* around and within the plant.

Rhizoctonia solani and *Fusarium* spp. are persistent root rot pathogens, which should not be neglected when introducing crop rotation as a control measure. The microbial population in the rhizosphere of the current study was low, except in 2016/17 with higher *F. chlamydosporum*, *F. oxysporum* and *Phoma* spp.. Crop rotations play a huge role in influencing soil microbial activity which contributes to plant health and this may have contributed to the low occurrence of pathogens.

Although, not directly related to this study, Zeng *et al.* (2016) reported that a double maize/soybean bacterial community was not affected by nitrogen fertilizer and this was attributed to the improvement of the rhizosphere microbial diversity due to crop rotation. Leguminous rotation system have reportedly increased beneficial organisms resulting in better yield and soil quality due to nutrient cycling (Liu *et al.*, 2007, 2009). Beneficial organisms such as *Bacillus cereus* and *B. subtilis'* strains have the ability exude antibiotics that can suppress *F. oxysporum* and *Pythium ultimum* in sorghum root rhizospheres (Idris *et al.,* 2007; Idris *et al.,* 2008).

Ergosterol analyses indicated significant increases in root colonization by fungi in legume rotations in 2017/18 season, however DB/SG was the only rotation system that was significantly different. Attribution can be given to the possibility of PAN8816 being a host for a variety of pathogens that occur and survive on dry bean roots.

Limited access to fertilizers has an effect on crop growth, yield and nutrient uptake (Bado *et al.*, 2011). However, there has been a shift from conventional farming to organic production systems in an attempt to reduce input costs and address environment issues. The use of crop rotation systems with legumes helps in limiting yield decline associated with reduced nutrient uptake and soil infertility (Bassegio *et al.*, 2015). Factors considered for disease suppression by macro- and micronutrients is their ability to interfere with the plant's physiology and directly influencing the causal pathogens (Dordas, 2008). Rhizosphere soil from the legume rotation systems in the current study showed the beneficial K obtained from the SG/SG, BA/SG and CP/SG and P provided by the CP/SG rotation systems. Phosphorus has an ability to influence plant growth in the early stages of a plant's life (Alvey *et al.*, 2001) and in turn reduces the effect of root rot (Dordas, 2008).

The current study indicated the effect of P Bray (1) on sorghum roots due to legumes on *Phoma* spp. and *F. oxysporum*. Costa *et al.* (2001) in a two-year study, found no effect on soil P in crop rotations in Parana under no-till. Santos *et al.* (2012) on the other hand, detected higher P concentrations in rotations with pearl millet and pigeon pea in Brazil. This was attributed to a lack of soil tillage, fertilizer applications, maintenance of crop residues resulting in higher accumulation of organic matter and nutrients in the top soil. These results agreed with that of the current study. Where P was found under cowpea/sorghum rotation systems in which less seed is produced and attribution is given to the translocation of nutrients to the roots to be mineralised, creating a pool of beneficial nutrients in the soil while crops with more seeds translocate their nutrients to the seed as opposed to the roots (Bloem and Barnard, 2010).

Literature emphasizes how a rotation system with legumes improves yield and soil fertility due to the nitrogen fixation ability as compared to monoculture (Bagayonko *et al.*, 1992, Stevenson and van Kennel, 1996, Kirkegaard *et al.*, 2008) however, the N-effect level was not confirmed in this study. Stevenson and van Kessel (1996) reported that yield increases in wheat with pea rotation systems

were mainly based on increased plant health which contributed 91% compared to only 9% from the N nutrition. Cook (1990) concluded that benefits from N nutrition arose from healthier root systems being able to utilize existing soil N more efficiently.

Yield varied with both seasons, relative to monoculture sorghum or sorghum subsequent to fallow plots with NS5511 yielding higher than PAN8816 and PAN8706W despite the high visual root rot severity detected. This suggested that NS5511 may be a more root rot tolerant sorghum cultivar compared to the other two cultivars. A study conducted in China showed an increase in yield when maize-soybean were rotated under no-till as compared to a double maize/soybean rotation and monoculture maize (Fan *et al.*, 2012). Bloem and Barnard (2010) reported sorghum grain yield increases of 7 and 13% after rotation with cowpea and soybean respectively as opposed to monoculture. Bado *et al.* (2011) reported similar results as sorghum grain yields increased from 0.9 $t.ha^{-1}$ in sorghum monoculture to 1.7 and 2.0 $t.ha^{-1}$ when rotated with groundnut and cowpea. These results correspond with those of Ncube *et al.* (2007). Horst and Hardter (1994) also reported higher yield and nutrient accumulation in maize/cowpea rotation systems compared to monoculture maize in Ghana. Cereal grain and stover yields of sorghum and millet increased by 18% and 25% with cowpea rotation as opposed to continuous cereal monoculture indicating the important role that legumes may play in ensuring yield stability (Kouyate *et al.*, 2000).

In the current study, higher yield was obtained in PAN8706W under SB/SG rotations in comparison to bambara while the inverse was recorded in PAN8816. The yield responses in the two cultivars could be attributed to the large diversity in legume-cereal rotation systems (McLaren, 2002), and the response of cultivars variations in certain nutrients in the soil (Kayode and Agboola, 1985). According to literature soybean is regarded as a good crop productivity and soil quality improver (Smith *et al.*, 2007).

Crop rotations in the current study had no significant effect on the soil nutrients content and pH except K and P. The pH values ranged between 5.64–5.76. However, this was not a limiting factor for plant growth as sorghum is more tolerant of alkaline salts than other grain crops and can therefore be successfully cultivated on soils with a pH (KCl) between 5.5 and 8.5 (du Plessis, 2008). High soil pH is beneficial for plant growth as it improves nutrient absorption and results in minimal need for lime application as a result of toxic content removal from the soil (Fageria and Zimmermann, 1998). Bagayoko *et al.* (2000) detected higher pH in cowpea rhizosphere soil in comparison to the soil from the rest of the field. Rotations with pearl millet and cowpea were also reported to have improved the uptake of Ca and Mg due to higher soil pH. However, Rosa *et al.* (2009) did not find any significant effect of crop rotation on soil pH under no-tillage where values ranged from 5.7–5.9. Costa *et al.* (2001) and Souza *et al.* (2013) reported the same results after two years of rotations in which their results correlated with those of the current study.

Regression relationships were observed between nutrients, sorghum root rot severity and pathogenic soil fungi and, although not significant, they still showed the effect rotation systems with legumes have in improving microbial diversity and soil quality. Pathogenic fungi declined with subsequent seasons. K, Mn, N NO_3 and P were able to reduce a few pathogens causing root rot with the different cultivars. Copper and Mn are important, as they lignify the cell wall. Brown *et al.* (1984) emphasised on the effect that Mn lignification has on wheat's take-all disease. As NO_3 increased in the soil, it reduced root rot severity of sorghum caused by *M. phaseolina* in the current study. This was in agreement with Fihlo and Dhingra (1980) who found reduced *M. phaseolina* levels with the increments of NO_3 which was attributed to its increase in microbial population which reduced the viability of *M. phaseolina* sclerotia, resulting in leakage of nutrients from the sclerotia. Increased K and Mg either increased susceptibility of roots to colonisation by general fungi or stimulated pathogen activity in the rhizosphere. K is important as it is involved in the cellular functions that are involved in reducing disease severity. When it is sufficient, it has the ability to stimulate development of thick cell walls which are able to resist pathogen penetration into the plant (Huber and Amy, 1985).

2.5 Conclusion

The findings of the current study indicated that yield and root rot were not significantly affected by legume-based rotations with sorghum. Certain root rot pathogens were, however, affected by the treatment*cultivar interaction. High ergosterol levels were detected under dry bean/sorghum rotation systems although the specific pathogens involved are not specified by this methodology. A broader study could be conducted targeting a wider range of soilborne pathogens instead of the more common species, while also targeting more than one locality over and extended period as the three–year rotation system used here. NS5511 yielded higher than PAN870W and PAN8816 and it was less susceptible to root rot even though it had higher visual root rot severity compared to the other two cultivars. Nutrient deficiency and toxicity vary amongst different plant varieties. It is vital to know the fertility of the soil and ensuring that there are sufficient nutrients needed for crop production and growth.

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Table 1: Representation of rotation systems with three sorghum cultivars and legumes, fallow and monoculture to reduce root and grain mold (one replicate)

Crop planted on plot during 2015/16	Split of treatments planted on plot during 2016/17	Split of treatments planted during 2017/18	Rotation system evaluated over a three year period
Plot 1: Bambara	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment 1: Bambara/Sorghum/Sorghum
	Bambara X6 rows	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment 2: Bambara/Bambara/Sorghum
Plot 2: Fallow	Pan8706W X2 rows Pan8816 x2 rows NS5511 X2 rows	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment3: Fallow/Sorghum/Sorghum
	Fallow X6 rows	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment4: Fallow/Fallow/Sorghum
Plot 3: Dry bean	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment 5: Dry bean/Sorghum/Sorghum
	Dry Bean x 6 rows	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment 6: Dry bean/Dry bean/Sorghum
Plot 4: Soybean	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment 7: Soybean/Sorghum/Sorghum
	Soybean x 6 rows	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment 8: Soybean/Soybean/Sorghum
Plot 5: Sorghum	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment 9: Sorghum/Sorghum/Sorghum
	Sorghum x6 rows (Pan8816)	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment 10: Sorghum/Sorghum/Sorghum
Plot 6: Cowpea	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment 11: Cowpea/Sorghum/Sorghum
	Cowpea X6 rows	Pan8706W X2 rows Pan8816 X2 rows NS5511 X2 rows	Treatment 12: Cowpea/Cowpea/Sorghum

Table 2: Sequences and names of species-specific primers used to quantify colonization of sorghum roots by specific root pathogens (Schoeman, 2016)

Species	Target sequence (5' – 3')	
	Forward primer	**Reverse primer**
C. eragrostidis	GCC CAA AGA CTC GCC TTA AA	GAT GGA TTG CTG GCC TCT TTA G
E. pedicellatum	AGC CGG CCT ACT GGT TTC	CCT ACC TGA TCC GAG GTC AA
F.chlamydosporum	CAC ATA TTC AAC GCC AAG ACA C	TGT ATC TTC TTC TCT TCA CCC TTC
F. equiseti	TTA CAC TCA TAA CCT TCT CAT GC	CAA TGA TGA GAA TAG CGC AAT CG
F. graminearum	CCC TCT TCC CAC AAA CCA TT	GCT TCC TAT TGA CAG GTG GTT A
F. oxysporum	CTC TCC TCG ACA ATG AGC AT	GGT CTG TGA AAC GAT GTC AGT A
F. verticillioides	CGC GTT TCT GCC CTC TC	TCG GAT GGT TAG TGA CTG CT
M. phaseolina	GCA ATC CTG TCG GAC TGT T	GCG ATG CCG ATA CCA AGA T
Phoma spp.	GCT CTG GTG TCT ACA ATG G	GTC AGT TCT AGT ACC TCG TTG AAG
R. solani	TGT TAT GCT TGG TTC CAC TCG	GGA CTA TTG GAA GCG GTT CAT C

Table 3: Analysis of variance of recorded sorghum yields ($t.ha^{-1}$) obtained over a three-year legume/sorghum rotation system with three sorghum cultivars.

Source	**2016/17**				
	d.f	s.s	m.s	v.r	F.pr
Rep	2	0.3617	0.1809	0.44	
Treatment	5	4.4038	0.8808	2.13	0.144
Residual	10	4.1325	0.4133	1.59	
Cultivar	2	6.1418	3.0709	11.85	<.001
Treatment x cultivar	10	2.9626	0.2963	1.14	0.375
Residual	23	5.9629	0.2593		
Total	52	23.9584			

Source	**2017/18**				
	d.f	**s.s**	**m.s.**	**v.r**	**F.pr**
Rep	2	0.7851	0.3926	0.74	
Year1[1]	5	2.4535	0.4907	0.92	**0.506**
Residual	10	5.3270	0.5327	1.43	
Split[2]	1	2.9238	2.9238	7.83	0.016
Year1 x Split	5	2.1083	0.4217	1.13	0.396
Residual	12	4.4785	0.3732	1.01	
Cultivar	2	10.2434	5.1217	13.93	<.001
Year1 x cultivar	10	1.9296	0.1930	0.52	0.864
Split x cultivar	2	0.6141	0.3070	0.83	0.440
Year1 x split x cultivar	10	2.1930	0.2193	0.60	0.809
Residual	48	17.6509	0.3677		
Total	107	50.7072			

- BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow

Rotation system[1]	**Consecutive years of sorghum**	**NS5511**				**PAN8706W**				**PAN8816**			
		2016/17		**2017/18**		**2016/17**		**2017/18**		**2016/17**		**2017/18**	
		Yield	**Mean**	**Yield**	**Mean**	**Yield**	**Mean**	**Yield**	**Mean**	**Yield**	**Mean**	**Yield**	**Mean**
BA-SG-SG	2	2.425		2.49		1.638		1.59		2.355		1.45	
CP-SG-SG	2	2.431		2.23		1.633		1.90		3.121		1.81	
DB-SG-SG	2	2.467	2.367a	2.26		1.324	1.59 1b	1.86		2.089	2.222a	1.67	
FW-SG-SG	2	3.054		2.12		1.795		2.24		2.422		1.27	
SB-SG-SG	2	2.013		1.57		1.745		2.03		1.842		1.50	
SG-SG-SG	(2)	1.815		2.32	2.35a	1.408		1.64	2.12a	1.630		1.59	1.61b
BA-BA-SG	1			2.59				2.27				1.91	
CP-CP-SG	1			2.80				2.50				2.19	
DB-DB-SG	1			2.14				2.06				0.80	
FW-FW-SG	1			2.62				2.71				2.10	
SB-SB-SG	1			2.42				2.12				1.68	
SG-SG-SG	(1)			2.61				2.57				1.39	
$LSD_{cultivar\ (P=0.05)}$			*0.351*		*0.287*								

Table 4: Analysis of variance of selected macro- and micronutrient concentrations in the soil observed in six legume/sorghum crop rotation systems and three sorghum cultivars during 2016/17 and 2017/18.

2016/17

Source	pH					NO_3					NH_4				
	d.f	s.s	m.s.	v.r	F.pr	d.	s.s	m.s.	v.r	F.pr	d.f	s.s	m.s.	v.r	F.pr
Rep	2	0.27603	0.13802	4.88		2	2.193	1.097	0.39		2	0.1186	0.0593	2.64	
Treatment	5	0.03085	0.00617	0.22		5	28.229	5.646	1.99	0.166	5	0.0723	0.0144	0.64	0.672
Residual	10	0.28277	0.02828			10	28.352	2.835			10	0.2247	0.0224		
Total	17	0.58965				17	58.774				17	0.4156			

Source	P					K					Ca				
	d.f	s.s	m.s.	v.r	F.pr	d.	s.s	m.s.	v.r	F.pr	d.f	s.s	m.s.	v.r	F.pr
Rep	2	103.44	51.72	4.36		2	5203.0	2601.5	6.59		2	61391	30695	20.09	
Treatment	5	46.28	9.26	0.78	0.586	5	731.8	146.4	0.37	0.857	5	40044	8009	0.52	0.753
Residual	10	118.56	11.86			10	3947.7	394.8			10	15275	15276		
Total	17	268.28				17	9882.5				17	80671			

Source	Mg					Na					Fe				
	d.f	s.s	m.s.	v.r	F.pr	d.	s.s	m.s.	v.r	F.pr	d.f	s.s	m.s.	v.r	F.pr
Rep	2	191702	95851	25.35		2	65.33	32.67	0.90		2	1.8629	0.9315	3.21	
Treatment	5	7540	1508.	0.40	0.839	5	345.17	69.03	1.91	0.180	5	1.2125	0.2425	0.84	0.553
Residual	10	37807	3781			10	362.00	36.20			10	2.9029	0.2903		
Total	17	237050				17	772.50				17	5.9784			

Source	Cu					Mn					Zn				
	d.f	s.s	m.s.	v.r	F.pr	d.	s.s	m.s.	v.r	F.pr	d.f	s.s	m.s.	v.r	F.pr
Rep	2	0.48124	0.24062	38.34		2	1206.0	603.0	1.76		2	0.5056	0.2528	1.51	
Treatment	5	0.04311	0.00862	1.37	0.312	5	1537.9	307.6	0.90	0.518	5	0.8221	0.1644	0.98	0.474
Residual	10	0.06275	0.00627			10	3426.4	342.6			10	1.6747	0.1675		
Total	17	0.58711				17	6170.2				17	3.0024			

Table 4 (cont):

2017/18

Source	pH					NO_3					NH_4				
	d.f	s.s	m.s.	v.r	F.pr	d.	s.s	m.s.	v.r	F.pr	d.f	s.s	m.s.	v.r	F.pr
Rep	2	0.21308	0.10654	4.17		2	0.4916	0.2458	1.20		2	0.1508	0.0754	1.22	
Treatment	5	0.01611	0.00322	0.13	0.983	5	0.6771	0.1354	0.66	0.660	5	0.1179	0.0235	0.38	0.850
Residual	10	0.25519	0.02552			10	2.0409	0.2041			10	0.6175	0.0617		
Total	17	0.48438				17	3.2096				17	0.8862			

Source	P					K					Ca				
	d.f	s.s	m.s.	v.r	F.pr	d.	s.s	m.s.	v.r	F.pr	d.f	s.s	m.s.	v.r	F.pr
Rep	2	21.778	10.889	6.71		2	3392.1	1696.1	15.07		2	57453	28726	51.73	
Treatment	5	65.778	13.156	8.11	0.003	5	2459.6	491.9	4.37	0.023	5	38383	7677	1.38	0.309
Residual	10	16.222	1.622			10	1125.2	112.5			10	55533	5553		
Total	17	103.778				17	6976.9				17	66845			

Source	Mg					Na					Fe				
	d.f	s.s	m.s.	v.r	F.pr	d.	s.s	m.s.	v.r	F.pr	d.f	s.s	m.s.	v.r	F.pr
Rep	2	172237	86118	79.20		2	1085.78	542.89	9.08		2	5.6311	2.8156	16.44	
Treatment	5	6319	1264	1.16	0.391	5	163.78	32.76	0.55	0.737	5	0.9860	0.1972	1.15	0.396
Residual	10	10874	1087			10	598.22	59.82			10	1.7129	0.1713		
Total	17	189430				17	1847.78				17	8.3300			

Source	Cu					Mn					Zn				
	d.f	s.s	m.s	v.r	F.pr	d.f	s.s	m.s	v.r	F.pr	d.f	s.s	m.s	v.r	F.pr
Rep	2	0.57760	0.28880	65.24		2	1936.33	968.17	32.89		2	0.4069	0.2035	1.45	
Treatment	5	0.02853	0.00570	1.29	0.342	5	183.07	36.61	1.24	0.358	5	0.6280	0.1256	0.89	0.520
Residual	10	0.04426	0.00442			10	294.36	29.44			10	1.4043	0.1404		
Total	17	0.65040				17	2413.76				17	2.4392			

Table 5: Impact of a three-year legume/sorghum-based crop rotation system on soil nutrient status

Nutrient	Rotation system[1]	2015/16[2]	2016/17[3]	2017/18[4]
N NO_3 ($mg.kg^{-1}$)	BA-SG-SG	6.18	4.12	2.13
	CP-SG-SG		3.48	2.03
	DB-SG-SG		3.11	1.66
	FW-SG-SG		5.69	1.95
	SB-SG-SG		6.61	1.69
	SG-SG-SG		3.85	1.65
P = 0.05			*Ns*	*Ns*
NH_4 ($mg.kg^{-1}$)	BA-SG-SG	0.80	1.73	1.35
	CP-SG-SG		1.67	1.37
	DB-SG-SG		1.80	1.43
	FW-SG-SG		1.62	1.52
	SB-SG-SG		1.72	1.57
	SG-SG-SG		1.78	1.52
P = 0.05			*Ns*	*Ns*
P ($mg.kg^{-1}$)	BA-SG-SG	15	23.33	14.67b
	CP-SG-SG		21.00	18.00a
	DB-SG-SG		20.00	12.00c
	FW-SG-SG		19.00	13.67bc
	SB-SG-SG		18.67	13.00bc
	SG-SG-SG		21.67	13.33bc
P = 0.05			*Ns*	*LSD = 2.317*
K ($mg.kg^{-1}$)	BA-SG-SG	138	177.7	119.3ab
	CP-SG-SG		156.3	118.3ab
	DB-SG-SG		163.3	97.7c
	FW-SG-SG		167.0	103.3bc
	SB-SG-SG		163.7	107.0bc
	SG-SG-SG		167.0	132.7a
P = 0.05			*Ns*	*LSD = 19.30*
Ca ($mg.kg^{-1}$)	BA-SG-SG	1240	1490	1610
	CP-SG-SG		1517	1527
	DB-SG-SG		1420	1473
	FW-SG-SG		1487	1500
	SB-SG-SG		1577	1487
	SG-SG-SG		1523	1553
P = 0.05			*Ns*	*Ns*
Mg ($mg.kg^{-1}$)	BA-SG-SG	580	703	701.0
	CP-SG-SG		683	688.3
	DB-SG-SG		669	643.7
	FW-SG-SG		686	670.0
	SB-SG-SG		734	674.3
	SG-SG-SG		699	692.7
P = 0.05			*Ns*	*Ns*
Na ($mg.kg^{-1}$)	BA-SG-SG	25	53.3	57.7
	CP-SG-SG		56.3	52.0
	DB-SG-SG		53.7	49.3
	FW-SG-SG		50.3	51.0
	SB-SG-SG		57.0	53.0
	SG-SG-SG		64.3	48.3
P = 0.05			*Ns*	*Ns*

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow
[2] – Soil samples taken during October 2015
[3] – Soil samples taken during October 2016
[4] – Soil samples taken during November 2017

Table 5 (cont.)

Nutrient	Rotation system[1]	2015/16[2]	2016/17[3]	2017/18[4]
pH	BA-SG-SG	6.28	5.73	5.69
	CP-SG-SG		5.64	5.67
	DB-SG-SG		5.76	5.74
	FW-SG-SG		5.66	5.67
	SB-SG-SG		5.66	5.69
	SG-SG-SG		5.69	5.74
P = 0.05			***Ns***	***Ns***
Fe ($mg.kg^{-1}$)	BA-SG-SG	Not available	6.40	6.75
	CP-SG-SG		6.20	6.89
	DB-SG-SG		6.91	6.33
	FW-SG-SG		6.89	6.41
	SB-SG-SG		6.57	6.97
	SG-SG-SG		6.43	6.63
P = 0.05			***Ns***	***Ns***
Cu ($mg.kg^{-1}$)	BA-SG-SG	Not available	2.24	2.20
	CP-SG-SG		2.24	2.21
	DB-SG-SG		2.32	2.16
	FW-SG-SG		2.37	2.23
	SB-SG-SG		2.27	2.28
	SG-SG-SG		2.25	2.17
P = 0.05			***Ns***	***Ns***
Mn ($mg.kg^{-1}$)	BA-SG-SG	Not available	68.6	40.9
	CP-SG-SG		57.6	40.9
	DB-SG-SG		86.3	35.3
	FW-SG-SG		79.5	34.4
	SB-SG-SG		71.1	41.7
	SG-SG-SG		66.1	42.5
P = 0.05			***Ns***	***Ns***
Zn ($mg.kg^{-1}$)	BA-SG-SG	Not available	4.72	4.64
	CP-SG-SG		4.29	4.71
	DB-SG-SG		4.81	4.16
	FW-SG-SG		4.44	4.31
	SB-SG-SG		4.23	4.51
	SG-SG-SG		4.59	4.44
P = 0.05			***Ns***	***Ns***

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow
[2] – Soil samples taken during October 2015
[3] – Soil samples taken during October 2016
[4] – Soil samples taken during November 2017

Table 6: Analysis of variance of sorghum root rot severity observed in a three-year legume/sorghum rotation system with three sorghum cultivars.

Source	2016/17				
	d.f	s.s	m.s.	v.r	F.pr
Rep	2	607.1	303.6	0.240	
Rotation system	5	9480.8	1896.2	1.480	0.280
Residual	10	12827.1	1282.7	1.760	
Cultivar	2	285892.	142946.3	196.54	<.001
Rotation system x	10	11015.0	1101.5	1.510	0.195
Residual	24	17455.1	727.3		
Total	53	337277.			

Source	2017/18				
	d.f	s.s	m.s.	v.r	F.pr
Rep	2	49288	24644	8.91	
Year1[1]	5	13816	2763	1.00	0.466
Residual	10	27665	2767	1.34	
Split[2]	1	2.00	2.00	0.00	0.975
Year1 x Split	5	6022	1204	0.58	0.713
Residual	12	24797	2066	1.08	
Cultivar	2	811793	405897	211.47	<.001
Year1 x Cultivar	10	11810	1181	0.62	0.793
Split x Cultivar	2	3426	1713	0.89	0.416
Year1 x Split x Cultivar	10	4654	465	0.24	0.990
Residual	48	92133	1919		
Total	107	1045406			

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow

[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

Rotation system[1]	Consecutive years of sorghum	NS5511					PAN8706W					PAN8816				
		2015/16	2016/17		2017/18		2015/16	2016/17		2017/18		2015/16	2016/17		2017/18	
		Root rot severity	Root rot severity	Mean	Root rot severity	Mean	Root rot severity	Root rot severity	Mean	Root rot severity	Mean	Root rot severity	Root rot severity	Mean	Root rot severity	Mean
BA-SG-SG	2	-	170.0		363.3		-	0.00		110.0		-	103.7		306.7	
CP-SG-SG	2	-	240.0		385.0		-	0.00		176.7		-	124.7		313.3	
DB-SG-SG	2	-	173.0	177.2a	346.7		-	0.00	0.0c	176.7		-	118.7	105.0b	306.7	
FW-SG-SG	2	-	180.0		333.3		-	0.00		126.7		-	79.7		273.3	
SB-SG-SG	2	-	143.0		366.7		-	0.00		136.7		-	100.0		290.0	
SG-SG-SG	(2)	-	156.0		383.3	358.5a		0.00		140.0	152.5c	190[2]	103.3		330.0	300.3b
BA-BA-SG	1	-	-		340.0		-	-		160.0		-	-		296.7	
CP-CP-SG	1	-	-		370.0		-	-		160.0		-	-		310.0	
DB-DB-SG	1	-	-		333.3		-	-		166.7		-	-		256.7	
FW-FW-SG	1	-	-		353.3		-	-		160.0		-	-		286.7	
SB-SB-SG	1	-	-		356.7		-	-		163.3		-	-		300.0	
SG-SG-SG	(1)	-	-		370.0		-	-		153.3			-		333.3	
$LSD_{cultivar\ (P=0.05)}$														*18.55*		*20.76*

Table 7: Analysis of variance of ergosterol concentration (µg.g^{-1}) in sorghum roots as observed over a three-year legume/sorghum rotation system with three sorghum cultivars.

Source	2016/17				
	d.f	s.s	m.s.	v.r	F.pr
Rep	2	1273	6363	5.930	
Rotation system	5	6920	1384	1.290	0.341
Residual	10	1072	1072	0.850	
Cultivar	2	1465	733	0.580	0.566
Rotation system x	10	2700	2699	2.150	0.061
Residual	24	3015	1256		
Total	53	8898			

Source	2017/18				
	d.f	s.s	m.s.	v.r	F.pr
Rep	2	1239163	619581	1.83	
Year1[1]	5	1783408	356682	1.05	0.439
Residual	10	3385596	338560	3.22	
Split[2]	1	7.00	7	0.00	0.994
Year1 x Split	5	568817	113763	1.08	0.418
Residual	12	1261274	105106	1.02	
Cultivar	2	570686	285343	2.78	0.072
Year1 x Cultivar	10	2298366	229837	2.24	0.031
Split x Cultivar	2	26789	13394	0.13	0.878
Year1 x Split x Cultivar	10	758853	75885	0.74	0.685
Residual	48	4929676	102702		
Total	107	16822633			

Rotation system[1]	Consecutive years of sorghum	NS5511				PAN8706W				PAN8816			
		2016/17		2017/18		2016/17		2017/18		2016/17		2017/18	
		Ergosterol	Mean	Ergosterol	Mean	Ergosterol	Mean	Ergosterol	Mean	Ergosterol	Mean	Ergosterol	Mean
BA-SG-SG	2	59.5		600		48.1		1716		77.1		696	
CP-SG-SG	2	32.3		492		64.9		1579		56.4		363	
DB-SG-SG	2	73.2	75.8	407		65.3	65.9	798		68.5	63.9	281	
FW-SG-SG	2	150.7		341		66.3		61		37.0		515	
SB-SG-SG	2	91.5		1595		61.7		1063		88.2		2508	
SG-SG-SG	(2)	47.5		1101	234	88.9		2703	193	56.1		1085	363
BA-BA-SG	1	-		382		-		411		-		298	
CP-CP-SG	1	-		1483		-		748		-		1022	
DB-DB-SG	1	-		1133		-		1593		-		1210	
FW-FW-SG	1	-		323		-		1267		-		2779	
SB-SB-SG	1	-		380		-		509		-		527	
SG-SG-SG	(1)	-		551		-		2614		-		2754	
LSD $_{year*cultivar\ (P=0.05)}$					*507.4*								

Table 8: Analysis of variance of selected soil borne root rot pathogens as observed in the roots of three sorghum cultivars in six legume/sorghum rotation systems during 2016/17.

Source	d.f	*Curvularia eragrostidis*				*Exserohilum pedicellatum*				*Fusarium chlamydosporum*			
		s.s	**m.s.**	**v.r**	**F.pr**	**s.s**	**m.s.**	**v.r**	**F.pr**	**s.s**	**m.s.**	**v.r**	**F.pr**
Rep	2	0.106	0.053	0.590		1979	989	0.690		6682	3341206	1.460	
Rotation system (R)	5	0.320	0.064	0.710	0.628	5892	1178	0.820	0.560	2170	4338964	1.890	0.183
Residual (A)	10	0.900	0.090	1.240		14300	1430	1.340		2293	2293296	0.890	
Cultivar ©	2	0.081	0.041	0.560	0.576	1651	826	0.770	0.472	24987338	1249366	4.840	0.017
R x C	10	0.760	0.076	1.050	0.435	10852	1085	1.020	0.457	31329156	3132916	1.210	0.332
Residual (B)	24	1.733	0.072			25580	1066			62008458	2583686		
Total	53	3.900				60255				169635146			

Source	d.f	*Fusarium equiseti*				*Fusarium graminearum*				*Fusarium oxysproum*			
		s.s	**m.s.**	**v.r**	**F.pr**	**s.s**	**m.s.**	**v.r**	**F.pr**	**s.s**	**m.s.**	**v.r**	**F.pr**
Rep	2	2243994	1121997	1.710		2.279	1.140	0.48		1382.5	691.2	0.87	
Rotation system (R)	5	3061315	612263	0.930	0.500	8.975	1.795	0.76	0.598	10032.7	2006.5	2.51	0.101
Residual (A)	10	6575035	657503	0.780		23.606	2.361	1.09		7979.2	797.9	2.31	
Cultivar ©	2	1365648	682824	0.810	0.458	2.254	1.127	0.52	0.600	1594.0	797.0	2.31	0.121
R x C	10	7071214	707121	0.840	0.600	24.628	2.463	1.14	0.374	9023.7	902.4	2.62	0.026
Residual (B)	24	20311524	846313			51.770	2.157			8278.5	344.9		
Total	53	40628730				113.512				38290.6			

Table 8 (cont.)

Source	**d.f**	***Fusarium verticillioides***				***Macrophomina phaseolina***				***Phoma* spp.**			
		s.s	**m.s.**	**v.r**	**F.pr**	**s.s**	**m.s.**	**v.r**	**F.pr**	**s.s**	**m.s.**	**v.r**	**F.pr**
Rep	2	15.85	7.92	0.40		0.06248	0.0312	0.86		165325	82663	0.96	
Rotation system (R)	5	53.51	10.70	0.54	0.742	0.10284	0.0205	0.57	0.725	63065	12613	0.15	0.977
Residual (A)	10	197.81	19.78	1.13		0.36323	0.0363	1.39		863486	86349	1.64	
Cultivar ©	2	1.19	0.59	0.03	0.967	0.03048	0.0152	0.58	0.566	878584	439292	8.32	0.002
R x C	10	207.52	20.75	1.18	0.348	0.27803	0.0278	1.06	0.425	1645397	164540	3.12	0.011
Residual (B)	24	420.64	17.53			0.62742	0.0261			1267055	52794		
Total	53	896.51				1.46448				4882913			

Source	**d.f**	***Rhizoctonia solani***			
		s.s	**m.s.**	**v.r**	**F.pr**
Rep	2	99800	49900	1.00	
Rotation system (R)	5	248640	49728	1.00	0.466
Residual (A)	10	497898	49790	1.00	
Cultivar ©	2	99736	49868	1.00	0.382
R x C	10	497973	49797	1.00	0.471
Residual (B)	24	1195374	49807		
Total	53	2639421			

Table 9: Analysis of variance of selected soil borne root rot pathogens observed in the roots of three sorghum cultivars in six legume/sorghum rotation systems during 2017/18.

Source	d.f	***Curvularia eragrostidis***				***Exeserohilum pedicellatum***				***Fusarium chlamydosporum***			
		s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr
Rep	2	44.495	22.248	7.51		2121.77	1060.88	7.58		30623.7	15311.9	28.98	
Year1[1]	5	21.832	4.366	1.47	0.281	703.77	140.75	1.01	0.462	3798.5	759.7	1.44	0.292
Residual	10	29.612	2.961	0.71		1399.42	139.94	7.58		5283.8	528.4	0.61	
Split[2]	1	2.800	2.800	0.67	0.429	26.18	26.18	1.42	0.257	1102.0	1102.0	1.27	0.282
Year1 x Split	5	24.358	4.872	1.17	0.381	86.23	17.25	0.93	0.493	6333.3	1266.7	1.46	0.274
Residual	12	50.175	4.181	1.38		221.52	18.46	0.61		10441.0	870.1	1.08	
Cultivar	2	3.050	1.525	0.50	0.607	40.68	20.34	0.67	0.518	2002.6	1001.3	1.25	0.297
Year1 x Cultivar	10	52.859	5.286	1.75	0.097	370.86	37.09	1.22	0.305	8281.0	828.1	1.03	0.433
Split x Cultivar	2	10.114	5.057	1.67	0.199	71.20	35.60	1.17	0.320	1812.3	906.1	1.13	0.332
Year1 x Split x Cultivar	10	16.356	1.636	0.54	0.852	243.09	24.31	0.80	0.631	6358.6	635.9	0.79	0.637
Residual	48	145.189	3.025			1462.74	30.47			38572.8	803.6		
Total	107	400.840				6747.45				114609.6			
Source	d.f	***Fusarium equiseti***				***Fusarium graminearum***				***Fusarium oxysporum***			
		s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr
Rep	2	927844	463922	0.90		0.00	0.00	0.00	0	151345	75672	22.57	
Year1[1]	5	2124057	424811	0.83	0.558	0.00	0.00	0.00	0.00	16763	3353	1.00	0.465
Residual	10	5135685	513568	1.03		0.00	0.00	0.00		33525	3353	0.51	
Split[2]	1	476215	476215	0.96	0.347	0.00	0.00	0.00	0.00	22844	22844	3.49	0.086
Year1 x Split	5	2502236	500447	1.00	0.456	0.00	0.00	0.00	0.00	16460	3292	0.50	0.769
Residual	12	5976219	498018	1.00		0.00	0.00	0.00		78606	6551	0.95	
Cultivar	2	861446	430723	0.86	0.428	0.00	0.00	0.00	0.00	25928	12964	1.88	0.164
Year1 x Cultivar	10	5122106	512211	1.03	0.437	0.00	0.00	0.00	0.00	78282	7828	1.13	0.358
Split x Cultivar	2	1271267	635634	1.27	0.289	0.00	0.00	0.00	0.00	10421	5211	0.75	0.476
Year1 x Split x Cultivar	10	4732913	473291	0.95	0.500	0.00	0.00	0.00	0.00	51120	5112	0.74	0.683
Residual	48	2396569	499285			0.00	0.00			331503	6906		
Total	107	5309568				0.00				816795			

Table 9 (cont.)

Source	d.f	*Fusarium verticillioides*				*Macrophomina phaseolina*				*Phoma* spp.			
		s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr
Rep	2	759.75	379.88	10.29		11.854	5.927	1.00		869687	434844	0.81	
Year1[1]	5	184.64	36.93	1.00	0.465	29.634	5.927	1.00	0.465	2794852	558970	1.04	0.446
Residual	10	369.28	36.93	2.54		59.268	5.927	17.20		5375258	537526	0.99	
Split[2]	1	13.11	13.11	0.90	0.361	0.345	0.345	1.00	0.337	565036	565036	1.04	0.327
Year1 x Split	5	74.00	14.80	1.02	0.448	1.723	0.345	1.00	0.458	2734633	546927	1.01	0.453
Residual	12	174.22	14.52	0.76		4.134	0.345	0.26		6499006	541584	1.00	
Cultivar	2	81.17	40.59	2.14	0.129	3.427	1.714	1.30	0.282	1173839	586919	1.09	0.345
Year1 x Cultivar	10	165.15	16.51	0.87	0.568	17.136	1.714	1.30	0.258	5607251	560725	1.04	0.425
Split x Cultivar	2	16.87	8.44	0.44	0.644	1.846	0.923	0.70	0.501	1097788	548894	1.02	0.369
Year1 x Split x Cultivar	10	192.95	19.30	1.02	0.445	9.231	0.923	0.70	0.719	5460386	546039	1.01	0.446
Residual	48	912.29	19.01			63.281	1.318			25868035	538917		
Total	107	2943.44				201.878				58045773			

Source	d.f	*Rhizoctonia solani*			
		s.s	m.s.	v.r	F.pr
Rep	2	94.793	47.397	5.78	
Year1[1]	5	40.662	8.132	0.99	0.469
Residual	10	81.974	8.197	1.02	
Split[2]	1	16.232	16.232	2.03	0.180
Year1 x Split	5	31.337	6.267	0.78	0.581
Residual	12	96.117	8.010	2.52	
Cultivar	2	13.524	6.762	2.12	0.131
Year1 x Cultivar	10	33.293	3.329	1.05	0.421
Split x Cultivar	2	7.735	3.868	1.22	0.306
Year1 x Split x Cultivar	10	21.777	2.178	0.68	0.733
Residual	48	152.746	3.182		
Total	107	590.192			

Table 10: *Curvularia eragrostidis* (pg.$\mu\ell^{-1}$) observed in sorghum roots over a three-year legume/sorghum rotation system with three sorghum cultivars.

Cultivar	Rotation system	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Concentration (pg.$\mu\ell^{-1}$)	Concentration (pg.$\mu\ell^{-1}$)	Cultivar Mean	Concentration (pg.$\mu\ell^{-1}$)	Mean
	BA-SG-SG	2	-	0.02		1.70	
	CP-SG-SG	2	-	0.01		0.00	
	DB-SG-SG	2	-	0.00	0.11	2.35	
	FW-SG-SG	2	-	0.01		2.50	
	SB-SG-SG	2	-	0.02		0.00	
NS5511	SG-SG-SG	(2)	-	0.62		0.00	1.05
	BA-BA-SG	1	-	-		0.00	
	CP-CP-SG	1	-	-		0.60	
	DB-DB-SG	1	-	-		1.01	
	FW-FW-SG	1	-	-		3.89	
	SB-SB-SG	1	-	-		0.59	
	SG-SG-SG	(1)	-	-		0.00	
	BA-SG-SG	2	-	0.05		3.46	
	CP-SG-SG	2	-	0.25		0.00	
	DB-SG-SG	2	-	0.04	0.06	0.00	
	FW-SG-SG	2	-	0.00		0.45	
	SB-SG-SG	2	-	0.00		0.00	
PAN8706W	SG-SG-SG	(2)		0.00		0.44	0.65
	BA-BA-SG	1	-	-		0.45	
	CP-CP-SG	1	-	-		2.13	
	DB-DB-SG	1	-	-		0.00	
	FW-FW-SG	1	-	-		0.35	
	SB-SB-SG	1	-	-		0.00	
	SG-SG-SG	(1)	-	-		0.54	
	BA-SG-SG	2	-	0.03		0.00	
	CP-SG-SG	2	-	0.03		0.08	
	DB-SG-SG	2	-	0.01	0.22	0.32	
	FW-SG-SG	2	-	0.00		0.30	
	SB-SG-SG	2	-	0.01		0.39	
PAN8816	SG-SG-SG	(2)	1894.67[2]	0.04		0.00	0.78
	BA-BA-SG	1	-	-		0.00	
	CP-CP-SG	1	-	-		1.78	
	DB-DB-SG	1	-	-		3.30	
	FW-FW-SG	1	-	-		1.61	
	SB-SB-SG	1	-	-		1.52	
	SG-SG-SG	(1)	1897.67	-		0.00	

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow
[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

Table 11: *Exserohilum pedicellatum* (pg.µℓ$^{-1}$) observed in sorghum roots over a three-year legume/sorghum rotation system with three sorghum cultivars.

Cultivar	Rotation system	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Concentration (pg.µℓ$^{-1}$)	Concentration (pg.µℓ$^{-1}$)	Cultivar Mean	Concentration (pg.µℓ$^{-1}$)	Mean
NS5511	BA-SG-SG	2	-	3.27	3.51	0.00	3.08
	CP-SG-SG	2	-	0.27		6.04	
	DB-SG-SG	2	-	6.32		0.50	
	FW-SG-SG	2	-	0.02		1.94	
	SB-SG-SG	2	-	5.85		0.22	
	SG-SG-SG	(2)	-	5.32		0.00	
	BA-BA-SG	1	-	-		0.01	
	CP-CP-SG	1	-	-		17.60	
	DB-DB-SG	1	-	-		5.58	
	FW-FW-SG	1	-	-		2.91	
	SB-SB-SG	1	-	-		2.21	
	SG-SG-SG	(1)	-	-		0.00	
PAN8706W	BA-SG-SG	2	-	82.42	15.14	0.86	2.42
	CP-SG-SG	2	-	0.01		1.46	
	DB-SG-SG	2	-	1.70		7.38	
	FW-SG-SG	2	-	0.00		0.03	
	SB-SG-SG	2	-	0.06		5.75	
	SG-SG-SG	(2)		6.67		0.16	
	BA-BA-SG	1	-	-		5.33	
	CP-CP-SG	1	-	-		3.63	
	DB-DB-SG	1	-	-		2.19	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		2.22	
	SG-SG-SG	(1)	-	-		0.00	
PAN8816	BA-SG-SG	2	-	4.40	3.32	0.00	3.92
	CP-SG-SG	2	-	0.89		11.34	
	DB-SG-SG	2	-	1.29		2.61	
	FW-SG-SG	2	-	0.31		0.03	
	SB-SG-SG	2	-	2.26		4.56	
	SG-SG-SG	(2)	1894.67[2]	10.79		4.78	
	BA-BA-SG	1	-	-		2.59	
	CP-CP-SG	1	-	-		8.83	
	DB-DB-SG	1	-	-		7.00	
	FW-FW-SG	1	-	-		0.92	
	SB-SB-SG	1	-	-		4.36	
	SG-SG-SG	(1)	1897.67	-		0.00	

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow
[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

Table 12: *Fusarium chlamydosporum* (pg.µℓ$^{-1}$) observed in sorghum roots over a three-year legume/sorghum rotation system with three sorghum cultivars.

Cultivar	Rotation system[1]	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Concentration (pg.µℓ$^{-1}$)	Concentration (pg.µℓ$^{-1}$)	Cultivar Mean	Concentration (pg.µℓ$^{-1}$)	Mean
NS5511	BA-SG-SG	2	-	1947.03	1834b	14.8	19.2
	CP-SG-SG	2	-	2309.05		8.8	
	DB-SG-SG	2	-	1497.20		20.8	
	FW-SG-SG	2	-	1318.51		17.0	
	SB-SG-SG	2	-	2099.40		0.0	
	SG-SG-SG	(2)	-	1835.21		0.0	
	BA-BA-SG	1	-	-		21.8	
	CP-CP-SG	1	-	-		91.4	
	DB-DB-SG	1	-	-		12.6	
	FW-FW-SG	1	-	-		17.4	
	SB-SB-SG	1	-	-		11.2	
	SG-SG-SG	(1)	-	-		14.2	
PAN8706W	BA-SG-SG	2	-	3200.79	3393a	14.7	11.8
	CP-SG-SG	2	-	6924.07		0.0	
	DB-SG-SG	2	-	2253.17		44.4	
	FW-SG-SG	2	-	2226.44		0.0	
	SB-SG-SG	2	-	3167.82		2.1	
	SG-SG-SG	(2)		2585.57		12.0	
	BA-BA-SG	1	-	-		20.9	
	CP-CP-SG	1	-	-		19.0	
	DB-DB-SG	1	-	-		17.1	
	FW-FW-SG	1	-	-		11.6	
	SB-SB-SG	1	-	-		0.0	
	SG-SG-SG	(1)	-	-		0.0	
PAN8816	BA-SG-SG	2	-	2228.30	2103b	0.0	8.9
	CP-SG-SG	2	-	2114.93		0.0	
	DB-SG-SG	2	-	1658.99		16.0	
	FW-SG-SG	2	-	2968.68		0.0	
	SB-SG-SG	2	-	1800.26		15.8	
	SG-SG-SG	(2)	1894.67[2]	1849.17		15.7	
	BA-BA-SG	1	-	-		20.8	
	CP-CP-SG	1	-	-		0.0	
	DB-DB-SG	1	-	-		11.6	
	FW-FW-SG	1	-	-		27.5	
	SB-SB-SG	1	-	-		0.0	
	SG-SG-SG	(1)	1897.67	-		0.0	
LSD $_{cultivar\ (P=0.05)}$			-		*18*		*Ns*

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow
[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

Table 13: *Fusarium equiseti* (pg.µℓ$^{-1}$) observed in sorghum roots over a three-year legume/sorghum rotation system with three sorghum cultivars.

Cultivar	Rotation system	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Concentration (pg.µℓ$^{-1}$)	Concentration (pg.µℓ$^{-1}$)	Cultivar Mean	Concentration (pg.µℓ$^{-1}$)	Mean
NS5511	BA-SG-SG	2	-	79.20	56.70	47	31
	CP-SG-SG	2	-	103.16		10.	
	DB-SG-SG	2	-	11.72		37	
	FW-SG-SG	2	-	0.00		3	
	SB-SG-SG	2	-	73.37		0	
	SG-SG-SG	(2)	-	72.72		2.	
	BA-BA-SG	1	-	-		0	
	CP-CP-SG	1	-	-		21	
	DB-DB-SG	1	-	-		44	
	FW-FW-SG	1	-	-		146	
	SB-SB-SG	1	-	-		0	
	SG-SG-SG	(1)	-	-		31	
PAN8706W	BA-SG-SG	2	-	759.29	400.38	59	220
	CP-SG-SG	2	-	41.35		60	
	DB-SG-SG	2	-	0.00		0	
	FW-SG-SG	2	-	453.24		47	
	SB-SG-SG	2	-	346.08		2436	
	SG-SG-SG	(2)		802.32		36	
	BA-BA-SG	1	-	-		0	
	CP-CP-SG	1	-	-		0	
	DB-DB-SG	1	-	-		0	
	FW-FW-SG	1	-	-		0	
	SB-SB-SG	1	-	-		0	
	SG-SG-SG	(1)	-	-		0	
PAN8816	BA-SG-SG	2	-	0.00	387.33	53	30
	CP-SG-SG	2	-	132.73		28	
	DB-SG-SG	2	-	126.44		0	
	FW-SG-SG	2	-	161.05		23	
	SB-SG-SG	2	-	1903.78		0	
	SG-SG-SG	(2)	1894.67	0.00		10	
	BA-BA-SG	1	-	-		159	
	CP-CP-SG	1	-	-		0	
	DB-DB-SG	1	-	-		88	
	FW-FW-SG	1	-	-		0	
	SB-SB-SG	1	-	-		0	
	SG-SG-SG	(1)	1897.67	-		0	

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow
[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

Table 14: *Fusarium graminearum* ($pg.\mu\ell^{-1}$) observed in sorghum roots over a three-year legume/sorghum rotation system with three sorghum cultivars.

Cultivar	Rotation system	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Concentration ($pg.\mu\ell^{-1}$)	Concentration ($pg.\mu\ell^{-1}$)	Cultivar Mean	Concentration ($pg.\mu\ell^{-1}$)	Mean
NS5511	BA-SG-SG	2	-	0.00	0.46	0.00	0.00
	CP-SG-SG	2	-	0.00		0.00	
	DB-SG-SG	2	-	0.00		0.00	
	FW-SG-SG	2	-	2.73		0.00	
	SB-SG-SG	2	-	0.00		0.00	
	SG-SG-SG	(2)	-	0.00		0.00	
	BA-BA-SG	1	-	-		0.00	
	CP-CP-SG	1	-	-		0.00	
	DB-DB-SG	1	-	-		0.00	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		0.00	
	SG-SG-SG	(1)	-	-		0.00	
PAN8706W	BA-SG-SG	2	-	0.00	0.00	0.00	0.00
	CP-SG-SG	2	-	0.00		0.00	
	DB-SG-SG	2	-	0.00		0.00	
	FW-SG-SG	2	-	0.00		0.00	
	SB-SG-SG	2	-	0.00		0.00	
	SG-SG-SG	(2)		0.00		0.00	
	BA-BA-SG	1	-	-		0.00	
	CP-CP-SG	1	-	-		0.00	
	DB-DB-SG	1	-	-		0.00	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		0.00	
	SG-SG-SG	(1)	-	-		0.00	
PAN8816	BA-SG-SG	2	-	0.00	0.41	0.00	0.00
	CP-SG-SG	2	-	0.00		0.00	
	DB-SG-SG	2	-	2.44		0.00	
	FW-SG-SG	2	-	0.00		0.00	
	SB-SG-SG	2	-	0.00		0.00	
	SG-SG-SG	(2)	1894.67	0.00		0.00	
	BA-BA-SG	1	-	-		0.00	
	CP-CP-SG	1	-	-		0.00	
	DB-DB-SG	1	-	-		0.00	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		0.00	
	SG-SG-SG	(1)	1897.67	-		0.00	

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow

[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

Table 15: *Fusarium oxysporum* (pg.µℓ$^{-1}$) observed in sorghum roots over a three-year legume/sorghum rotation system with three sorghum cultivars.

Cultivar	Rotation system[1]	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Concentration (pg.µℓ$^{-1}$)	Concentration (pg.µℓ$^{-1}$)	Cultivar Mean	Concentration (pg.µℓ$^{-1}$)	Mean
NS5511	BA-SG-SG	2	-	9.55b	5.00	0.00	43.1
	CP-SG-SG	2	-	3.07b		48.50	
	DB-SG-SG	2	-	2.30b		6.20	
	FW-SG-SG	2	-	0.63b		0.00	
	SB-SG-SG	2	-	14.44b		0.00	
	SG-SG-SG	(2)	-	0.00b		45.50	
	BA-BA-SG	1	-	-		0.00	
	CP-CP-SG	1	-	-		135.90	
	DB-DB-SG	1	-	-		70.90	
	FW-FW-SG	1	-	-		126.80	
	SB-SB-SG	1	-	-		3.20	
	SG-SG-SG	(1)	-	-		79.9	
PAN8706W	BA-SG-SG	2	-	24.23b	7.52	27.80	30.50
	CP-SG-SG	2	-	11.57b		0.00	
	DB-SG-SG	2	-	0.19b		39.20	
	FW-SG-SG	2	-	7.00b		0.00	
	SB-SG-SG	2	-	0.68b		26.60	
	SG-SG-SG	(2)		1.45b		0.00	
	BA-BA-SG	1	-	-		0.00	
	CP-CP-SG	1	-	-		76.60	
	DB-DB-SG	1	-	-		0.00	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		196.30	
	SG-SG-SG	(1)	-	-		0.00	
PAN8816	BA-SG-SG	2	-	86.29a	17.57	0.00	5.80
	CP-SG-SG	2	-	7.37b		6.80	
	DB-SG-SG	2	-	1.37b		14.10	
	FW-SG-SG	2	-	10.24b		0.00	
	SB-SG-SG	2	-	0.00b		0.00	
	SG-SG-SG	(2)	1894.67[2]	0.17b		0.00	
	BA-BA-SG	1	-	-		29.10	
	CP-CP-SG	1	-	-		11.60	
	DB-DB-SG	1	-	-		0.00	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		7.90	
	SG-SG-SG	(1)	1897.67	-		0.00	
$LSD_{treatment*cultivar\ (P=0.05)}$			-	*37.341*		*Ns*	

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow

[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

Table 16: *Fusarium verticillioides* (pg.µℓ$^{-1}$) observed in sorghum roots over a three-year legume/sorghum rotation system with three sorghum cultivars.

Cultivar	Rotation system[1]	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Concentration (pg.µℓ$^{-1}$)	Concentration (pg.µℓ$^{-1}$)	Cultivar Mean	Concentration (pg.µℓ$^{-1}$)	Mean
	BA-SG-SG	2	-	8.47		0.41	
	CP-SG-SG	2	-	2.98		3.60	
	DB-SG-SG	2	-	0.69	3.07	1.95	
	FW-SG-SG	2	-	3.54		1.03	
	SB-SG-SG	2	-	2.03		0.00	
NS5511	SG-SG-SG	(2)	-	0.69		6.13	3.08
	BA-BA-SG	1	-	-		0.04	
	CP-CP-SG	1	-	-		12.41	
	DB-DB-SG	1	-	-		6.65	
	FW-FW-SG	1	-	-		1.35	
	SB-SB-SG	1	-	-		1.31	
	SG-SG-SG	(1)	-	-		2.05	
	BA-SG-SG	2	-	1.97		1.68	
	CP-SG-SG	2	-	5.40		0.27	
	DB-SG-SG	2	-	0.00	3.06	5.69	
	FW-SG-SG	2	-	1.22		0.00	
	SB-SG-SG	2	-	6.47		1.48	
PAN8706W	SG-SG-SG	(2)		3.29		0.00	1.48
	BA-BA-SG	1	-	-		2.95	
	CP-CP-SG	1	-	-		5.30	
	DB-DB-SG	1	-	-		0.00	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		0.40	
	SG-SG-SG	(1)	-	-		0.00	
	BA-SG-SG	2	-	0.64		0.00	
	CP-SG-SG	2	-	0.98		3.85	
	DB-SG-SG	2	-	2.63	2.75	0.02	
	FW-SG-SG	2	-	3.18		0.00	
	SB-SG-SG	2	-	4.45		0.58	
PAN8816	SG-SG-SG	(2)	1894.67[2]	4.60		0.78	1.07
	BA-BA-SG	1	-	-		2.97	
	CP-CP-SG	1	-	-		1.43	
	DB-DB-SG	1	-	-		0.36	
	FW-FW-SG	1	-	-		0.27	
	SB-SB-SG	1	-	-		2.54	
	SG-SG-SG	(1)	1897.67	-		0.00	

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow

[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

Table 17: *Macrophomina phaseolina* ($pg.\mu\ell^{-1}$) observed in sorghum roots over a three-year legume/sorghum rotation system with three sorghum cultivars.

Cultivar	Rotation system[1]	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Concentration ($pg.\mu\ell^{-1}$)	Concentration ($pg.\mu\ell^{-1}$)	Cultivar Mean	Concentration ($pg.\mu\ell^{-1}$)	Mean
	BA-SG-SG	2	-	0.00		1.16	
	CP-SG-SG	2	-	0.00		0.00	
	DB-SG-SG	2	-	0.00	0.06	0.00	
	FW-SG-SG	2	-	0.00		0.00	
	SB-SG-SG	2	-	0.36		0.00	
NS5511	SG-SG-SG	(2)	-	0.00		0.00	0.10
	BA-BA-SG	1	-	-		0.00	
	CP-CP-SG	1	-	-		0.00	
	DB-DB-SG	1	-	-		0.00	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		0.00	
	SG-SG-SG	(1)	-	-		0.00	
	BA-SG-SG	2	-	0.14		4.07	
	CP-SG-SG	2	-	0.10		0.00	
	DB-SG-SG	2	-	0.03	0.07	0.00	
	FW-SG-SG	2	-	0.03		0.00	
	SB-SG-SG	2	-	0.00		0.00	
PAN8706W	SG-SG-SG	(2)		0.14		0.00	0.49
	BA-BA-SG	1	-	-		1.76	
	CP-CP-SG	1	-	-		0.00	
	DB-DB-SG	1	-	-		0.00	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		0.00	
	SG-SG-SG	(1)	-	-		0.00	
	BA-SG-SG	2	-	0.00		0.00	
	CP-SG-SG	2	-	0.00		0.00	
	DB-SG-SG	2	-	0.00	0.02	0.00	
	FW-SG-SG	2	-	0.00		0.00	
	SB-SG-SG	2	-	0.06		0.00	
PAN8816	SG-SG-SG	(2)	1894.67[2]	0.04		0.00	0.12
	BA-BA-SG	1	-	-		1.44	
	CP-CP-SG	1	-	-		0.00	
	DB-DB-SG	1	-	-		0.00	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		0.00	
	SG-SG-SG	(1)	1897.67	-		0.00	

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow

[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

Table 18: *Phoma* (pg.µℓ⁻¹) observed in sorghum roots over a three-year legume/sorghum rotation system with three sorghum cultivars.

Cultivar	Rotation system	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Concentration (pg.µℓ⁻¹)	Concentration (pg.µℓ⁻¹)	Cultivar Mean	Concentration (pg.µℓ⁻¹)	Mean
NS5511	BA-SG-SG	2	-	210.15c	277.13	37	235
	CP-SG-SG	2	-	168.08c		13	
	DB-SG-SG	2	-	288.49c		11	
	FW-SG-SG	2	-	215.69c		2596	
	SB-SG-SG	2	-	373.62bc		17	
	SG-SG-SG	(2)	-	406.74bc		25	
	BA-BA-SG	1	-	-		21	
	CP-CP-SG	1	-	-		9	
	DB-DB-SG	1	-	-		37	
	FW-FW-SG	1	-	-		33	
	SB-SB-SG	1	-	-		17	
	SG-SG-SG	(1)	-	-		4	
PAN8706W	BA-SG-SG	2	-	791.26ab	577.18	22	17
	CP-SG-SG	2	-	1065.87a		3	
	DB-SG-SG	2	-	424.66bc		45	
	FW-SG-SG	2	-	387.56bc		29	
	SB-SG-SG	2	-	447.71bc		13	
	SG-SG-SG	(2)		345.99c		15	
	BA-BA-SG	1	-	-		24	
	CP-CP-SG	1	-	-		10	
	DB-DB-SG	1	-	-		26	
	FW-FW-SG	1	-	-		0	
	SB-SB-SG	1	-	-		0	
	SG-SG-SG	(1)	-	-		13	
PAN8816	BA-SG-SG	2	-	152.21c	351.69	0	11
	CP-SG-SG	2	-	193.17c		5	
	DB-SG-SG	2	-	424.08bc		10	
	FW-SG-SG	2	-	522.41bc		0	
	SB-SG-SG	2	-	369.73c		9	
	SG-SG-SG	(2)	1894.67	448.56bc		28	
	BA-BA-SG	1	-	-		7	
	CP-CP-SG	1	-	-		1	
	DB-DB-SG	1	-	-		20	
	FW-FW-SG	1	-	-		24	
	SB-SB-SG	1	-	-		5	
	SG-SG-SG	(1)	1897.67	-		24	
LSD $_{treatment*cultivar\ (P=0.05)}$			-	*421.523*		*Ns*	

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow
[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

Table 19: *Rhizoctonia solani* ($pg.\mu\ell^{-1}$) observed in sorghum roots over a three-year legume/sorghum rotation system with three sorghum cultivars.

Cultivar	Rotation system[1]	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Concentration ($pg.\mu\ell^{-1}$)	Concentration ($pg.\mu\ell^{-1}$)	Cultivar Mean	Concentration ($pg.\mu\ell^{-1}$)	Mean
	BA-SG-SG	2	-	0.00		0.04	
	CP-SG-SG	2	-	0.00		1.73	
	DB-SG-SG	2	-	0.00	0.14	0.05	
	FW-SG-SG	2	-	0.00		0.00	
	SB-SG-SG	2	-	0.85		0.00	
NS5511	SG-SG-SG	(2)	-	0.00		0.61	43.1
	BA-BA-SG	1	-	-		0.00	
	CP-CP-SG	1	-	-		1.85	
	DB-DB-SG	1	-	-		2.20	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		0.58	
	SG-SG-SG	(1)	-	-		5.73	
	BA-SG-SG	2	-	0.40		0.06	
	CP-SG-SG	2	-	0.03		0.41	
	DB-SG-SG	2	-	0.00	0.07	0.20	
	FW-SG-SG	2	-	0.00		0.00	
	SB-SG-SG	2	-	0.00		0.41	
PAN8706W	SG-SG-SG	(2)		0.00		0.00	30.50
	BA-BA-SG	1	-	-		0.39	
	CP-CP-SG	1	-	-		0.31	
	DB-DB-SG	1	-	-		0.46	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		0.21	
	SG-SG-SG	(1)	-	-		0.02	
	BA-SG-SG	2	-	0.04		0.14	
	CP-SG-SG	2	-	0.28		0.86	
	DB-SG-SG	2	-	546.68	91.27	0.06	
	FW-SG-SG	2	-	0.00		0.00	
	SB-SG-SG	2	-	0.00		0.08	
PAN8816	SG-SG-SG	(2)	1894.67[2]	0.65		0.39	5.80
	BA-BA-SG	1	-	-		1.99	
	CP-CP-SG	1	-	-		0.70	
	DB-DB-SG	1	-	-		0.05	
	FW-FW-SG	1	-	-		0.00	
	SB-SB-SG	1	-	-		0.07	
	SG-SG-SG	(1)	1897.67	-		4.46	

[1] - BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow
[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

Table 20: Concentrations ($mg.kg^{-1}$) at which micro- and macronutrients resulted in the lowest level of soil borne pathogenic fungi in the roots of three sorghum cultivars grown in legume/sorghum rotation systems from 2015/16 to 2017/18.

Micro/macro nutrient	Cultivar	Pathogen	Nutrient concentrations ($mg.kg^{-1}$) at which pathogen occurred at lowest concentrations in the roots	Reference figure
Ca	PAN8816	*F. equiseti*	±1440-1525	4A
	NS5511	*F. verticillioides*	±1425-1490	4B
	NS5511	*M. phaseolina*	±1425-1511	4C
	PAN8706W	*C.eragrostidis*	±1490-1540	4D
	PAN8706W	*E. pedicellatum*	±1547-1600	4E
	NS5511	*F. oxysporum*	±1500 -1600	4F
	Pan8706W	*F. verticillioides*	±1525 -1580	4G
Cu	PAN8816	*F.chlamydosporum*	±2.25-2.3	5A
	PAN8706W	*E. pedicellatum*	±2.22-2.23	5B
K	PAN8706W	*E.pedicellatum*	±157-165	6A
	PAN8706W	*F.chlamydosporum*	±168-173	6B
	PAN8706W	*F. equiseti*	>155	6C
	PAN8706W	*F. oxysporum*	±163-168	6D
	PAN8816	*F. oxysporum*	±160-165	6D
Mg	PAN8816	*F. equiseti*	±680 -700	7A
	PAN8706W	*F. equiseti*	±700-740	7A
	NS5511	*F. oxysporum*	±670 - 680	7B
	PAN8706W	*M. phaseolina*	±700-740	7C
	NS5511	*M.phaseolina*	±675-700	7C
	PAN8706W	*C.eragrostidis*	±645-680	7D
	PAN8706W	*F.verticillioides*	±678- 690	7E
Mn	PAN8706W	*C.eragrostidis*	±75-80	8A
	PAN8706W	*F.chlamydosporum*	±78-80	8B
	PAN8706W	*F. equiseti*	±70-80	8C
	NS5511	*C.eragrostidis*	±35-40	8D
	NS5511	*F.chlamydosporum*	±35-40	8E
N NO_3	PAN8816	*F.equiseti*	±3.8-5.5	9A
	PAN8706W	*F.equiseti*	±5-7	9A
	PAN8706W	*M.phaseolina*	±3-7	9B
Na	NS5511	*C.eragrostidis*	±50-53	10A
Pbray(1)	PAN8706W	*E.pedicellatum*	±19-20	11A
	PAN8706W	*M.phaseolina*	±18	11B
pH	PAN8706W	*F.equiseti*	±5.7-5.76	12A
Zn	PAN8816	*F.equiseti*	±4.4-4.78	13A

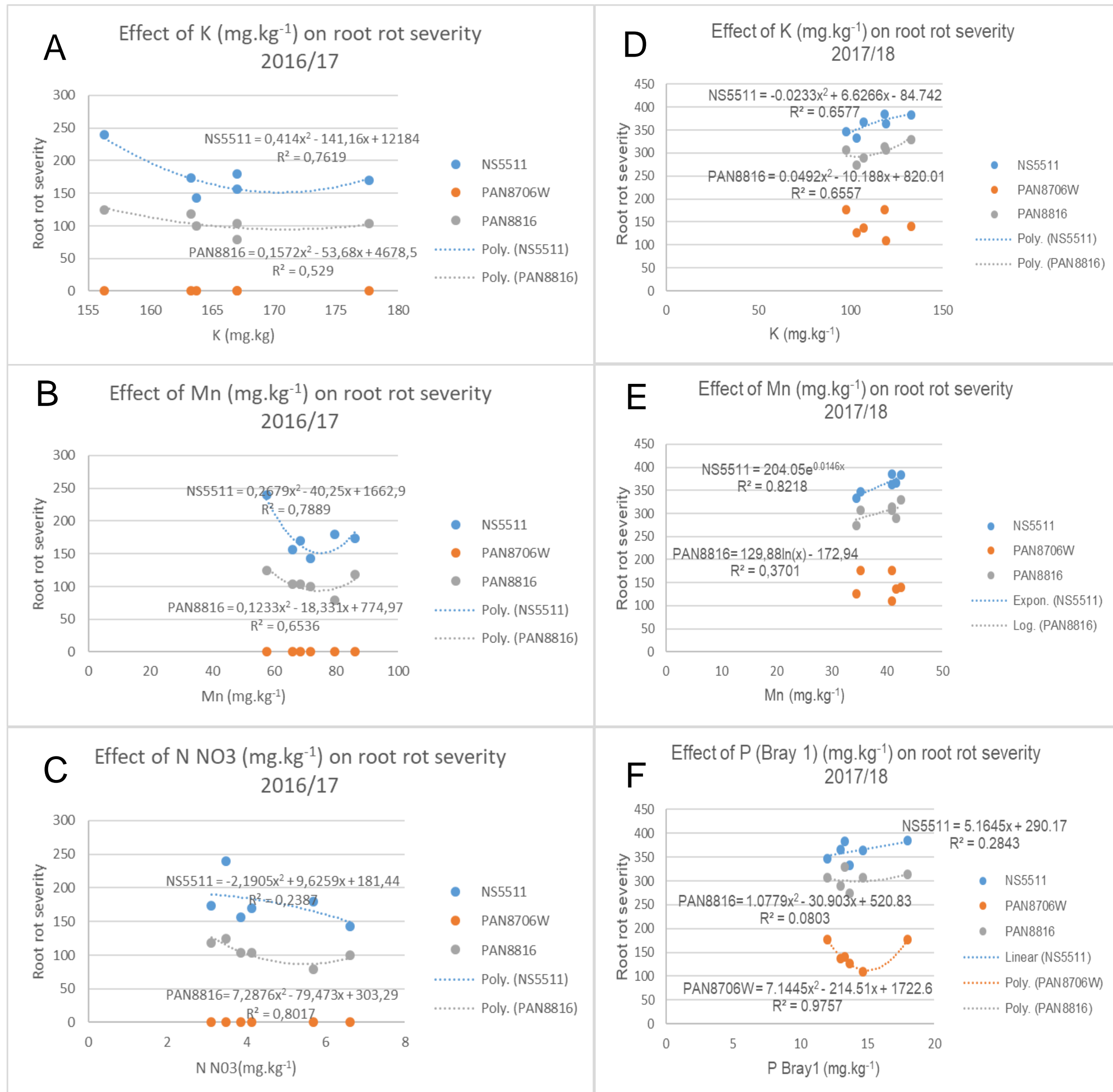

Figure 3: Significant relationships observed between macro- and micronutrients in the soil and sorghum root rot severity in three sorghum cultivars during 2016/17 (A-C) and 2017/18 (D-F).

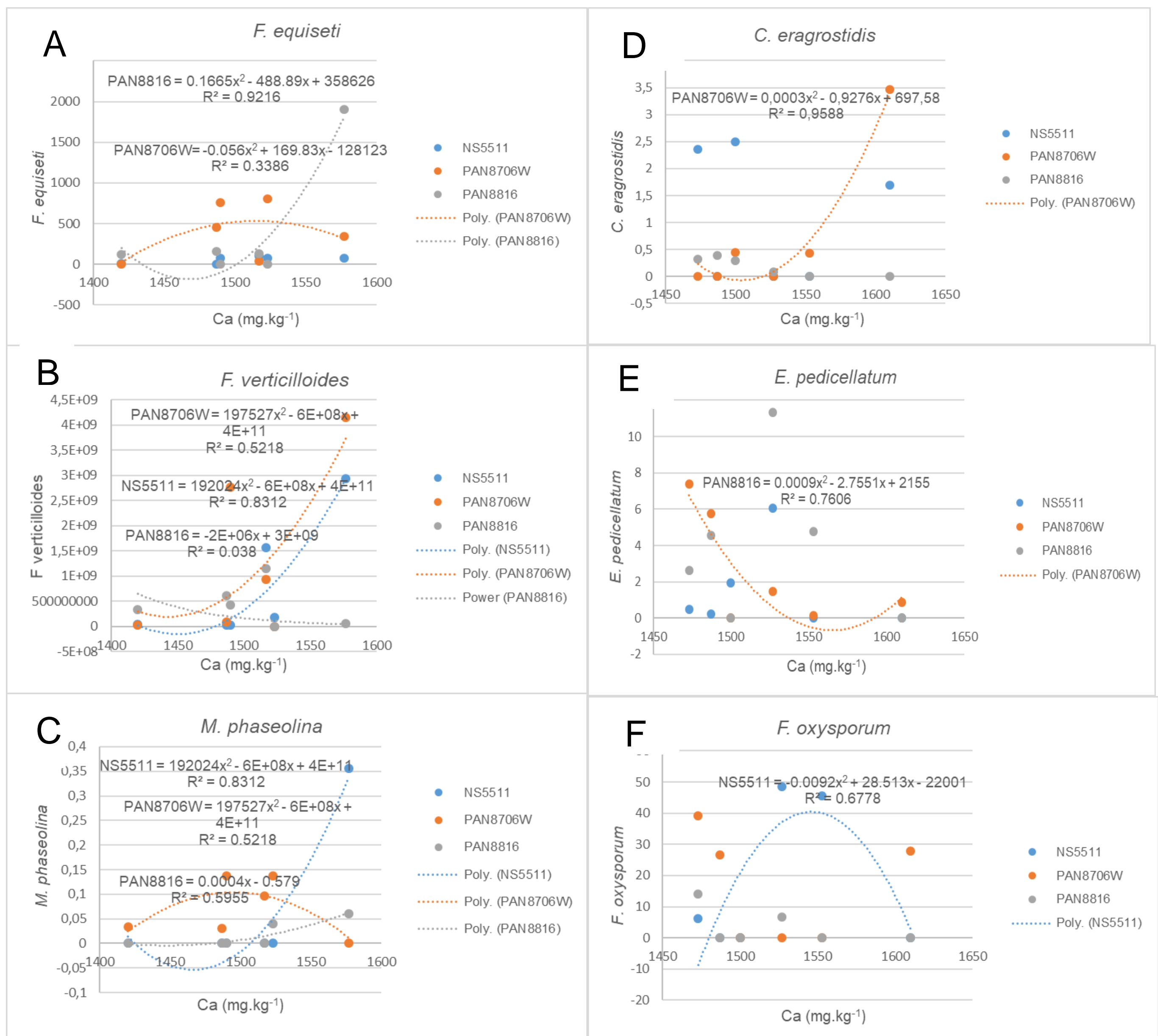

Figure 4: Relationships observed between Ca concentration (mg.kg-1) in the soil and pathogenic soil fungi concentrations (pg.uℓ-1) in the roots of three sorghum cultivars (R^2 > 0.6 = significant) during 2016/17 (A–C) and 2017/18 (D–G).

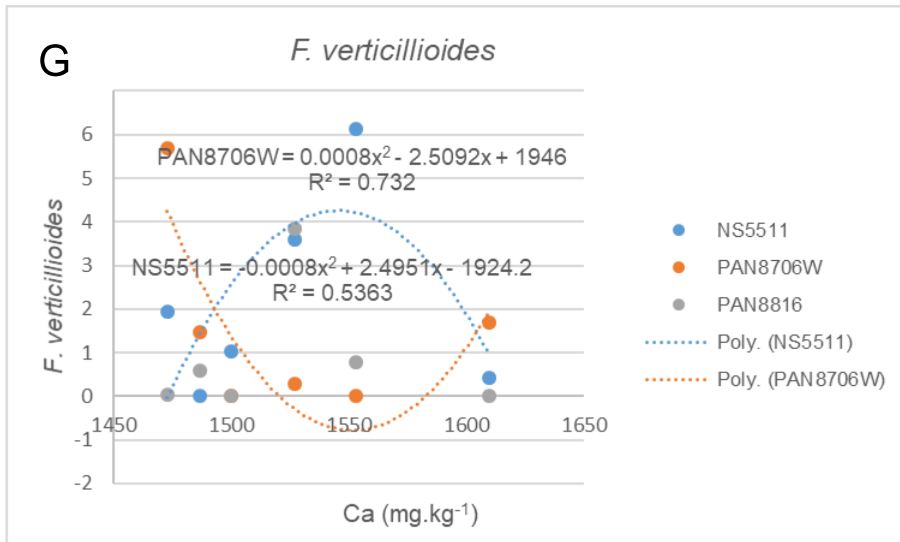

Figure 4 (cont): Relationships observed between Ca concentration (mg.kg^{-1}) in the soil and pathogenic soil fungi concentrations pg.uℓ$^{-1}$ in the roots of three sorghum cultivars ($R^2 > 0.6$ = significant) during 2016/17 (A–C) and 2017/18 (D–G).

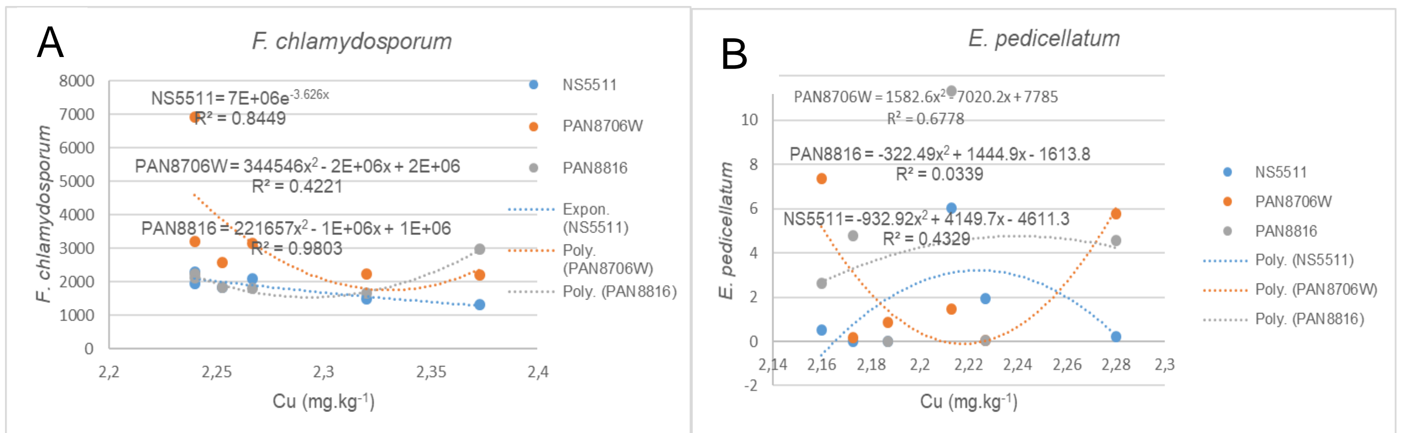

Figure 5: Relationships observed between Cu concentration (mg.kg^{-1}) in the soil and pathogenic soil fungi concentrations pg.uℓ$^{-1}$) in the roots of three sorghum cultivars ($R^2 > 0.6$ = significant) during 2016/17 (A) and 2017/18 (B).

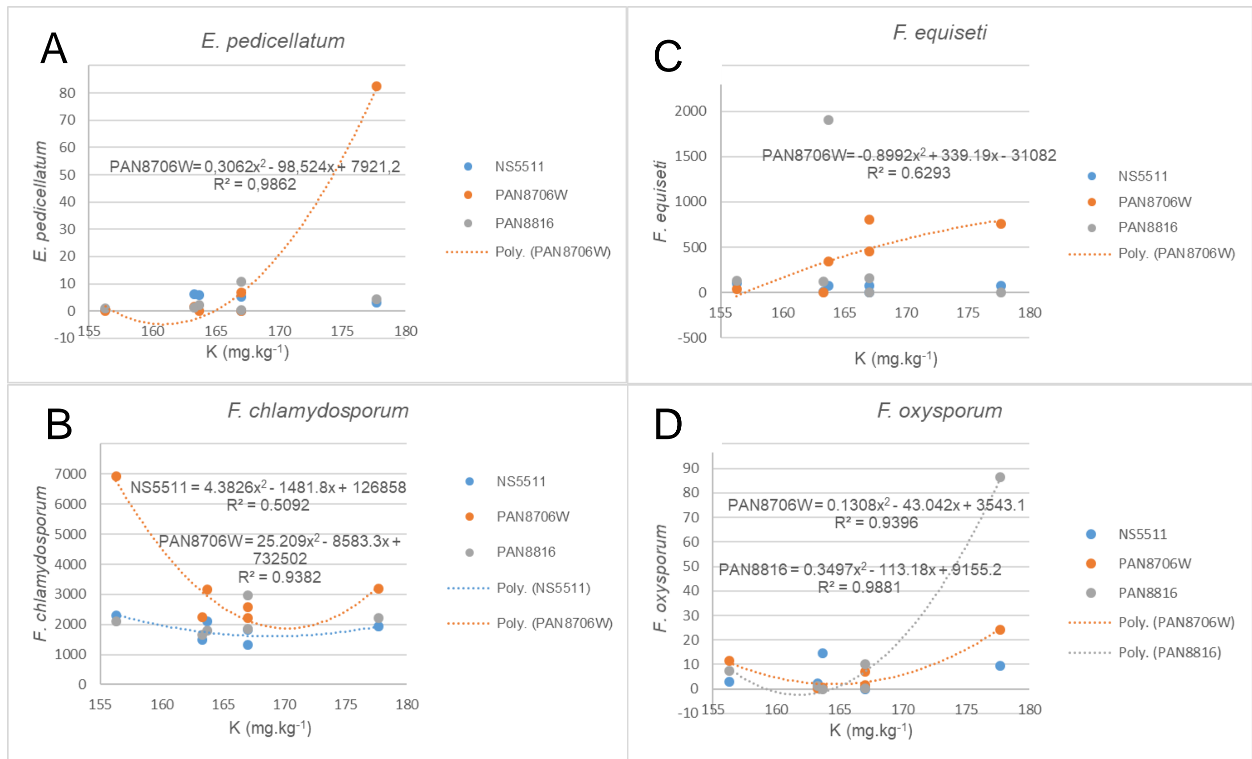

Figure 6: Relationships observed between K concentration (mg.kg^{-1}) in the soil and pathogenic soil fungi concentrations (pg.uℓ$^{-1}$) in the roots of three sorghum cultivars (R^2 > 0.6 = significant) in 2016/17.

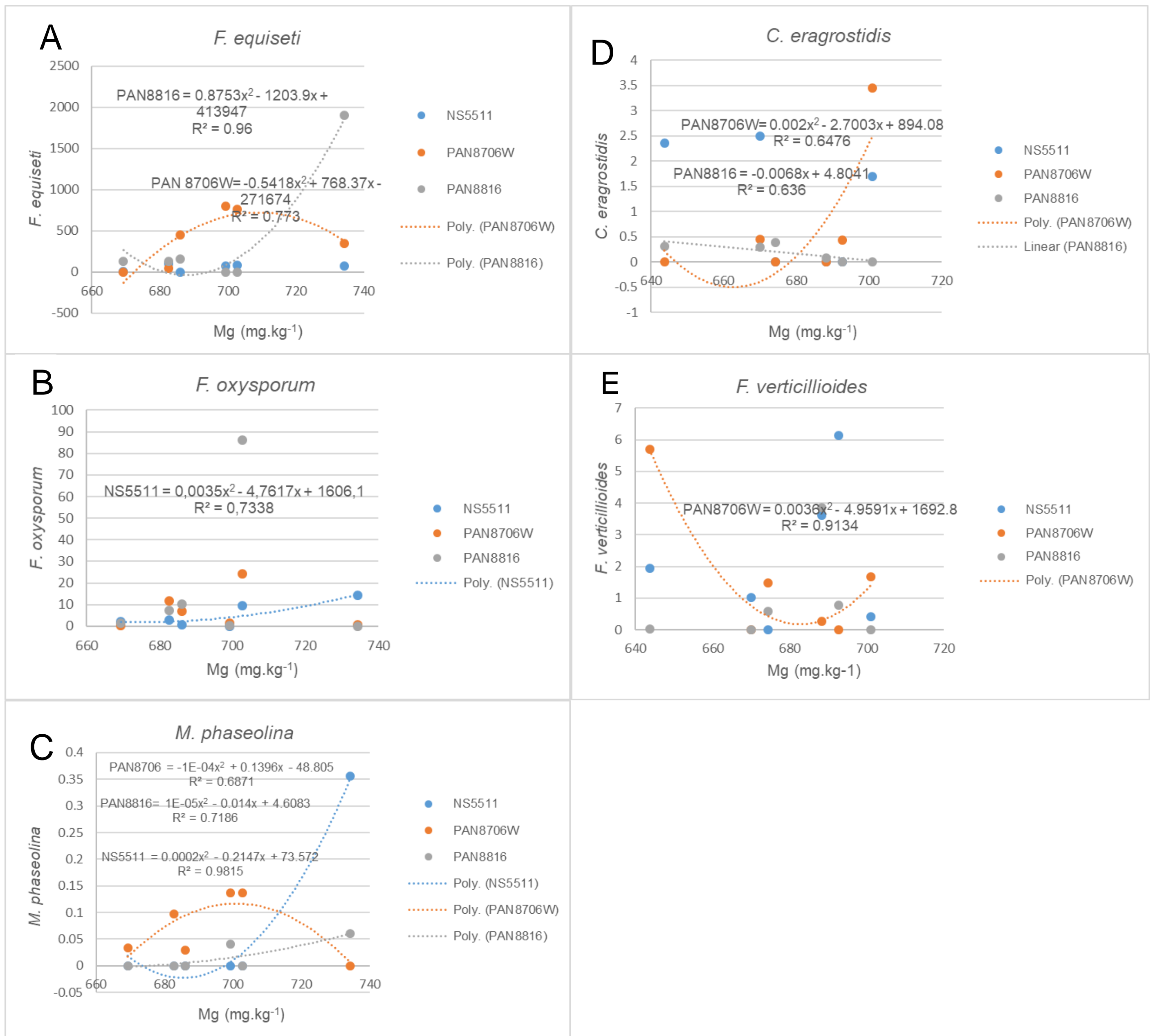

Figure 7: Relationships observed between Mg concentration (mg.kg^{-1}) in the soil and pathogenic soil fungi concentrations (pg.uℓ$^{-1}$) in the roots of three sorghum cultivars ($R^2 > 0.6$ = significant) during 2016/17 (A–C) and 2017/18 (D–E).

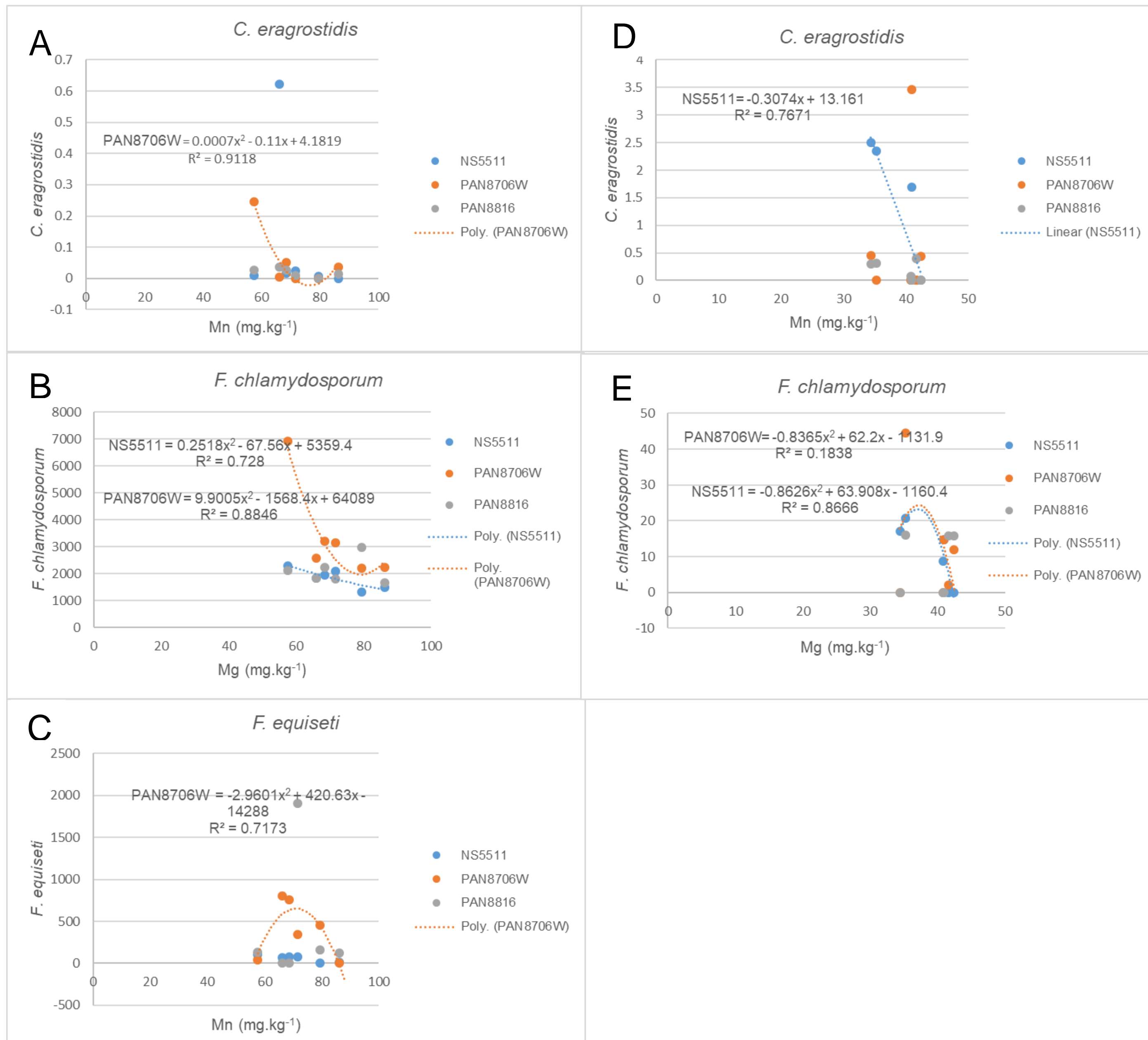

Figure 8: Relationships observed between Mn concentration (mg.kg^{-1}) in the soil and pathogenic soil fungi concentrations (pg.uℓ$^{-1}$) in the roots of three sorghum cultivars ($R^2 > 0.6$ = significant) in 2016/17 (A–C) and 2017/18 (D–E).

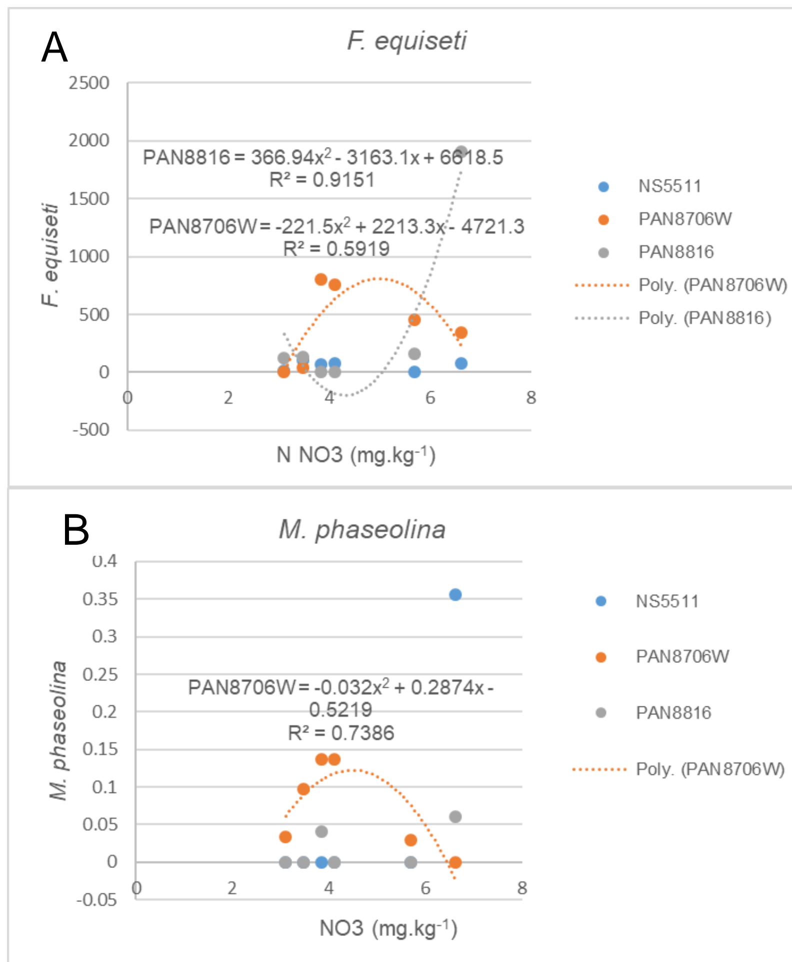

Figure 9: Relationships observed between NO_3 concentration ($mg.kg^{-1}$) in the soil and pathogenic soil fungi concentrations ($pg.u\ell^{-1}$) in the roots of three sorghum cultivars ($R^2 > 0.6$ = significant) in 2016/17 (A–B)

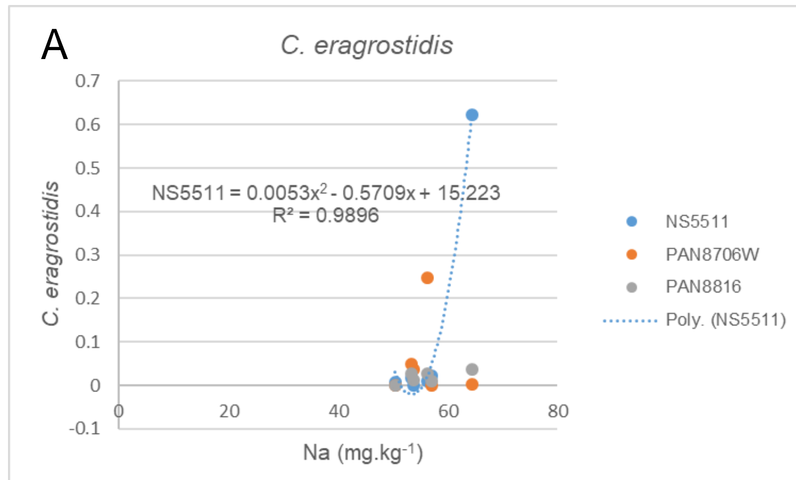

Figure 10: Relationships observed between Na concentration (mg.kg^{-1}) in the soil and pathogenic soil fungi concentrations (pg.uℓ$^{-1}$) in the roots of three sorghum cultivars ($R^2 > 0.6$ = significant) in 2016/17.

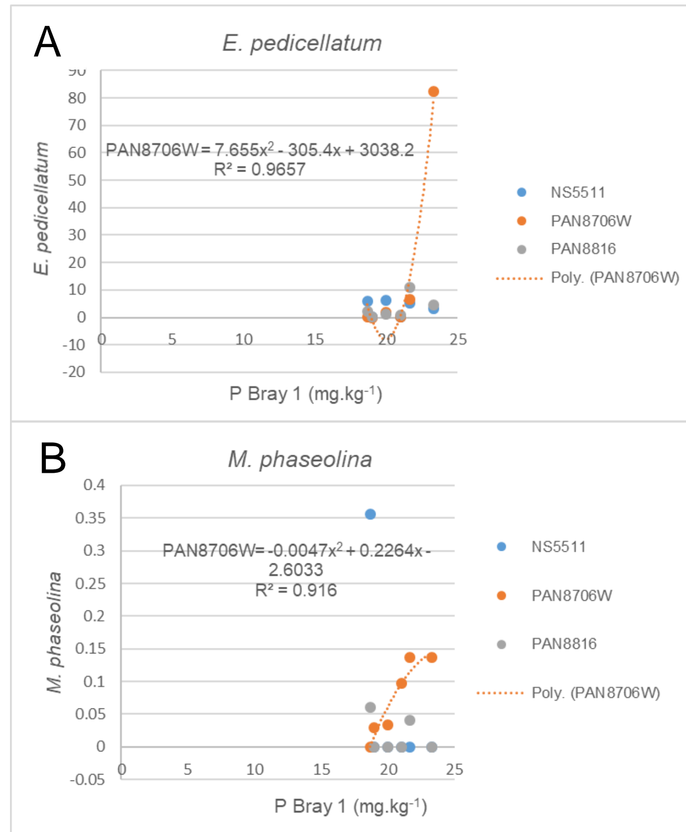

Figure 11: Relationships observed between P Bray (1) concentration (mg.kg^{-1}) in the soil and pathogenic soil fungi concentrations (pg.uℓ$^{-1}$) in the roots of three sorghum cultivars (R^2 > 0.6 = significant) in 2016/17.

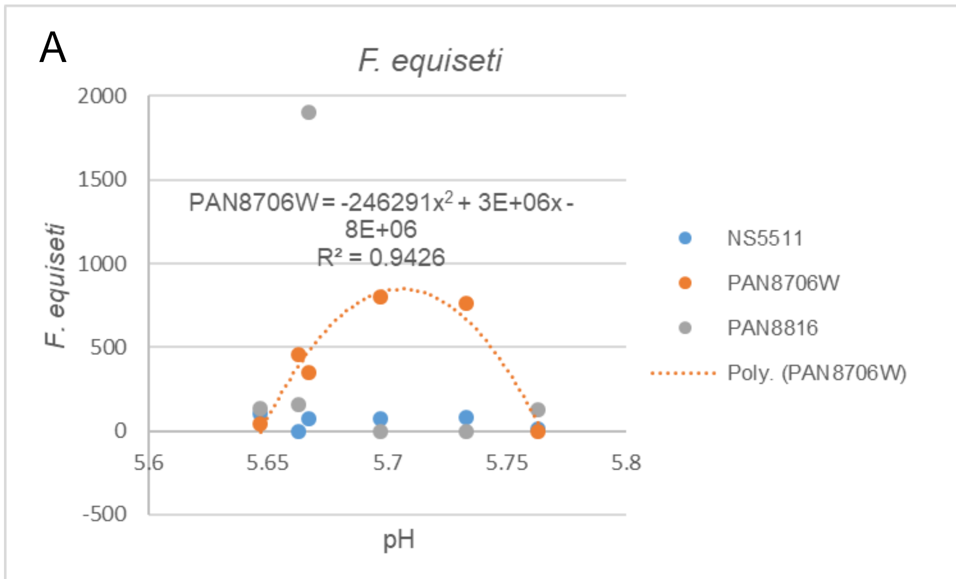

Figure 12: Relationships observed between pH concentration in the soil and pathogenic soil fungi concentrations (pg.uℓ$^{-1}$) in the roots of three sorghum cultivars ($R^2 > 0.6$ = significant) in 2016/17.

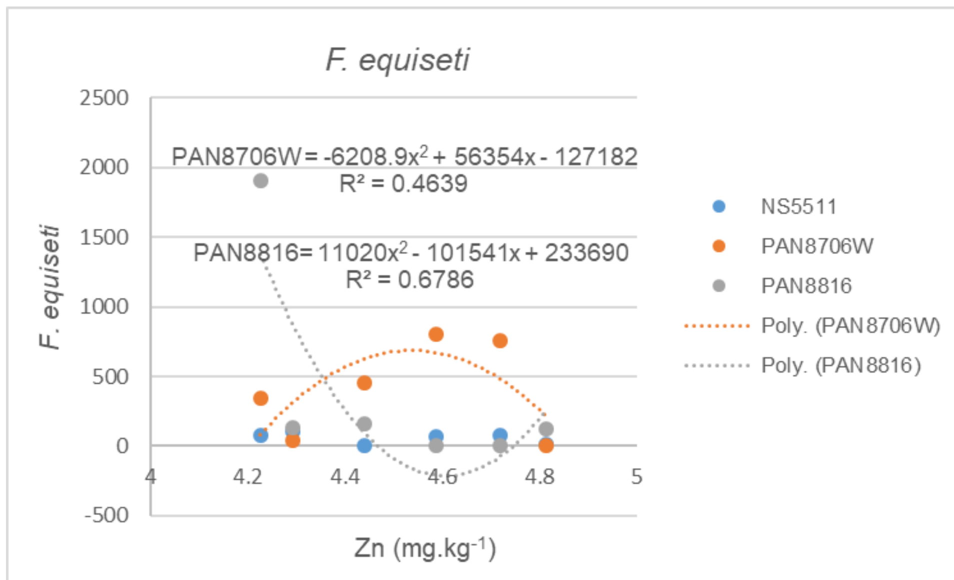

Figure 13: Relationships observed between Zn ($mg.kg^{-1}$) concentration in the soil and pathogenic soil fungi concentrations ($pg.u\ell^{-1}$) in the roots of three sorghum cultivars ($R^2 > 0.6$ = significant) in 2016/17.

Chapter 3

The impact of crop rotation on grain mold severity of selected sorghum cultivars

Abstract

Sorghum is a native crop, cultivated mostly in semi-arid regions. It serves as a staple food in Africa but its vulnerability to diseases such as grain mold results in yield losses and reduced market and nutritional value. Sorghum grain mold (SGM) also produces mycotoxins, which are harmful to humans and animals, sometimes leading to death. This study aimed at determining the colonization of *F. graminearum* Species Complex (*Fg*SC) on three sorghum cultivars and their response to grain mold pathogens under fallow, monoculture and legume rotation systems over a three season period (2015/16; 2016/17 and 2017/18). Ten plants were collected each season from a 10 m designated inner row per cultivar, per treatment for threshing. The threshed grains were visually rated for grain mold on a 1-5 scale. Total biofungal mass as a measure of colonization was quantified using ergosterol analysis while *Fg*SC was quantified using qPCR analyses. No significant treatment or treatment*cultivar interaction was observed for threshed grain mold rating (Tgmr) in the second season (2016/17). Significant cultivar differences were obtained with PAN8706W (mean rating score = 2.7) yielding higher visual Tgmr than NS5511 (2.2) and PAN8816 (2.3). Ergosterol quantification was highly affected by the crop planted during the first season and number of following seasons planted with sorghum (year 1*'number of seasons of sorghum' interaction) during the 2017/18 season. The sorghum monoculture treatment in both rotation systems yielded higher ergosterol levels (1973 $\mu g.g^{-1}$ and 1623 $\mu g.g^{-1}$), followed by soybean/sorghum/sorghum (SB/SG/SG, 1722 $\mu g.g^{-1}$). Lower ergosterol content was observed in the fallow/sorghum/sorghum (FW/SG/SG, 306 $\mu g.g^{-1}$) rotation system followed by bambara/bambara/sorghum (BA/BA/SG, 364 $\mu g.g^{-1}$) and soybean/soybean/sorghum (SB/SB/SG, 472 $\mu g.g^{-1}$). *Fg*SC DNA concentration was higher in PAN8706W (73.68 $pg.\mu l^{-1}$) than in PAN8816 and NS5511 (2.5 $pg.\mu l^{-1}$ and 6.75 $pg.\mu l^{-1}$ respectively) in 2016/17. No significant differences were detected for *Fg*SC in the 2017/18 season. Higher Tgmr were recorded in PAN8706W although no pathogens were quantified using ergosterol quantification. This confirms the general consensus internationally that Tgmr should not be the only assessment measure of sorghum grain mold as it lacks the ability to quantitatively detect internal infection and can provide a skewed indication of disease severity. A positive correlation between Tgmr and ergosterol levels were obtained

in PAN8706W. Crop rotation has the potential to reduce grain mold on sorghum cultivars and inoculum on the soil. Differential legume systems showed variation in colonisation by pathogens which are dependent on cultivar, pathogen survival mode and environmental conditions. The current study, however, indicated that consecutive seasons of legume cultivation had no additional benefit on the disease reduction.

3.1 Introduction

Sorghum is an essential, economical food and feed crop in African and Asian populations (Al-Jedabi, 2009), with well-managed crops generally yielding 1.7-4.8 t.ha^{-1} sorghum grain (Ncube *et al.*, 2007). An estimated yield loss of approximately 30% is, however, experienced annually due to pests and diseases (Chandrashekar and Satyanarayana, 2006). Disease incidence and severity are dependent on factors such as soil type, weather conditions, fertility, drainage, insect damage, cultural practices and host plant genetics (Logrieco *et al.*, 2002). Grain mold is considered one of the major constraints associated with production and profit loss in sorghum producing areas (Menkir *et al.*, 1996, Idris *et al.*, 2007, Al-Jedabi, 2009, Rao *et al.*, 2012, Mpofu and McLaren, 2014).

A complex number of fungi are involved in diseases affecting sorghum grain (Waliyar *et al.*, 2007). Sorghum grain mold (SGM) pathogens are also associated with mycotoxin production (Castor and Frederiksen, 1980, Thakur *et al.,* 2006, Waliyar *et al.*, 2007, Ambekar *et al.*, 2011). This include fungi such as *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Cladosporium* spp., *Colletotrichum* spp., *Curvularia* spp., *Phoma* spp. and *Fusarium* spp., including *Fusarium moniliforme* (*sensu lato*), *Fusarium thapsinum* and *Fusarium verticillioides* (Williams and Rao, 1981, Thakur *et al.*, 2006). SGM thrives well in areas with moderate temperatures and high humidity (75–100%) from anthesis to harvest (Forbes *et al.*, 1992, Melake-Berhna *et al.*, 1996, Bandyopadhyay *et al.*, 2000). The disease is associated with field fungi, which are regarded as true parasites on the living tissue. SGM can, however, be easily confused with grain weathering (GW) (Thakur *et al.*, 2006). Grain weathering is associated with many field fungi that parasitize the non-living tissue and is thought to be part of the general post-harvest grain deterioration process (Forbes *et al.*, 1992).

Fusarium graminearum (sensu lato) is a pathogen that attacks maize, sorghum and other cereals, resulting in complete destruction of the entire grain and its market value. It was split into two taxa,

F. graminearum Group 1 and Group 2, based on fertility, disease association (Leslie and Summerell, 2006) and phylogenetic differences (Aoki and O'Donnell, 1999). Sixteen phylogenetic species have been distinctively labelled under *F. graminearum* (*sensu lato)* forming what is currently known as *Fusarium graminearum* Species Complex (*Fg*SC). The effects of *Fg*SC are detrimental to host plants, animals and humans (Bennett and Klich, 2003). This pathogen complex reduces grain size, market and nutritional value, prevents the exportation of grain and causes severe grain discolouration, which have an impact on sorghum production and yield (William and McDonald, 1983, Thakur *et al.*, 2006). Depending on the pathogen, infection of sorghum results in pink, white, orange, grey or black discolouration on the grain surface (Williams and Rao, 1981, Bandyopadhyay *et al.*, 2000). Severely infected grains are covered fully by mold, while those partially infected may look normal and uninfected (Waliyar *et al.*, 2007). They produce mycotoxin that contaminate food and feed at high concentrations leading to health implications in humans and animals.

Grain mold pathogens that produce mycotoxins are a health hazard to humans and animals, limiting the use of grain sorghum as food and feed (Castor and Frederiksen, 1980, Thiel *et al.*, 1992, Chu and Li, 1994). Food and feed security in developing countries is of paramount importance and this is challenged by these constraints on cereal crops. Mycotoxins are major contributors to losses of food and feed in the Sub-Saharan Africa (Udomkun *et al.*, 2016). It is essential to avoid using contaminated grain during malt processing, as pathogens will proliferate in the beer, resulting in toxic metabolic products that pose a threat to consumers (Ilori *et al.*, 1991). Grain grown under subsistence farming systems is sent directly to local markets, raising a high health risk to consumers due to the lack of strict regulations that address quality issues (Shephard, 2008, WHO/FAO, 2012).

According to Tarr (1962) inoculum is ever present in the soil, which may support grain infection depending on climatic conditions during flowering and grain fill stages of the crop. Management of SGM is important in enhancing food quality and grain health, with genetic resistance being the primary strategy for grain mold management to date (Rosenow and Frederiksen, 1982, Cuevas *et al.*, 2016). It has however been a struggle to find durable resistance in sorghum cultivars worldwide, due to the complex number of fungi involved, the control mechanism in plants and differential adaptation to geographic locations and climate conditions (Waniska *et al.*, 2001, Prom *et al.*, 2012). Effective control of grain mold has thus far been found in the central and western parts of Africa, Sudan and Uganda due to the discovery of promising cultivars found to be resistant

to multiple fungal infections. Such resistance has resulted in low grain mold severities, high germination rate and photoperiod insensitivity (Prom *et al*., 2011). Organic farming has increasingly showed its impact in the reduction of grain mold compared to conventional and integrated farming systems (Lampkin, 1990). Its nutritional benefit stems from rotating cereals with N_2 fixing legumes, crop residues and animal manure (Karlen *et al*., 1994).

Crop rotation systems provide a preferred, alternative approach to monoculture practices for sustainable crop production in agriculture. According to Bullock (1992), positive effects are achieved when different crops are grown in succession, alternated with periods of fallow. Cost reduction as well as the reduced reliance on external inputs *viz.* synthetic fertilizers, herbicides and pesticides (Tarekegn *et al*., 2004, Zegada-Lizarazu and Monti, 2011) are considered to be some of the many benefits associated with crop rotation systems. Primary benefits associated with crop rotation, however, include better weed, pest and disease control (Emmond and Ledingham, 1972, Nel and Lamprecht, 2011) while assisting in the prevention of soil fertility exhaustion needed for plant growth (Brankatschk and Finkbeiner, 2015, Orio *et al*., 2016). Other beneficial rotational effects include maintenance of good root health, lower inoculum levels of soil borne pathogens (Cook, 1993) and yield increases that farmers can capitalise from (Nel and Lamprecht, 2011). Such positive yield effects have been attributed to the recycling of crop residue as an organic matter in the soil, promoting plant growth (Masri and Ryan, 2006, Brankatschk and Finkbeiner, 2015).

Little to no information is, however, internationally available on whether crop rotation systems might aid in reducing the incidence and severity of SGM. An ecofallow system (a method of farming that diminishes weeds and conserves water by rotating crops and reducing or eliminating tillage) initiated by Smika and Wicks (1968) showed a decline in foliar diseases and stalk rot of sorghum planted on wheat residues and *vice versa* as opposed to conventional tillage. A reduction in sorghum stalk rot incidence, from 39% to 11% severity, was observed. Crop rotation assists in reducing disease build-up. Lower stalk rot severities of sorghum were recorded under minimum tillage as opposed to conventional tillage, resulting in significantly higher grain yields (Flett, 1996). Marley and Ajayi (1999) reported that SGM was found to be lower at milk stage, 10 days after fungicide application. However, the application of these fungicides can induce stress on crops leading to the production of mycotoxins.

This study aimed at (i) evaluating three selected sorghum cultivars for their response to grain mold pathogens under various legume based crop rotation systems relative to fallow and monoculture systems and (ii) quantifying the occurrence of *F. graminearum* Species Complex in selected cultivars and rotation systems.

3.2 Materials and methods

3.2.1 Field trial

Field trials were planted during 2015/16, 2016/17 and 2017/18 at Potchefstroom (ARC-Grain Crops, South Africa) as described in Chapter 2, section 2.2.1. The trial consisted of cropping systems with sorghum in monoculture and in rotation combinations with dry bean, soybean, cowpea, bambara or fallow. Three sorghum cultivars were included i.e. PAN8816, (red) NS5511 (brown, tan) and PAN8706W (white, tan). During 2015/16, the baseline crops/treatments (legume, monoculture or fallow) were established. The following season (2016/17), the trial was planted in a split plot layout, with rotation system planted as main plot, and sorghum cultivars as sub-plot. During 2018/19 an additional factor was included, which entailed each of the plots being split again, to accommodate a crop rotation system that entailed 1) two consecutive seasons of sorghum (e.g. legume/sorghum/sorghum) and 2) two consecutive seasons of the legume followed with one season of sorghum. The trial was replicated three times and was maintained under dryland conditions with minimum tillage and no fertiliser input. Dimethenamid (75 $g.l^{-1}$) was applied directly after planting at a rate of 2000 $ml.ha^{-1}$ for pre-emergence grass control, followed by Basagran® (Bendioxide - thiadiazine 480 $g.l^{-1}$) post-emergence as required at 1.5 $l.ha^{-1}$ for broadleaf weeds. Hand weeding was also done when required. Decis® (deltamethrin, 25 $g.l^{-1}$) was applied at 250 $ml.ha^{-1}$ for pest control, including stalk borer and aphids.

3.2.2 Field sampling

At physiological maturity 10 plants of the designated inner row of each cultivar were harvested. Harvested panicles were threshed and grain weight was obtained. Grain moisture was determined with a Twist Grain pro moisture meter (Draminski Elektronics, London, U.K). Yield ($t.ha^{-1}$) was calculated at 12.5% moisture using the equation provided (Chapter 2, Section 2.2.3). Ten random sorghum grain samples were collected per cultivar per plot for grain mold quantification. Grain

samples were stored in brown bags in a cold room at 10˚C until grain mold ratings could be performed.

3.2.3 Visual disease rating and sample processing

Grain samples were visually rated for grain mold severity. Three sorghum grain sub-samples of 30 g each were weighed per sample collected. Each of the three sub-samples were individually rated for threshed grain mold severity, after which the average score of the three samples were determined. A scale of 1-5 was used in scoring grain mold, where 1 = no deterioration, 2 = 10% of grain surface deteriorated, 3 = moderate deterioration with 11–25% of the grain surface deteriorated, 4 = considerable deterioration with 26–50% of the grain surface deteriorated and 5 = extensive deterioration with more than 50% of the grain surface deteriorated (Audilakshmi *et al.*, 2007).

3.2.4 DNA Extraction and qPCR quantification in grain samples in 2016/17 and 2017/18 seasons.

The three above sub-samples were pooled after determining threshed grain mold rating (Tgmr) and used for DNA extraction and qPCR analyses. An IKA® All basic grinder (Germany) was used to grind the grain which was kept in cold room at 10˚C until further use. A modified method of Möller *et al.* (1992) was used to extract DNA from grain samples as described in Chapter 2, section 2.2.5.3, after which qPCR analyses were conducted for *F. graminearum* (*sensu lato*) (as described in Chapter 2, Section 2.2.5.4).

3.2.5 Ergosterol quantification

Five g were used from each of the three above sub-samples after threshing to quantify ergosterol in the sorghum grain using the modified method of Jambunathan *et al.* (1991) (as described in Chapter 2, Section 2.2.6).

3.2.6 Statistical analysis

Visual ratings, *Fg*SC and ergosterol were analysed as a split plot in the 2016/17 seasons and as a split-split plot analysis in 2017/18 season as indicated in Chapter 2, Section 2.2.7. Relationships

between variables were analysed using regression analyses including simple, non-linear and multivariate regression methodologies on excel.

3.3 Results

3.3.1 Visual grain mold severity

Analysis of variance indicated no significant treatment or treatment*cultivar interaction in the second season (2016/17 i.e. the season subsequent to the initial cropping system) (Table 21). Although not significant, lower visual grain mold rating was observed in BA/SG/SG rotation system of PAN8816 in comparison to the other systems included (Table 23). A significant difference (P=0.05; LSD = 0.25) was only observed amongst the cultivars in the third season (2017/18) (Table 22 and 23). PAN8706W had the highest visual Tgmr (mean=2.7) compared to the other two cultivars even though the difference was low. Lower mean ratings were recorded in NS5511 (2.2) and PAN8816 (2.3) which did not differ significantly from one another.

3.3.2 Ergosterol quantification

Highly significant (R^2=0.99 and 0.98) linear relationships were recorded between detected peak areas and the actual concentrations of the standards to provide a reliable standard curve for the two seasons respectively (Figure 14 and 15). In general, the average ergosterol content of the various cultivars and treatments varied between 16.6 $\mu g.g^{-1}$ and 111.6 $\mu g.g^{-1}$ in 2016/17, and 61 $\mu g.g^{-1}$ and 2779 $\mu g.g^{-1}$ in 2017/18 (Table 24a). Higher levels of ergosterol content were accordingly observed during 2017/18 compared to the previous season. ANOVA indicated no significant difference in ergosterol concentration between treatment or treatment*cultivar in 2016/17 season (Table 21). Analysis of variance indicated that the ergosterol concentration was significantly affected by the year 1*number of seasons of sorghum interaction during the 2017/18 season (Table 22) indicating that the three-year crop rotation system implemented significantly affected the ergosterol concentration observed in the grains during the last season of the trial. As stated under 2.2.7. ‘Year 1’ indicates the crop that was planted during the first year of the trial (2015/16) and ‘number of seasons of sorghum’ whether the crop rotation system consisted of two consecutive seasons of sorghum planted or only one season of sorghum planted. The highest ergosterol content was observed in the sorghum monoculture treatment in both rotation systems (1973 $\mu g.g^{-1}$ and 1623 $\mu g.g^{-1}$), with the second highest ergosterol content observed in the

SB/SG/SG (1722 μg.g^{-1}) (Table 24b). The lowest ergosterol content was observed in the FW/SG/SG (306 μg.g^{-1}) rotation system followed by BA/BA/SG (364 μg.g^{-1}) and SB/SB/SG (472 μg.g^{-1}).

Regression analyses were conducted to determine the relationship between Tgmr and ergosterol content in three sorghum cultivars. No relationships were detected between Tgmr and ergosterol concentration in either PAN8816 or NS5511 in both seasons (2016/17 and 2017/18). Significant relationships were detected in both seasons (2016/17 - polynomial and 2017/18 - linear) between Tgmr and ergosterol content in PAN8706W (R^2=0. 77 and 0.86) (Figure 16 and 17). The higher the visual Tgmr, the higher the ergosterol concentration, however at 100 μg.g^{-1} the level of ergosterol tended to decrease with increasing Tgmr (Figure 16). A linear regression was obtained for 2017/18.

3.3.3 qPCR analysis

Analysis of variance (Table 21) indicated that only cultivars differed significantly in *Fusarium graminearum* Species Complex *(Fg*SC*)* DNA content of grains in the 2016/17 season (P=0.05; LSD=35.07). The cultivar with highest *Fg*SC DNA concentration was PAN8706W (73.68 pg.μl^{-1}). NS5511 and PAN8816 *Fg*SC DNA concentrations did not differ significantly from one another (2.5 pg.μl^{-1} and 6.75 pg.μl^{-1} respectively) (Table 25). DB/SG/SG yielded higher *F. graminearum* concentration (139.3 pg.μl^{-1}) than monoculture sorghum (19.8 pg.μl^{-1}) and FW/SG/SG (30 pg.μl^{-1}) rotation systems in PAN8706W even though not significant (Table 25). Higher levels of *F. graminearum* concentration were observed under BA/SG/SG (18.9 pg.μl^{-1}) in comparison to monoculture sorghum (0.1 pg.μl^{-1}) and FW/SG/SG (2.4 pg.μl^{-1}) in PAN8816. No significant differences were observed during 2017/18 (Table 25).

3.4 Discussion

Susceptibility to grain mold differs with cultivar and the coincidence of host growth stages with favourable conditions for infection and colonization (Tarekegn *et al.*, 2004). Selection criteria in cultivar development are important and in addition to disease resistance, include yield potential, adaptability regarding soil and climatic conditions and the length of growing season in the specific area (Wanjekeche *et al.*, 2015).

Threshed grain mold rating (Tgmr) has been the common means of quantifying grain mold in cereals. It allows for a descriptive and visual estimation of disease severity based on the researcher observation (Bandyopadhyay and Mughogho, 1988). Fungal infection of grain is generally indicated by the discolouration on the pericarp without revealing the extent of damage caused internally (Seitz *et al*., 1983, Jambunathan *et al*., 1991). Based on the Tgmr results from this study, PAN8706W had the highest grain mold severity compared to the brown and red grain pericarp cultivars (NS5511 and PAN8816 respectively). Generally, grain mold is thought to be more severe in white grain, as opposed to brown and red grain cultivars, due to the absence of tannins which occur at higher concentrations in the coloured pericarps (Jambunathan *et al*., 1991). This result in increased visibility of symptoms even though the actual severity in white and coloured pericarp grains may be similar (Jambunathan *et al*., 1991, Forbes *et al*., 1992). Based on visual screenings, PAN8706W could be thought to be less tolerant to grain mold pathogens. Although Tgmr can be a reliable measure of grain mold detection, as it is time efficient and affordable (Audilakshmi *et al*., 1999), it must, however, not be the only disease evaluation technique used, as it can be misleading (Williams and Rao, 1981). Coetzee (2015) detected lower grain mold concentrations in PAN8706W when ergosterol was used as an evaluation criterion despite high visual infection, with intermediate ergosterol content in the less visibly infected NS5655 (brown grain pericarp cultivar). It is thus evident that a resistant cultivar can be classified as susceptible due to the fungus growing on the periphery of the pericarp without causing significant internal damage (Audilakshmi *et al*., 1999, Mpofu and McLaren, 2014). Menkir *et al.* (1996) reported that a hard endosperm in white sorghum cultivars and high tannin concentration in brown cultivars cannot always be used as measures of resistance to grain mold. This explains why Tgmr should not be the only assessment measure of sorghum grain mold as it lacks the ability to quantitatively detect internal infection and can provide a skewed indication of disease severity.

Ergosterol quantification is a reliable assessment measure as it measures the total fungal biomass (viable and non-viable) within grain tissues (Seitz *et al*., 1977). Compared to Tgmr, ergosterol quantification is specific, stable, sensitive and quantitative (Seitz *et al*., 1979). A significant correlation has previously been recorded between Tgmr and ergosterol concentration (Bandyopadhyay and Mughogho, 1988, Forbes *et al*., 1989, Jambunathan *et al*., 1991). In the current study, the presence or absence of a relationship was dependant on the cultivar investigated. In both 2016/17 and 2017/18 significant relationships were observed in PAN8706W,

which was not the case with NS5511 or PAN8816. During 2016/17 a polynomial curve was fitted, as the ergosterol content increased with the increasing Tgmr until 100 $\mu g.g^{-1}$, after which a decline was observed. In 2017/18 a linear regression was recorded, with an increase in ergosterol concentration coinciding with an increase in Tgmr levels. The difference in trends observed between the two seasons might be attributed to higher levels of ergosterol being detected during the 2017/18 season (as high as 2700 $\mu g.g^{-1}$) compared to the highest concentration of 120 $\mu g.g^{-1}$ achieved for PAN8706W during 2016/17. This allowed for a better correlation to be drawn over a wider range of fungal concentrations. The increase in ergosterol content with increasing Tgmr observed during 2017/18, could be due to pathogens that survived in the preceding season multiplying with favourable conditions (Sumner and Bell, 1986).

Colonization of grain as indicated by *Fg*SC DNA concentrations was more evident in PAN8706W than in PAN8816 and NS5511. No *Fg*SC DNA was detected in the 2017/18 season. This could be attributed to the fact that crop rotation reduces inoculum over years (Fry, 1982). Crop rotation could have had an effect on *Fg*SC colonization as indicated by DNA levels. The ergosterol content and *Fg*SC DNA results did not correlate. High levels of ergosterol were observed in some legume crops rotated with single or double sorghum, yet no pathogens were detected under qPCR analyses indicating that ergosterol was the result of organisms other than *Fg*SC. This could mean that the crops harbour other grain mold pathogens in addition to the *Fg*SC tested (Hugo, 1995). Doupnik and Boosalis (1980) indicated that minimum tillage could also lead to high unimportant sorghum diseases due to the retention of plant residues in the field. Mpofu and McLaren (2014) reported *Curvularia lunata*, *Phoma sorghina* and *Fusarium thapsinum* as the predominant grain mold pathogens as opposed to *F. graminearum*. It has been reported that in mixed inoculation fields, *F. graminearum* tends to occur in lower concentrations than other pathogens. Reid *et al*. (1999) and Stewart *et al*. (2002) found that the growth of *F. verticillioides* interfered with that of *F. graminearum* when co-inoculated. A complex of fungi could have co-occurred on the cultivars, hence no detection of *F. graminearum* even though colonization may have occurred. Some fungi may adapt to wide range of environmental conditions and crop rotation may not be as effective in reducing their inoculum potential (Sumner and Bell, 1986).

Crop rotation had an effect on ergosterol content in different sorghum cultivars. Ergosterol levels were significantly higher in sorghum monoculture and SB/SG/SG interactions. The infection in sorghum monoculture was expected because continuous cultivation results in depletion of micronutrients in the soil, low yield and increase in inoculum (Logrono and Lothrop, 1997,

Wanjekeche *et al*., 2015). Hugo (1995) reported high incidence of *F. graminearum* in monoculture maize roots and SB/maize/maize rotation systems. This was in agreement with the results on the current study. High ergosterol levels could also be attributed to residues of sorghum crops in the field as they reportedly result in temporary unavailability of soil nitrogen (Robinson, 1966). Another reason could be that these crops (sorghum and soybean) are dryland-adapted and compete with each other for water and nutrients. Low ergosterol levels occurred in SB/SB/SG and BA/BA/SG rotation systems as well as FW/SG/SG interaction. This could be due to fertility advantage of the legume crops and the undisturbed soil in the fallow system resulting in higher nutrient concentrations and moisture (Egesa *et al*., 2016). Fallow rotation system has been traditionally used as a soil fertility maintenance technique for decades. A double legume rotation system could have contributed to a certain extend based on their ability to fix nitrogen and reduce inoculum.

Compared to other legumes, superior yields were observed where soybean was rotated with maize in north-western Kenya. These yield increases were attributed to the high nitrogen fixing ability of soybeans (Mwangi and Wanjekenche, 1997), leaving the soil in excellent physical condition with available nitrogen for the next crop (Robinson, 1996). A study in Indiana also reported improved maize yield with soybean as a preceding crop under no tillage systems (Franzluebbers *et al*., 1995), which was attributed to the longer rotational periods of legumes and the moisture retaining ability that comes with a minimal or a no tillage system. A study was conducted where a sorghum-sudan hybrid and fallow reduced the mixed population of two nematodes in the field (*Meloidogyne and Heterodera glycine*), in turn increasing the soybean yields (Weaver *et al*., 1995). Bambara has reportedly been rotated with maize, millet, sorghum, cassava, yam, peanut and cowpea (Ezueh, 1997, Sangare, 2012). It is believed to have an ability of resisting pest infection which could be related to the results obtained in this study where ergosterol content and Tgmr were found at lower levels.

3.5 Conclusion

Crop rotation has the ability to reduce grain mold in sorghum cultivars and increase yield quality. Although *F. graminearum (sensu lato)* was detected in all samples of 2016/17 and 2017/18 (albeit at very low levels), none of the crop rotation systems had a significant effect on the concentration at which the fungal complex was detected. Double legume crops did not provide any additional benefit in terms Tgmr and ergosterol reduction. Colonization of grain was evident even though *F. graminearum* (*sensu lato*) was detected at very low levels suggesting that other fungal complexes

could be involved in the colonization. This study revealed the importance of using different assessment techniques in detecting fungal biomass without bias. The study showed that *F. graminearum* (*sensu lato*) might not be major grain mold causal pathogen on its own as the grain was colonized but the level of the pathogen presence was very low. However taking, into consideration of the El Nino that occurred in the previous year, the pathogen could have been under unfavourable conditions to reproduce as it would when conditions are favourable. In future studies, prominent grain mold pathogens such as *Curvularia lunata*, *Phoma sorghina* and *Fusarium thapsinum* and a wider range of grain mold pathogens should be tested for potential grain deterioration.

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Table 21: Analysis of variance of visual grain mold rating ergosterol concentration and *Fusarium graminearum* observed in the grains of three sorghum cultivars utilised in six legume/sorghum based crop rotation system and fallow during 2016/17.

Source	d.f	Threshed grain mold rating				Ergosterol (Grains)				*Fusarium graminearum*			
		s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr
Rep	2	0.7	0.4	1.3		12631	6315	2.6		67370	33680	1.6	
Rotation system (R)	5	0.8	0.2	0.6	0.69	78530	1571	0.7	0.67	10864	21730	1.0	0.47
Residual (A)	10	2.6	0.3	1.0		24301	2430	1.0		21713	21710	0.8	
Cultivar ©	2	0.1	0.1	0.3	0.77	76840	3842	1.6	0.22	57366	28683	11.0	<.001
R x C	10	1.9	0.2	0.7	0.74	21564	2156	0.9	0.54	19115	19110	0.7	0.69
Residual (B)	24	7.0	0.3			56634	2360			62376	25990		
Total	53	12.8				13067				17817			

Table 22: Analysis of variance of visual grain mold rating ergosterol concentration and *Fusarium graminearum* observed in the grains of three sorghum cultivars utilised in six legume/sorghum based crop rotation system and fallow during 2017/18.

Source	d.f	**Threshed grain mold rating**				**Ergosterol (Grains)**				***Fusarium graminearum***		
		s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r
Rep	2	0.7	0.3	1.2		10343	51713	0.0		11575	57875	1.3
Year1[1]	5	1.2	0.2	0.8	0.56	13696	27391	1.7	0.23	23576	47152	1.0
Residual	10	2.9	0.3	1.2		16513	16513	2.1		46071	46071	1.2
Split[2]	1	0.2	0.2	1.0	0.35	36051	36051	0.5	0.51	37890	37890	1.0
Year1 x Split	5	0.4	0.1	0.3	0.89	18339	36677	4.7	0.013	20697	41394	1.0
Residual	12	2.9	0.2	0.9		94175	78480	0.4		47648	39707	1.0
Cultivar	2	4.4	2.2	7.8	0.001	56636	28318	1.4	0.25	73150	36575	1.0
Year1 x Cultivar	10	2.8	0.3	1.0	0.45	14608	14608	0.7	0.70	39264	39264	1.0
Split x Cultivar	2	0.7	0.3	1.2	0.31	22794	11397	0.6	0.57	82898	14494	1.0
Year1 x Split x Cultivar	10	0.5	0.1	0.2	0.99	11155	11155	0.6	0.84	40085	40085	1.0
Residual	48	13.6	0.3			96372	20078			19275	40155	
Total	107	30.2				18851				44106		

Table 23: Visual grain mold rating over a three-year legume/sorghum and fallow/sorghum rotation system

Cultivar	Rotation system[1]	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Visual grain mold rating	Visual grain mold rating	Mean	Visual grain mold rating	Mean[3]
	BA-SG-SG	2	-	2.0		2.6	
	CP-SG-SG	2	-	2.3		2.0	
	DB-SG-SG	2	-	2.0		2.3	
	FW-SG-SG	2	-	2.3	2.1	2.0	
	SB-SG-SG	2	-	2.0		2.6	
	SG-SG-SG	(2)	-	2.0		2.0	2.2b
NS5511	BA-BA-SG	1	-	-		2.3	
	CP-CP-SG	1	-	-		2.0	
	DB-DB-SG	1	-	-		2.0	
	FW-FW-SG	1	-	-		2.0	
	SB-SB-SG	1	-	-		2.6	
	SG-SG-SG	(1)	-	-		2.0	
	BA-SG-SG	2	-	2.0		2.7	
	CP-SG-SG	2	-	2.0		2.7	
	DB-SG-SG	2	-	2.3		2.3	
	FW-SG-SG	2	-	2.3	2.2	2.3	
	SB-SG-SG	2	-	2.0		2.3	
	SG-SG-SG	(2)		2.6		3.0	
PAN8706W	BA-BA-SG	1	-	-		2.7	2.69a
	CP-CP-SG	1	-	-		3.0	
	DB-DB-SG	1	-	-		3.0	
	FW-FW-SG	1	-	-		2.7	
	SB-SB-SG	1	-	-		2.7	
	SG-SG-SG	(1)	-	-		3.0	
	BA-SG-SG	2	-	1.6		2.3	
	CP-SG-SG	2	-	2.3		2.3	
	DB-SG-SG	2	-	2.3		2.3	
	FW-SG-SG	2	-	2.0	2.1	2.0	
	SB-SG-SG	2	-	2.3		2.3	
	SG-SG-SG	(2)	190[2]	2.0		2.3	2.3b
PAN8816	BA-BA-SG	1	-	-		2.3	
	CP-CP-SG	1	-	-		2.3	
	DB-DB-SG	1	-	-		2.3	
	FW-FW-SG	1	-	-		2.3	
	SB-SB-SG	1	-	-		2.7	
	SG-SG-SG	(1)		-		2.3	
$LSD_{cultivar}$ *(P=0.05)*			-	*Ns*	-	-	*0.25*

[1] – BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow

[2] - PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

[3] - Values affected by the same letter in the same column are not significantly different at $p < 0.005$ according to Fisher's test.

Table 24: Ergosterol concentration (µg.g^{-1}) observed in sorghum grain over a three-year legume/sorghum and fallow/sorghum rotation system

Cultivar	Rotation system[1]	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Ergosterol (µg.g^{-1})	Ergosterol (µg.g^{-1})	Cultivar Mean	Ergosterol (µg.g^{-1})	Mean
NS5511	BA-SG-SG	2	-	111.6	74.9	600	732
	CP-SG-SG	2	-	92.2		492	
	DB-SG-SG	2	-	16.6		407	
	FW-SG-SG	2	-	65.5		341	
	SB-SG-SG	2	-	78.0		1595	
	SG-SG-SG	(2)	-	85.4		1101	
	BA-BA-SG	1	-	-		382	
	CP-CP-SG	1	-	-		1483	
	DB-DB-SG	1	-	-		1133	
	FW-FW-SG	1	-	-		323	
	SB-SB-SG	1	-	-		380	
	SG-SG-SG	(1)	-	-		551	
PAN8706W	BA-SG-SG	2	-	51.2	76.1	1716	1255
	CP-SG-SG	2	-	63.3		1579	
	DB-SG-SG	2	-	81.0		798	
	FW-SG-SG	2	-	116.1		61	
	SB-SG-SG	2	-	55.7		1063	
	SG-SG-SG	(2)		89.1		2703	
	BA-BA-SG	1	-	-		411	
	CP-CP-SG	1	-	-		748	
	DB-DB-SG	1	-	-		1593	
	FW-FW-SG	1	-	-		1267	
	SB-SB-SG	1	-	-		509	
	SG-SG-SG	(1)	-	-		2614	
PAN8816	BA-SG-SG	2	-	32.3	50.2	696	1170
	CP-SG-SG	2	-	79.9		363	
	DB-SG-SG	2	-	40.8		281	
	FW-SG-SG	2	-	62.6		515	
	SB-SG-SG	2	-	43.1		2508	
	SG-SG-SG	(2)	-	42.5		1085	
	BA-BA-SG	1	-	-		298	
	CP-CP-SG	1	-	-		1022	
	DB-DB-SG	1	-	-		1210	
	FW-FW-SG	1	-	-		2779	
	SB-SB-SG	1	-	-		527	
	SG-SG-SG	(1)	-	-		2754	
LSD $_{treatment*cultivar\ (P=0.05)}$			-	*Ns*	*Ns*	*Ns*	*Ns*

[1] – BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow

Table 24 (cont): Significant differences observed in ergosterol concentration (µg.g^{-1}) in sorghum grain during 2017/18 in legume/sorghum based rotation systems spanning three season where rotations with two consecutive seasons of sorghum were compared to rotations systems with one season of sorghum only.

Crop planted during[1]	**Two consecutive seasons of sorghum**	**One season of sorghum in crop**
BA	1004abcd	364d
CP	812bcd	1084abcd
DB	495cd	1312abcd
FW	306d	1456abc
SB	1722ab	472cd
SG	1623ab	1973a
LSD$_{(Year\ 1*seasons\ of\ sorghum)}$	*1091.1*	

[1] – BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow

Table 25: *Fusarium graminearum* (pg.µℓ$^{-1}$) observed in sorghum grain over a three-year legume/sorghum and fallow/sorghum rotation system

Cultivar	Rotation system[1]	Consecutive years of sorghum	2015/16	2016/17		2017/18	
			Concentration (pg.µℓ$^{-1}$)	Concentration (pg.µℓ$^{-1}$)	Cultivar Mean	Concentration (pg.µℓ$^{-1}$)	Mean
NS5511	BA-SG-SG	2	-	7.1	2.5b	5	196
	CP-SG-SG	2	-	1.5		8	
	DB-SG-SG	2	-	0.9		14	
	FW-SG-SG	2	-	0.1		13	
	SB-SG-SG	2	-	2.5		231	
	SG-SG-SG	(2)	-	3.2		4	
	BA-BA-SG	1	-	-		3	
	CP-CP-SG	1	-	-		11	
	DB-DB-SG	1	-	-		11	
	FW-FW-SG	1	-	-		24	
	SB-SB-SG	1	-	-		27	
	SG-SG-SG	(1)	-	-		0	
PAN8706W	BA-SG-SG	2	-	78.7	73.68a	7	24
	CP-SG-SG	2	-	76.2		11	
	DB-SG-SG	2	-	139.3		13	
	FW-SG-SG	2	-	30.0		23	
	SB-SG-SG	2	-	98.0		54	
	SG-SG-SG	(2)		19.8		15	
	BA-BA-SG	1	-	-		12	
	CP-CP-SG	1	-	-		52	
	DB-DB-SG	1	-	-		25	
	FW-FW-SG	1	-	-		23	
	SB-SB-SG	1	-	-		40	
	SG-SG-SG	(1)	-	-		8	
PAN8816	BA-SG-SG	2	-	18.9	6.75b	1	19
	CP-SG-SG	2	-	9.7		12	
	DB-SG-SG	2	-	3.7		15	
	FW-SG-SG	2	-	2.4		10	
	SB-SG-SG	2	-	5.6		51	
	SG-SG-SG	(2)	1894.67[2]	0.1		15	
	BA-BA-SG	1	-	-		5	
	CP-CP-SG	1	-	-		12	
	DB-DB-SG	1	-	-		9	
	FW-FW-SG	1	-	-		44	
	SB-SB-SG	1	-	-		56	
	SG-SG-SG	(1)	1897.67	-		5	
LSD $_{cultivar\ (P=0.05)}$			-	*Ns*	*35.07*	*Ns*	*Ns*

[1] BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow
[2] PAN8816 was the only cultivar planted on the plot allocated for sorghum during the first season (2015/16)

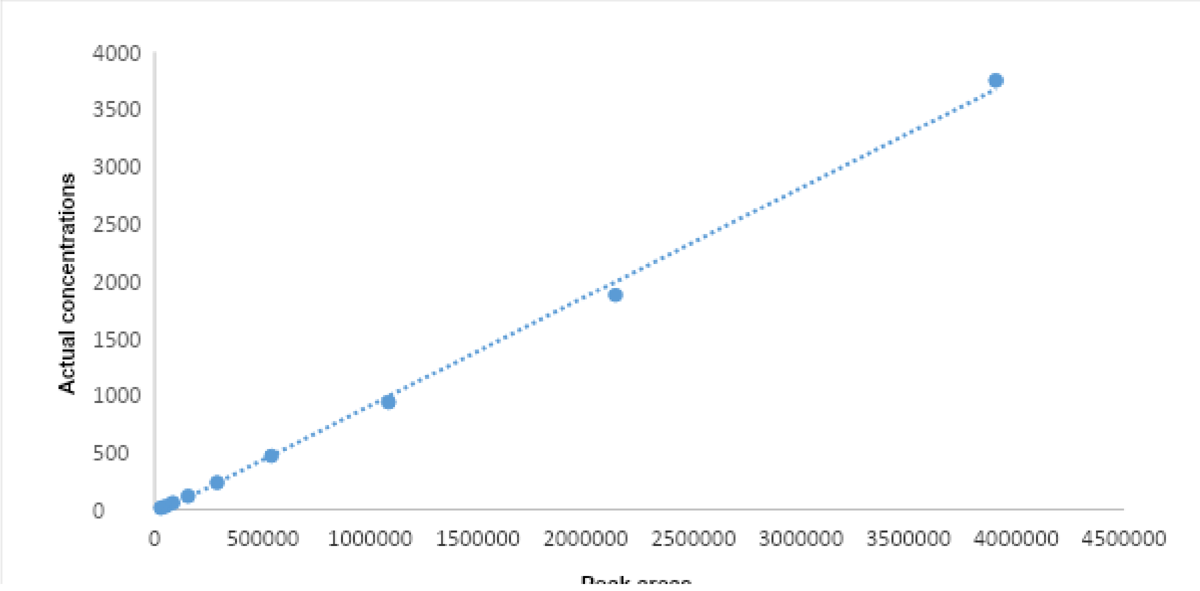

Figure 14: A linear standard curve obtained between detected peak areas and the actual concentrations of the ergosterol standards in 2016/17 season.

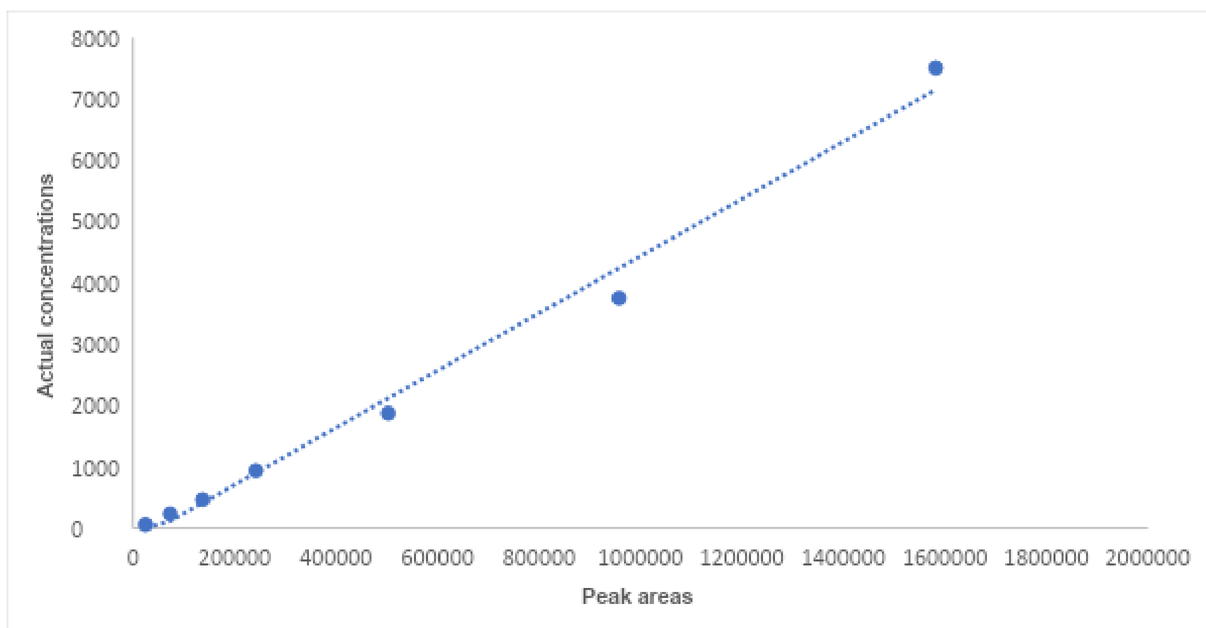

Figure 15: A linear standard curve obtained between detected peak areas and the actual concentrations of the ergosterol standards in 2017/18 season.

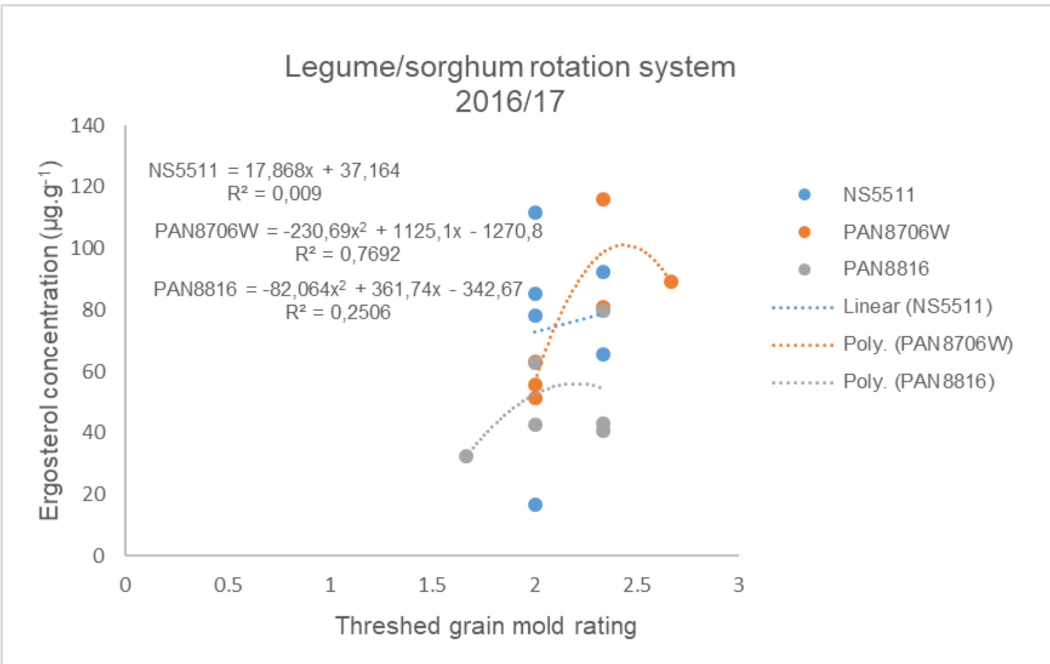

Figure 16: Relationship between threshed grain mold rating and ergosterol concentration of sorghum in sorghum/legume rotation systems for 2016/17 season at Potchefstroom.

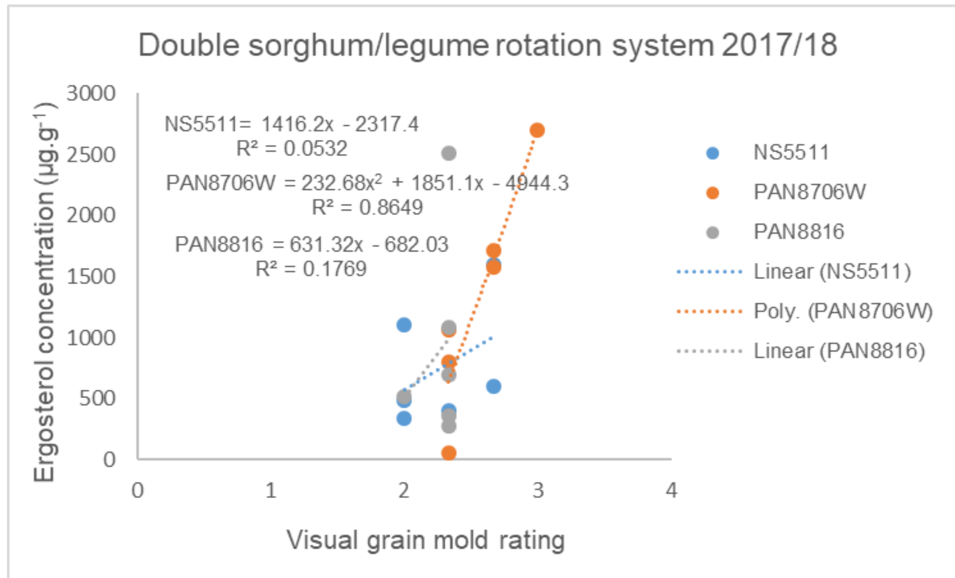

Figure 17: Relationship between threshed grain mold rating and ergosterol concentration of sorghum in two sorghum/legume rotation systems for 2017/18 season at Potchefstroom

Chapter 4

Changes in grain mycotoxin concentrations associated with various degrees of root health as observed under legume based crop rotation systems

Abstract

Food security is increasingly becoming a more interesting topic in South Africa and other developing countries due to poor food and feed supplies for humans and animals. Lack of proper food quality monitoring infrastructures result in consumption of mycotoxin infected food, leading to health scares and death. This study aimed at determining whether legume based rotation systems assist in reducing deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) concentrations in sorghum grain and to establish whether the mycotoxin concentration in sorghum grain might be due to translocation of mycotoxins from the root system to sorghum grain. Ten plants were collected during each of three seasons from a 10 m designated inner row per cultivar, per rotation treatment for threshing and assessment of root rot severity. The threshed grains were visually rated for grain mold on a 1-5 scale and roots were visually assessed on a scale of 0-4. Total fungal biomass as a measure of colonization was quantified through ergosterol quantification in grains and roots, while *Fusarium graminearum* Species Complex was quantified using qPCR analyses. Mycotoxins were quantified using a LC-MS/MS method. Neither treatment or treatment*cultivar interaction was significant for visual grain mold rating in the 2016/17 season. Highest visual grain mold rating was recorded during the 2017/18 season in cultivars with PAN8706W (mean=2.7) yielding a higher rating than PAN8816 (2.3) and NS5511 (2.2). Similarly, neither treatment or treatment*cultivar interaction was significant in both 2016/17 and 2017/18 in the roots. Significant differences in root rot severity were detected in both the 2016/17 and 2017/18 in cultivars with NS5511 (2016/17=177.5 and 2017/18=358.5) having higher root rot severity than PAN8606W (2016/17=0.0; 2017/18=152.5) and PAN8816 (2016/17=105.0b and 2017/18= 300. 3b) respectively. Grain ergosterol content was higher in the 2017/18 season compared with the 2016/17 season. Ergosterol content level was significantly affected by the initial cropping cycle (year 1)*number of seasons of sorghum interaction during the 2017/18 season with sorghum monoculture treatment in both rotation systems (1973 $\mu g.g^{-1}$ and 1623 $\mu g.g^{-1}$) yielding higher levels of ergosterol followed by SB/SG/SG (1722 $\mu g.g^{-1}$). Lower ergosterol content was observed in FW/SG/SG (306 $\mu g.g^{-1}$) followed by BA/BA/SG (364 $\mu g.g^{-1}$) and SB/SB/SG

(472 µg.g^{-1}) rotation system. Ergosterol quantification in roots did not differ significantly in 2016/17 season. An increase in ergosterol level was detected in 2017/18 with all rotation systems not differing from each other except PAN8816 under the dry bean rotation system which yielded higher ergosterol levels (1049.2a µg.g^{-1}). Significant differences between grain sorghum cultivars were recorded with PAN8706W (73.68 pg.µl^{-1}) resulting in higher *Fg*SC DNA levels than NS5511 and PAN8816 (2.5 pg.µl^{-1} and 6.75 pg.µl^{-1} respectively) in the 2016/17 season. Analysis of variance showed no significant differences in the three mycotoxins (DON, NIV and ZEA) tested in sorghum grains during 2016/17 season. DON and NIV were significantly different ($P<0.05$) in sorghum roots in 2016/17 season. Higher levels of DON in roots were detected in PAN8706W (70.08) than in NS5511 and PAN8816 (22.39b and 16.94b). NIV was higher in PAN8706W and PAN8816 (273a and 265a) compared to NS5511 (154.9b) in the roots in season 2016/17. Significant differences for DON and NIV were recorded in grains from different cultivars. DON concentration was higher in PAN8706W (62.22) than NS5511 (11.92) and PAN8816 (29.45) in 2017/18. NIV was more concentrated in PAN8816 (120.2) compared to PAN8706W (109.2) and NS5511 (92.5). ZEA concentration was significantly affected by the year 1*number of seasons of sorghum interaction under dry bean rotation systems. DON in the roots was affected by crop rotation system*treatment interaction with higher DON levels detected under fallow rotation system in PAN8706W and PAN8816 in two consecutive seasons of sorghum. Lower concentrations were detected in NS5511, PAN8706W and PAN8816 in sorghum, dry bean and bambara systems under two consecutive years of legumes. ZEA showed significant difference amongst sorghum cultivars with PAN8706W (1.3) yielding higher levels than NS5511 (0.3) and PAN8816 (0.2). A few relationships were recorded where a decline in different mycotoxins were recorded under PAN8816 compared to PAN8706W suggesting that PAN8816 was a better cultivar with respect to mycotoxin tolerance than PAN8706W under favourable conditions. These results suggest that the minimal mycotoxins detected in this study do not pose a threat as they are far below EU regulation limit, however they should be monitored over years to avoid accumulation that could potentially lead to health scares and deaths.

4.1 Introduction

The demand for food worldwide is currently higher than that produced (WHO, 2012). In addition, factors such as grain mold and mycotoxins negatively affect quality and value (ICRISAT, 1987). The importance of diseases vary worldwide due to economic losses and the food security threat that they pose.

Prominent fungi associated with mycotoxin production include *Alternaria* spp*., Aspergillus* spp*., Fusarium* spp*. and Penicillium* spp*..* Physical damage appears as softened and chalky endosperms, discolouration of pericarps and small grain size (Williams and Rao, 1981). Grain infected with *Fusarium* spp*.* appear white, pink or orange in colour whilst those infected with *Alternaria* spp*. and Curvularia* spp*.* appear black on the surface (William and Rao, 1981). *Fusarium graminearum* Species Complex (*Fg*SC) is one of the most important SGM causal agents with 16 biogeographically structured and phylogenetically distinct species (Starkey *et al.,* 2007, Davari *et al*., 2012). Climate and the interaction between host, pathogen and environment play a major role in the manifestation of species complex fungi and its response (Logrieco *et al*., 2002, Waliyar *et al*., 2007). Due to tremendous damage caused by mycotoxins, in some cases resulting in mortality of people and animals, maximum tolerable regulation levels of mycotoxins have been implemented. South African legislation regulates aflatoxins in all food products (Viljoen, 2003).

Mycotoxins are known as toxic secondary metabolites in grain contaminating fungi (Parry *et al*., 1995; Goswani and Kistler, 2004), and thought to be a defence mechanism trait, which fungi developed against insects and rodents. Mycotoxin producing fungi proliferate in the field (pre–harvest), during storage, processing, transportation and marketing (post–harvest) under favourable conditions (Menkir *et al*., 1996, Waliyar *et al*., 2007). They pose a health concern to humans and animals and their occurrence is common mostly in developing countries due to less variation in diet consumed by the population (Marroquín-Cardona *et al*., 2014). Grain grown under subsistence farming is sent straight to local market, raising a high health risk to the consumers due to lack of strict regulation and minimal attention to quality issues (Shephard, 2008, WHO/FAO, 2012).

The economic impact of mycotoxins on society include import and export restrictions, food recalls, detrimental impact on the livestock industry, human health decline from adverse effects

associated with mycotoxin consumption and monetary impact on human and animal health care (Marroquín-Cardona *et al*., 2014). Health associated effects include immune suppressions, cancers, neurological disorders, reproduction complications and extensive organ damage in humans and livestock (Bandyopadhyay *et al*., 2000). In grain, mycotoxins result in a deterioration in grain quality, discolouration of grain, reduction of nutritional value of grain, seed germination failure and affects the export of the grain and its products (Waliyar *et al*., 2007). Certain countries have implemented legislative regulation of the acceptable maximum levels of mycotoxin in maize and small cereals (Boutigny *et al*., 2012).

Crop rotation is one of the best cultural methods still used to reduce the incidence of grain mold and mycotoxin causing pathogens in the soils and their translocation in certain cereal crop including barley, maize, oat, sorghum and wheat (Dominy *et al.,* 2002). The ability of crop rotation in alleviating these pathogens depends entirely on type of crops planted and the amount of subsequent crop residue (Dill-Macky and Jones, 2000). According to Rousseau *et al.* (2007) rotations with two unrelated crops can help to reduce diseases. This was based on observations during a three-year soybean-maize rotation system where a decline in *Sclerotinia* stem rot caused by *S. sclerotiorum* in soybeans was observed.

Five important mycotoxins are globally recognized *viz.* aflatoxin, fumonisins, deoxynivalenol (DON), ochratoxins and zearalenone (ZEA) (Shephard, 2008). Mavhunga (2013) reported that maize and sorghum cultivars in South Africa are affected by DON, NIV and ZEA in levels that exceed the EU and USA legislative regulation limits set for the three mycotoxins. NIV was found to be the predominant toxin detected in all the samples tested. The current study is aimed at (i) determining whether legume based rotation systems assist in reducing deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) concentrations in sorghum grain (ii) to establish whether the mycotoxin concentration in sorghum grain might be due to translocation of mycotoxins from the root system to sorghum grain.

4.2 Materials and methods

4.2.1 Field trial

A field trial was planted during 2015/16, 2016/17 and 2017/18 at Potchefstroom (ARC-Grain Crops, South Africa) as described in Chapters 2 and 3, (Section 2.2.1 and 3.2.1). Trials consisted of various crop rotation systems of sorghum in combination with dry bean, soybean, cowpea, bambara or fallow. Three sorghum cultivars were included PAN8816, NS5511 and PAN8706W. Trials were planted in a split plot layout, with rotation crop as main plot, and sorghum cultivars as sub-plot. The trial was replicated three times and was maintained under dryland conditions with minimum tillage and no fertiliser input.

4.2.2 Field sampling and yield calculation

Ten randomly selected sorghum plants per cultivar per plot were sampled every season at soft dough stage to establish root rot severity (Chapter 2, section 2.2.3.) Samples were taken from a part of the inner row of each cultivar allocated for destructive sampling and did not form part of the row area allocated for yield calculation.
The sorghum cultivars were harvested by hand at physiological maturity (Chapter 3, Section 3.2.2) and random grain samples collected per cultivar per plot were used for grain mold (same method and results as Chapter 3) and mycotoxin quantification.

4.2.3 Visual disease rating and sample processing

4.2.3.1 Root rot severity

Roots were visually assessed for root rot severity based on the percentage of root discolouration. Root rot incidence was quantified as the percentage of the plants sampled per plot that demonstrated some degree of rot (visual discolouration) as described in Chapter 2 (Section 2.2.4). A root disease index (RDI) was used to record disease severity of each plant sampled based on an adjusted scale of 0–4 where, 0 = no symptoms, 1 = 1–24% rot, 2 = 25–49% rot, 3 = 50–75% rot and 4 = 75–100% rot (Soonthornpoct *et al.*, 2000). Disease severity in the sampled population was accordingly calculated as the product of disease incidence x RDI (Soonthornpoct *et al.*, 2000).

4.2.3.2 Grain mold severity

A sub-sample of the three sorghum cultivars per plot were used to visually rate the severity of grain mold on the grains. A scale of 1-5 was used in scoring grain mold (Audilakshmi *et al.*, 2007) as described in Chapter 3 (Section 3.2.3). The three sub-samples were used for DNA extraction and qPCR analysis as well as ergosterol quantification. An IKA® All basic grinder (Germany) was used to grind the grain which was kept in a brown bag in a cold room at 10˚C until further use.

4.2.4 Fungal target DNA qualification

4.2.4.1 Fungal reference isolates for qPCR analysis

A *F. graminearum* reference isolate was provided by Stellenbosch University, Plant Pathology Department, Stellenbosch as described in Chapter 2 (Section 2.2.5.1).

4.2.4.2 DNA extraction and qPCR in grain and root samples 2016/17 and 2017/18 seasons

A modified method of Möller *et al.* (1992) was used to extract DNA from grain and root samples as described in Chapter 2, (Section 2.2.5.3) after which qPCR analysis were conducted for *F. graminearum* as described in Chapter 2 (Section 2.2.5.4).

4.2.3.3 Ergosterol quantification

Five g of plant tissue was used from the three above sub-samples to quantify ergosterol in the sorghum root samples collected at soft dough stage and in grain subsequent to threshing using the modified method of Jambunathan *et al.* (1991) as described in Chapter 2, Section 2.2.6

4.2.5 Mycotoxin quantification

Mycotoxin standards (DON, NIV and ZEA) were purchased from Sigma Aldrich (Milan, Italy) and stored at 4°C in the dark. Water and the organic solvent (methanol) were high performance liquid chromatography (HPLC) grade from Merck (Darmstadt, Germany). Working standard solutions

were prepared by serially diluting each individual standard stock solution with a suitable solvent mixture (methanol/water, 70:30; v/v). Detection and quantification of DON, NIV and ZEA were determined using the modified Small *et al.* (2012) method. Fifty-four and 108 sorghum samples in 2016/17 and 2017/18 respectively, were analysed to detect the level of DON, NIV and ZEA in grain and root samples respectively. Grain and root samples were ground to fine powder using Cyclotech sample mill (Foss Tecator, Hoganas, Sweden). A 20 ml aliquot of methanol/water 70:30 v/v was added to 50 ml Falcon tubes containing 5 g sub-samples of the respective plant tissues. The tubes were shaken at 2000 rpm on Heidolph MultiReax Shaker (Labotec, South Africa) for 30 min at room temperature. Samples were centrifuged for 10 min at 2000 u.min^{-1}. Two ml of supernatant was drawn and filtered through a 0.45 µm RC syringe (Acrodisc® PALL PSF Syringe Filter, Premium Glass Fiber Prefilter) into a 2 ml Eppendorf tube and left overnight at 4°C. Extract was centrifuged for 10 min at maximum acceleration (g) ±14000 rcf. A 100 µl of sample and 900 µl methanol/water (70:30 v/v) were added into vials for analysis of DON, NIV and ZEA (10 x dilutions). Samples were vortexed prior to analysis. The samples were extracted in triplicates to ensure accuracy. The concentration of the mycotoxins standards were determined at the University of the Free State, Department of Microbial, Biochemical and Food Biotechnology.

4.3 Results

4.3.1 Root rot severity

Higher root rot severity was recorded in the 2017/18 season compared to the preceding 2016/17 season. NS5511 yielded significantly greater root rot severity in both 2016/17 (root rot severity score = 177.5) and 2017/18 (root rot severity score = 358.5), while PAN8706W resulted in the lowest root rot severity in both seasons (2016/17 = 0.0; 2017/18 = 152.5) (Table 6) as described in Chapter 2 (Section 2.3.3). Crop rotation system and the interaction with cultivar did not have a significant effect on root rot severity on both seasons (2016/17 and 2017/18). Lower root rot severity could be seen with soybean in comparison to cowpea in 2017/18 season (Table 6), although not significant as described in Chapter 2 (Section 2.3.3).

4.3.2 Grain mold severity

Neither treatment nor treatment*cultivar interaction was significant in the second season (2016/17) (Table 21) while a significant difference (P=0.05; LSD = 0.2518) was observed amongst

the cultivars in the third season (2017/18) (Table 22 and 23) as described in Chapter 3 (Section 3.3.1). Higher visual grain mold was detected in PAN8706W (rating = 2.7) in comparison to PAN8816 (rating = 2.3) and NS5511 (rating = 2.2).

4.3.3 Ergosterol quantification in grains and roots

Grain ergosterol content amongst cultivars varied between 16.6 $\mu g.g^{-1}$ and 111.6 $\mu g.g^{-1}$ in 2016/17, and 61 $\mu g.g^{-1}$ and 2779 $\mu g.g^{-1}$ in 2017/18 (Table 24a) as described in Chapter 3, (Section 3.3.2). Higher ergosterol content was recorded in the 2017/18 season compared to the 2016/17 season. ANOVA indicated no significant difference in ergosterol concentration between treatment or treatment*cultivar interaction in the 2016/17 season (Table 21; Chapter 3, Section 3.3.2). Analysis of variance indicated that the ergosterol concentration was significantly affected by the Year 1*number of seasons of sorghum interaction during the 2017/18 season (Table 22; Chapter 3, Section 3.3.2). The highest ergosterol content was observed in the sorghum monoculture treatment in both double sorghum/legume and double legume/sorghum interaction seasons (1973 $\mu g.g^{-1}$ and 1623 $\mu g.g^{-1}$), with the second highest ergosterol content observed in the SB/SG/SG (1722 $\mu g.g^{-1}$) (Table 24b). The lowest ergosterol content was observed in the FW/SG/SG (306 $\mu g.g^{-1}$) rotation system followed by BA/BA/SG (364 $\mu g.g^{-1}$) and SB/SB/SG (472 $\mu g.g^{-1}$).

Ergosterol quantification in roots did not differ significantly in the 2016/17 season as described in Chapter 2 (Section 2.3.4) despite the lower ergosterol levels that were expected in legume rotation systems, indicating that they did not have a significant effect on fungal colonisation of roots. Cultivars did not differ significantly amongst each other except PAN8816 under the dry bean rotation system which yielded higher ergosterol levels (1049.2 $\mu g.g^{-1}$) in the 2017/18 season.

4.3.4 qPCR analysis

Neither treatment nor treatment*cultivar interaction was significant in both 2016/17 and 2017/18 in the roots as shown in Chapter 2 (Table 14). Significant difference was obtained amongst the three sorghum cultivars in 2016/17 season, with high level of *Fusarium graminearum* Species Complex *(Fg*SC*)* DNA content in PAN8706W (73.68 $pg.\mu l^{-1}$). NS5511 and PAN8816 *Fg*SC DNA concentrations did not differ significantly (2.5 $pg.\mu l^{-1}$ and 6.75 $pg.\mu l^{-1}$ respectively) (Table 25) as described in Chapter 3 (Section 3.3.3). No significant differences between cultivars were observed during 2017/18 (Table 25).

4.3.5 Mycotoxin quantification

Analysis of variance showed no significant differences in the concentrations of the three mycotoxins (DON, NIV and ZEA) tested in sorghum grains during 2016/17 season (Table 26). This was however, different in the roots as both DON and NIV were significantly different (P= 0.05) amongst the sorghum cultivars (Table 26). High levels of DON concentrations in roots were detected in PAN8706W (70.08 $\mu g.kg^{-1}$) comparative to NS5511 and PAN8816 that were not significantly different (22.39 $\mu g.kg^{-1}$ and 16.94 $\mu g.kg^{-1}$) (Tables 26 and 31). NIV concentrations occurred in higher levels in PAN8706W and PAN8816 (273 $\mu g.kg^{-1}$ and 265 $\mu g.kg^{-1}$) in the roots as compared to NS5511 (154.9 $\mu g.kg^{-1}$) (Table 32). Although not significant, both DON and NIV grain concentrations were higher in the 2016/17 season as opposed to the subsequent 2017/18 season.

In the 2017/18 season, ANOVA showed accumulation of the three tested mycotoxins (Table 27). Significant difference of DON and NIV occurred in grains from different cultivars (Table 27, 28 and 29). DON concentration was higher in PAN8706W (62.22 $\mu g.kg^{-1}$) than NS5511 (11.92 $\mu g.kg^{-1}$) and PAN8816 (29.45 $\mu g.kg^{-1}$) that did not differ significantly from each other (Table 28). Although not significant, DON occurred in higher concentrations in DB/SG/SG (130.5 $\mu g.kg^{-1}$) compared to BA/SG/SG (24.8 $\mu g.kg^{-1}$), SG/SG/SG (33.7 $\mu g.kg^{-1}$) and CP/SG/SG (37.0 $\mu g.kg^{-1}$) under two consecutive years of planting sorghum. The FW/FW/SG yielded higher DON concentration than SG/SG/SG (38.4 $\mu g.kg^{-1}$) and DB/DB/SG (39.4 $\mu g.kg^{-1}$) both in PAN8706W (Table 28). NIV concentration accumulated more in PAN8816 (120.2 $\mu g.kg^{-1}$) when compared to PAN8706W (109.2 $\mu g.kg^{-1}$) and NS5511 (92.5 $\mu g.kg^{-1}$) (Table 29). ZEA concentration was significantly affected by the year 1*number of seasons of sorghum interaction (Table 27 and 30b). Dry bean had higher ZEA concentrations under two consecutive years of sorghum planted.

Analysis of variance showed that accumulation of DON in roots was affected by crop rotation system*treatment interaction (year1*split*cultivar) (Table 27 and 31). Higher DON concentrations were detected under fallow rotation sytems in PAN8706W and PAN8816 in two consecutive seasons of sorghum. Lower concentrations were detected in NS5511, PAN8706W and PAN8816 in sorghum monoculture, dry bean and bambara systems after two consecutive seasons of legumes. ZEA showed significant difference amongst sorghum cultivars with PAN8706W (1.3 $\mu g.kg^{-1}$) yielding higher than NS5511 (0.3 $\mu g.kg^{-1}$) and PAN8816 (0.2 $\mu g.kg^{-1}$) (Table 27 and 33).

4.3.6 Relationships between *Fg*SC and mycotoxins

A polynomial relationship was observed between *Fg*SC DNA concentration in PAN8816 and DON concentration in the 2016/17 season. As *Fg*SC increased between 0–10 pg.μl^{-1} so did DON up to 120 $\mu g.kg^{-1}$, subsequently decreasing despite increase *Fg*SC in the grains (Figure 18A). A similar pattern was observed in the roots in this cultivar between 0–5 pg.μl^{-1}. Although this range was small, DON concentrations higher than 50 $\mu g.kg^{-1}$ were recorded as the *Fg*SC DNA concentration increased (Figure 18B). Contrary to this, however, an increase in DON up to 100 $\mu g.kg^{-1}$ was observed in PAN8706W roots, which immediately declined with increasing *Fg*SC DNA. No correlation was observed in both grains and roots between *Fg*SC and DON in 2017/18 season.
No correlation between NIV and *Fg*SC DNA was detected in sorghum roots in the 2016/17 season. A negative correlation was however detected in PAN8816 grain between *Fg*SC DNA and NIV which decreased, tending towards 0 $\mu g.kg^{-1}$ (Figure 19A). A polynomial relationship in rotation systems including both one and two consecutive years of sorghum, in PAN8706W showed an increase in NIV concentrations between 140-160 $\mu g.kg^{-1}$ followed by a decrease with increasing *Fg*SC DNA levels ranging between 30-50 pg.μl^{-1}. Rotation systems with legumes and sorghum played a role in reducing NIV concentrations (Figure 19 B–C).

Correlations were recorded in PAN8816 grains between *Fg*SC DNA and ZEA concentration in the 2016/17 season with ZEA decreasing with increasing *Fg*SC concentration (Figure 20A). A polynomial regression curve in PAN8706W roots under two consecutive years of legumes planted showed an increase in ZEA with increasing *Fg*SC ranging from 30-50 pg.μl^{-1} (Figure 20B). Higher ZEA concentration was detected in PAN8706W under two consecutive seasons of sorghum in 2017/18 (Figure 20D). A neutral level, with no increase or decline with increasing *Fg*SC was recorded in PAN8816 under one year of sorghum in 2017/18.

4.4 Discussion

Food security comes under pressure with the increasing awareness of mycotoxins in food and feed and the resulting health implications for humans and animals and in some cases, death. Mycotoxin detection in cereals such as maize, wheat, rice and sorghum negatively impacts developing countries, in particular, due to their reliance on these cereals as staple food. Many developing countries including South Africa, are not sufficiently protected by food quality monitoring infrastructures due to the exportation of best food quality and the feeding of millions of

local consumers and animals by poor, rejected food and feed (Leslie and Logrieco, 2014). Accurate methods are required in order to ensure a sustainable production of high quality food and feed. Enzyme-linked Immunosorbent assays (ELISA) have been used as a screening tool to detect mycotoxins, however, it is thought to be unreliable as it can give false positives due to cross-reactivity (Mavhunga, 2013). LC-MS/MS is one of the more precise methods for mycotoxin quantification, as it allows for simultaneous multi-mycotoxin detection through a single extraction protocol (Coetzee, 2015).

The current study showed no significant difference amongst three tested mycotoxins in sorghum grain from the rotation systems during 2016/17 as opposed to 2017/18 season and this could be attributed to more favourable weather conditions and the presence of inoculum while the crops were still in the field (Mavhunga, 2013). DON was high in the roots of PAN8706W with a concentration level of 70.08 $ug.kg^{-1}$ t compared with NS5511 and PAN8816 (22.39 $ug.kg^{-1}$ and 16.94 $ug.kg^{-1}$) during the 2016/17 season although *F. graminearum* was not detected in the roots in both seasons (2016/17 and 2017/18). According to Leslie and Logrieco (2014) sorghum susceptibility to *F. graminearum* (*sensu lato*) differs between cultivars and the difference in the relationships between colonization by the pathogen and mycotoxin levels could be due to host genotype influencing mycotoxin production despite similar colonisation levels (Coetzee, 2015). van Rooyen (2019) detected *Fg*SC in sorghum roots in the initial stage of the soybean rotation system with a decline in subsequent season in all the systems suggesting a potential shift in fungal populations as a result of continuous rotation of crops (Janvier *et al.*, 2007). This could be due to partial or total control of root rot fungi in the rotation systems (Curl, 1963). Attribution could also be given to the fact that *F. graminearum* (has a limited ability to compete in more diverse soil micro-organism populations.

Translocation of DON was studied extensively on wheat infected with *F. culmorum* at the stem base (Purss, 1971, Snijders, 1990, Clement and Parry, 1998). The fungus colonised the stem tissues up to the 2nd and 3rd node without reaching the head and it was thought that translocation occurs in advance of fungal colonization (Beccari *et al.*, 2011). Similar results were reported (Xu *et al.*, 2008). This, however, is in contrast with the current study where *F. graminearum (sensu lato)* and DON were detected in the head with no significant difference in Tgmr and ergosterol in the 2016/17 season. The results correlated with that of (Mudge *et al.*, 2006), where it was reported that *F. graminearum* and *F. pseudograminearum* were detected in the wheat head following stem base inoculation without symptoms.

Higher DON levels were detected in 2017/18 season despite low or insignificant levels of *Fg*SC in grain and roots. Attribution could be given to the longer storage of grain in cold room with limited air circulation and moisture accumulation (Ayalew *et al*., 2006, Dejene *et al*., 2004) forced by budget constraints which delayed analyses. Lower DON concentrations were detected in roots and attribution could be given to the legume rotation system mostly bambara and dry bean in different cultivars in 2017/18 season. These low levels were detected under two consecutive years of legume rotation indicating the effect of legumes in reducing mycotoxin levels. These results correlated with those of van Rooyen (2019), where DON and NIV were reportedly lower in dry bean and soybean rotation systems. Bambara was reported to be less prone to pests and diseases making it a better legume alternative in controlling grain mold pathogens and mycotoxin production. It has been successfully rotated with maize, sorghum and millet (Ezueh, 1997, Sangare, 2012).

Generally, mycotoxin levels in this study were relatively low and attribution could be given to warmer temperatures experienced in Potchefstroom over the past 2–3 years whereas their production requires humid areas. Sorghum reportedly results in lower mycotoxin levels than maize (Bandyopadhyay *et al*., 2000, da Silva *et al*., 2006). Deoxynivalenol concentration levels detected were below the maximum level permitted by EU regulations currently applied as a guideline in local grains. Acceptable DON levels in South Africa range between 1000 $\mu g.kg^{-1}$ and 2000 $\mu g.kg^{-1}$ depending on where grain is used. The maximum level accepted for DON contamination in cereals ranges between 200 and 2000 $\mu g.kg^{-1}$ (FAO/WHO, 2012). DON detection however, should be taken into consideration even though the limit was below the permitted regulation, because long term consumption could lead to subsequent health issues in both humans and animals (Mavhunga, 2013).

Nivalenol is a volatile trichothecene mycotoxin, often overshadowed by the presence of DON (Yoshida and Nakajima, 2010). As a result, detecting its toxicity level has always been a challenge. NIV producing members of the *Fg*SC in sorghum include *F. acacia-mearnsii*, *F. cortaderiae* and *F. meridionale* (Mavhunga, 2013). The EU maximum limit of NIV has not been set yet, however Japan has set a daily NIV intake allowance of 0.4 $\mu g.kg^{-1}$ of body weight per day (Cast, 2003, Koppen *et al*., 2010). NIV accumulation was detected in sorghum roots with PAN8706W and PAN8816 (273 and 265 $\mu g.kg^{-1}$) and with no significant differences in grains in the 2016/17 season. In contrast, NIV was not detected in the roots during 2017/18 and attribution

could be given to the rotation systems although grains were less contaminated compared to the preceding season. The absence of *F. graminearum* in the roots could have also played a role in the reduction of NIV concentration which was supported by ergosterol quantification results. However, PAN8816 roots resulted in higher ergosterol levels under dry bean rotation systems which could explain higher NIV in PAN8816 grains (120.2 $\mu g.kg^{-1}$). This result suggests that cultivar PAN8816 could be susceptible to other pathogens other than *F. graminearum*.

The importance of ZEA contamination was reportedly found in sorghum malt and beer even in the absence of aflatoxin and fumonisins in Botswana, Nigeria and South Africa (Okoye, 1986, Odhav and Naicker, 2002, Nkwe *et al*., 2005) due to its heat stability (Cast, 2003, Yazar and Omurtag, 2008, Zinedine and Mañes, 2009). Zearalenone co-occurs with DON when produced by *F. graminearum* (*sensu stricta*) or *F. culmorum* (Reddy *et al*., 2010) but can also occur on its own (Aoyama *et al*., 2009). It is an estrogenic toxin affecting the fertility and reproductive ability of livestock. This study showed low occurrence of ZEA in both roots and grains of sorghum in the three-year crop rotation system. Its EU maximum tolerable level in food commodities is 100 $\mu g.kg^{-1}$. Tolerable intake of ZEA was recorded as 0.50 $\mu g.kg^{-1}$ bodyweight for consumption (Zinedine *et al*., 2007). Mavhunga (2013), detected ZEA in fewer samples that exceeded maximum EU limit than reported by Dutton *et al*. (2001) in South African commodities. The higher ZEA level in the current study was recorderd under dry bean rotation systems (2.6 $\mu g.kg^{-1}$) under two consecutive years of sorghum. Similar results were reported (van Rooyen, 2019). Coetzee (2015) reported that 60% and 80% of grain samples containing NIV and ZEA were detected in sorghum resonating with the findings that suggested NIV to be a predominant member of the *Fg*SC in local sorghum grain. Zearalenone with other mycotoxins such as aflatoxins and fumonisns are common in sorghum crops (Leslie and Logrieco, 2014). In the current study ZEA was recorded at low levels and it may be suggested that ZEA does not pose a local threat.

4.5 Conclusion

There is limited reviews reporting on the relationship between *Fg*SC and mycotoxins. In the current study a number of significant polynomial regression curves were recorded although these were based on the different cultivars. The relationship was significant in PAN8816 with all three the mycotoxins tested. The decline in mycotoxins in this cultivar with increasing *Fg*SC could suggest that this cultivar is less favourable for mycotoxin production in both grains and roots as reported by Coetzee (2015). Overall, mycotoxin levels in this study were low however, they should

be monitored to avoid accumulation that could lead to future health scares. There was a minimal effect of legume rotation systems on the translocation of mycotoxins from the roots to grains, however these still remain a favoured disease control strategy due to the numerous other benefits of prolonged exposure. The choice of legume remains challenging, as no single legume was connected to enhanced reduction in the disease parameters applied in the current study and effectiveness was dependent on the specific disease parameter in question. PAN8816 could potentially be an alternative sorghum cultivar to use in rotaion systems to reduce mycotoxin production as in this study did not seem to be affected by the presence of *Fg*SC.

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Table 26: Analysis of variance of mycotoxins observed in the roots and grains of the three sorghum cultivar used in six legume/sorghum based crop rotation system during 2016/17

Source	d.f	DON (Grains)				NIV (Grains)				ZEA (Grains)			
		s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr
Rep	2	1125.	562	0.16		27754178.	138770	2.43		0.3550	0.1775	2.58	
Rotation system (R)	5	20165	4033	1.13	0.406	45225600	904512	1.58	0.250	0.4950	0.0990	1.44	0.291
Residual (A)	10	35753.	3575	0.93		57100756	571007	1.08		0.6866	0.0687	0.56	
Cultivar ©	2	17808	8904	2.31	0.120	9799644	489982	0.92	0.410	0.1658	0.0829	0.68	0.517
R x C	10	42294	4229	1.10	0.401	76288622	762886	1.44	0.223	0.4309	0.0431	0.35	0.955
Residual (B)	24	92329.	3847.			127173333.	529888			2.9305	0.1221		
Total	53	209473				343342133				5.0640			
Source	**d.f**	**DON (Roots)**				**NIV (Roots)**				**ZEA (Roots)**			
		s.s	**m.s.**	**v.r**	**F.pr**	**s.s**	**m.s.**	**v.r**	**F.pr**	**s.s**	**m.s.**	**v.r**	**F.pr**
Rep	2	78	39	0.04		12194	6097	3.69		52.30	26.15	0.67	
Rotation system (R)	5	6002	1200	1.20	0.374	18536.	3707	2.24	0.130	150.61	30.12	0.78	0.588
Residual (A)	10	9970	997	0.51		16538	1654	0.18		387.58	38.76	1.29	
Cultivar ©	2	30775	15387	7.93	0.002	156959	78480	8.58	0.002	117.86	58.93	1.96	0.163
R x C	10	20677	2068	1.07	0.424	29586	2959	0.32	0.967	242.39	24.24	0.81	0.625
Residual (B)	24	46575	1941			219604.	9150			721.54	30.06		
Total	53	114077				453418				1672.29			

Table 27: Analysis of variance of mycotoxins observed in the roots and grains of three sorghum cultivars used in six legume/sorghum based crop rotation systems during 2017/18

Source	d.f	DON (Grains)				NIV (Grains)				ZEA (Grains)			
		s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr
Rep	2	79456	39728	58.30		137620.9	68810.5	27.35		1.5031	0.7515	1.02	
Year1[1]	5	5761	1152	1.69	0.224	5545.5	1109.1	0.44	0.811	2.9600	0.5920	0.81	0.57
Residual	10	6814	681	0.35		25156.5	2515.6	4.90		7.3477	0.7348	2.53	
Split[2]	1	744	744	0.38	0.550	115.9	115.9	0.23	0.643	1.0208	1.0208	3.51	0.08
Year1 x Split	5	9285	1857	0.95	0.486	4388.8	877.8	1.71	0.207	5.2985	1.0597	3.65	0.03
Residual	12	23537	1961	1.13		6161.4	513.4	1.06		3.4886	0.2907	0.58	
Cultivar	2	46937	23469	13.52	<.001	14004.4	7002.2	14.42	<.001	0.5601	0.2800	0.56	0.57
Year1 x Cultivar	10	15184	1518	0.87	0.562	4490.9	449.1	0.92	0.519	6.0871	0.6087	1.22	0.30
Split x Cultivar	2	397	199	0.11	0.892	1476.0	738.0	1.52	0.229	0.5535	0.2768	0.55	0.57
Year1 x Split x Cultivar	10	20483	2048	1.18	0.327	5983.7	598.4	1.23	0.296	4.1315	0.4132	0.83	0.60
Residual	48	83309	1736			23313.5	485.7			24.0120	0.5003		
Total	107	291907				228257.6				56.9629			
Source	d.f	DON (Roots)				NIV (Roots)				ZEA (Roots)			
		s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr	s.s	m.s.	v.r	F.pr
Rep	2	763.57	381.78	1.86		0.00	0.00	0.00	0	5.6519	2.8259	6.39	
Year1[1]	5	401.99	80.40	0.39	0.844	0.00	0.00	0.00	0.00	1.3836	0.2767	0.63	0.68
Residual	10	2057.84	205.78	5.69		0.00	0.00	0.00		4.4190	0.4419	1.28	
Split[2]	1	157.45	157.45	4.36	0.059	0.00	0.00	0.00	0.00	1.5099	1.5099	4.38	0.05
Year1 x Split	5	80.26	16.05	0.44	0.810	0.00	0.00	0.00	0.00	1.5224	0.3045	0.88	0.52
Residual	12	433.67	36.14	1.20		0.00	0.00	0.00		4.1344	0.3445	0.41	
Cultivar	2	76.28	38.14	1.27	0.291	0.00	0.00	0.00	0.00	27.5003	13.7502	16.31	<.00
Year1 x Cultivar	10	235.85	23.59	0.78	0.645	0.00	0.00	0.00	0.00	8.9913	0.8991	1.07	0.40
Split x Cultivar	2	14.36	7.18	0.24	0.789	0.00	0.00	0.00	0.00	0.2991	0.1496	0.18	0.83
Year1 x Split x Cultivar	10	642.38	64.24	2.13	0.040	0.00	0.00	0.00	0.00	3.0302	0.3030	0.36	0.95
Residual	48	1446.74	30.14			0.00	0.00			40.4671	0.8431		
Total	107	6310.38				0.00				98.9092			

Table 28: DON concentration (μg.kg^{-1}) in sorghum grains observed in three sorghum cultivars over a three-year legume/sorghum rotation system.

Cultivar	Rotation system[1]	Consecutive years of sorghum	2016/17		2017/18	
			Mycotoxin (ug.kg^{-1})	Cultivar Mean	Mycotoxin (ug.kg^{-1})	Cultivar Mean
NS5511	BA-SG-SG	2	103.1	113.4	0.0	11.92b
	CP-SG-SG	2	74.3		20.4	
	DB-SG-SG	2	123.6		14.6	
	FW-SG-SG	2	97.5		3.6	
	SB-SG-SG	2	148.1		16.9	
	SG-SG-SG	(2)	134		6.1	
	BA-BA-SG	1	-		18.8	
	CP-CP-SG	1	-		39.3	
	DB-DB-SG	1	-		0.0	
	FW-FW-SG	1	-		2.4	
	SB-SB-SG	1	-		20.9	
	SG-SG-SG	(1)	-		0.0	
PAN8706W	BA-SG-SG	2	40.1	69.6	24.8	62.22a
	CP-SG-SG	2	45		37.0	
	DB-SG-SG	2	78.8		130.5	
	FW-SG-SG	2	138.5		72.9	
	SB-SG-SG	2	79.8		69.0	
	SG-SG-SG	(2)	35.6		33.7	
	BA-BA-SG	1	-		83.0	
	CP-CP-SG	1	-		51.4	
	DB-DB-SG	1	-		39.4	
	FW-FW-SG	1	-		104.9	
	SB-SB-SG	1	-		61.6	
	SG-SG-SG	(1)	-		38.4	
PAN8816	BA-SG-SG	2	17.2	84.8	8.0	29.45b
	CP-SG-SG	2	140.2		28.6	
	DB-SG-SG	2	122.3		19.9	
	FW-SG-SG	2	71.7		3.9	
	SB-SG-SG	2	91.7		22.5	
	SG-SG-SG	(2)	65.9		62.0	
	BA-BA-SG	1	-		17.4	
	CP-CP-SG	1	-		27.4	
	DB-DB-SG	1	-		63.1	
	FW-FW-SG	1	-		37.4	
	SB-SB-SG	1	-		52.6	
	SG-SG-SG	(1)	-		10.5	
LSD $_{cultivar}$			-	-	-	*19.74*

Table 29: NIV concentration (µg.kg^{-1}) in sorghum grains observed in three sorghum cultivars over a three-year legume/sorghum rotation system.

Cultivar	Rotation system[1]	Consecutive years of sorghum	2016/17		2017/18	
			Mycotoxin (ug.kg^{-1})	Cultivar Mean	Mycotoxin (ug.kg^{-1})	Cultivar Mean
NS5511	BA-SG-SG	2	10533	9753.	86.9	92.5c
	CP-SG-SG	2	9780		92.8	
	DB-SG-SG	2	7733		90.8	
	FW-SG-SG	2	11453		89.9	
	SB-SG-SG	2	7527		86.7	
	SG-SG-SG	(2)	11493		96.1	
	BA-BA-SG	1	-		84.9	
	CP-CP-SG	1	-		95.1	
	DB-DB-SG	1	-		108.5	
	FW-FW-SG	1	-		87.5	
	SB-SB-SG	1	-		96.1	
	SG-SG-SG	(1)	-		94.5	
PAN8706W	BA-SG-SG	2	10700	9471	102.3	109.2b
	CP-SG-SG	2	10707		110.2	
	DB-SG-SG	2	9813		111.8	
	FW-SG-SG	2	6767		117.4	
	SB-SG-SG	2	8507		121.4	
	SG-SG-SG	(2)	10333		115.5	
	BA-BA-SG	1	-		117.7	
	CP-CP-SG	1	-		83.5	
	DB-DB-SG	1	-		113.1	
	FW-FW-SG	1	-		128.7	
	SB-SB-SG	1	-		88.1	
	SG-SG-SG	(1)	-		100.9	
PAN8816	BA-SG-SG	2	6247	8742	96.7	120.2a
	CP-SG-SG	2	9060		82.6	
	DB-SG-SG	2	9053		137.5	
	FW-SG-SG	2	9580		108.2	
	SB-SG-SG	2	7653		131.0	
	SG-SG-SG	(2)	10860		134.9	
	BA-BA-SG	1	-		119.0	
	CP-CP-SG	1	-		114.9	
	DB-DB-SG	1	-		143.0	
	FW-FW-SG	1	-		155.6	
	SB-SB-SG	1	-		118.2	
	SG-SG-SG	(1)	-		100.7	
LSD $_{cultivar}$			-	-	-	*10.44*

Table 30: ZEA concentration (µg.kg^{-1}) in sorghum grains observed in three sorghum cultivars over a three-year legume/sorghum rotation system.

Cultivar	Rotation system[1]	Consecutive years of sorghum	2016/17		2017/18	
			Mycotoxin (ug.kg^{-1})	Cultivar Mean	Mycotoxin (ug.kg^{-1})	Cultivar Mean
NS5511	BA-SG-SG	2	0.513	0.403	1.703	1.858
	CP-SG-SG	2	0.602		1.663	
	DB-SG-SG	2	0.401		3.997	
	FW-SG-SG	2	0.298		1.620	
	SB-SG-SG	2	0.488		1.707	
	SG-SG-SG	(2)	0.115		1.590	
	BA-BA-SG	1	-		1.620	
	CP-CP-SG	1	-		1.650	
	DB-DB-SG	1	-		1.670	
	FW-FW-SG	1	-		1.647	
	SB-SB-SG	1	-		1.737	
	SG-SG-SG	(1)	-		1.690	
PAN8706W	BA-SG-SG	2	0.226	0.268	2.243	1.681
	CP-SG-SG	2	0.28		1.900	
	DB-SG-SG	2	0.293		1.827	
	FW-SG-SG	2	0.292		1.207	
	SB-SG-SG	2	0.364		1.790	
	SG-SG-SG	(2)	0.151		1.207	
	BA-BA-SG	1	-		1.710	
	CP-CP-SG	1	-		1.707	
	DB-DB-SG	1	-		1.253	
	FW-FW-SG	1	-		1.733	
	SB-SB-SG	1	-		1.873	
	SG-SG-SG	(1)	-		1.727	
PAN8816	BA-SG-SG	2	0.101	0.325	1.653	1.768
	CP-SG-SG	2	0.403		2.430	
	DB-SG-SG	2	0.293		2.027	
	FW-SG-SG	2	0.251		1.640	
	SB-SG-SG	2	0.583		1.750	
	SG-SG-SG	(2)	0.318		1.640	
	BA-BA-SG	1	-		1.680	
	CP-CP-SG	1	-		1.677	
	DB-DB-SG	1	-		1.670	
	FW-FW-SG	1	-		1.683	
	SB-SB-SG	1	-		1.713	
	SG-SG-SG	(1)	-		1.653	
LSD cultivar			-	-	-	-

Table 30 (cont): Significant differences observed in ZEA concentration ($\mu g.kg^{-1}$) in sorghum grain during 2017/18 in legume/sorghum based rotation systems spanning three season where rotations with two consecutive seasons of sorghum were compared to rotations systems with one season of sorghum only.

Crop planted during[1]	**Two consecutive seasons of sorghum**	**One season of sorghum in crop**
BA	1.9 b	1.7b
CP	2.0ab	1.7b
DB	2.6a	1.5b
FW	1.5b	1.7b
SB	1.8b	1.8b
SG	1.5b	1.7b
*LSD(Year 1*seasons of sorghum)*	*0.7*	

[1] – BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow

Table 31: DON concentration (µg.kg^{-1}) in sorghum roots observed in three sorghum cultivars over a three-year legume/sorghum rotation system.

Cultivar	Rotation system[1]	Consecutive years of sorghum	2016/17		2017/18	
			Mycotoxin (ug.kg^{-1})	Cultivar Mean	Mycotoxin (ug.kg^{-1})	Cultivar Mean
NS5511	BA-SG-SG	2	0	22.4b	9.17	14.06
	CP-SG-SG	2	0		17.80	
	DB-SG-SG	2	25.6		19.10	
	FW-SG-SG	2	28.5		15.28	
	SB-SG-SG	2	50		9.67	
	SG-SG-SG	(2)	30.3		19.39	
	BA-BA-SG	1	-		14.84	
	CP-CP-SG	1	-		10.64	
	DB-DB-SG	1	-		17.40	
	FW-FW-SG	1	-		14.18	
	SB-SB-SG	1	-		12.29	
	SG-SG-SG	(1)	-		8.93	
PAN8706W	BA-SG-SG	2	120.6	70.1a	12.41	14.90
	CP-SG-SG	2	96.9		14.81	
	DB-SG-SG	2	33.4		21.20	
	FW-SG-SG	2	52.2		22.47	
	SB-SG-SG	2	68.5		11.44	
	SG-SG-SG	(2)	48.9		12.45	
	BA-BA-SG	1	-		14.15	
	CP-CP-SG	1	-		14.44	
	DB-DB-SG	1	-		9.22	
	FW-FW-SG	1	-		19.17	
	SB-SB-SG	1	-		16.07	
	SG-SG-SG	(1)	-		11.01	
PAN8816	BA-SG-SG	2	50.4	16.9b	18.97	16.10
	CP-SG-SG	2	9		14.21	
	DB-SG-SG	2	17.9		17.80	
	FW-SG-SG	2	19.9		22.53	
	SB-SG-SG	2	4		18.25	
	SG-SG-SG	(2)	0.4		15.17	
	BA-BA-SG	1	-		8.98	
	CP-CP-SG	1	-		12.31	
	DB-DB-SG	1	-		19.40	
	FW-FW-SG	1	-		14.47	
	SB-SB-SG	1	-		12.77	
	SG-SG-SG	(1)	-		18.39	
LSD $_{cultivar}$			-	*30.3*	-	-

Table 31 (cont): DON concentration (µg.kg^{-1}) in sorghum roots observed in three sorghum cultivars over a three-year legume/sorghum rotation system.

		Two consecutive seasons of SG			**One season of SG in a crop rotation system**		
	Split[2]	**1**			**2**		
Year 1[1]	**Cultivar[3]**	**NS5511**	**PAN8706W**	**PAN8816**	**NS5511**	**PAN8706W**	**PAN8816**
BA		9.17b	12.41ab	18.97ab	14.84ab	14.15ab	8.98b
CP		17.8ab	14.81ab	14.21ab	10.64ab	14.44ab	12.31ab
DB		19.1ab	21.2ab	17.8ab	17.4ab	9.22b	19.4ab
FW		15.28ab	22.47a	22.53a	14.18ab	19.17ab	14.47ab
SB		9.67ab	11.44ab	18.25ab	12.29ab	16.07ab	12.77ab
SG		19.39ab	12.45ab	15.17ab	8.93b	11.01ab	18.39ab
LSD *(year1*seasons of sorghum*cultivar)*					*12.994*		

[1]= BA = bambara; CP = cowpea; DB = dry bean; SB = soybean; SG = sorghum; FW = fallow
[2]= Two consecutive seasons of SG and One season of SG in a crop rotation system[3]= NS5511, PAN8816 and Pan8706W

Table 32: NIV concentration (µg.kg^{-1}) in sorghum roots observed in three sorghum cultivars over a three-year legume/sorghum rotation system.

Cultivar	Rotation system[1]	Consecutive years of sorghum	2016/17		2017/18	
			Mycotoxin (ug.kg^{-1})	Cultivar Mean	Mycotoxin (ug.kg^{-1})	Cultivar Mean
NS5511	BA-SG-SG	2	141	154.9b	0.00	0.00
	CP-SG-SG	2	140		0.00	
	DB-SG-SG	2	190		0.00	
	FW-SG-SG	2	179		0.00	
	SB-SG-SG	2	176		0.00	
	SG-SG-SG	(2)	103		0.00	
	BA-BA-SG	1	-		0.00	
	CP-CP-SG	1	-		0.00	
	DB-DB-SG	1	-		0.00	
	FW-FW-SG	1	-		0.00	
	SB-SB-SG	1	-		0.00	
	SG-SG-SG	(1)	-		0.00	
PAN8706W	BA-SG-SG	2	243	273.0a	0.00	0.00
	CP-SG-SG	2	291		0.00	
	DB-SG-SG	2	276		0.00	
	FW-SG-SG	2	267		0.00	
	SB-SG-SG	2	286		0.00	
	SG-SG-SG	(2)	275		0.00	
	BA-BA-SG	1	-		0.00	
	CP-CP-SG	1	-		0.00	
	DB-DB-SG	1	-		0.00	
	FW-FW-SG	1	-		0.00	
	SB-SB-SG	1	-		0.00	
	SG-SG-SG	(1)	-		0.00	
PAN8816	BA-SG-SG	2	246	265.2a	0.00	0.00
	CP-SG-SG	2	345		0.00	
	DB-SG-SG	2	282		0.00	
	FW-SG-SG	2	239		0.00	
	SB-SG-SG	2	230		0.00	
	SG-SG-SG	(2)	249		0.00	
	BA-BA-SG	1	-		0.00	
	CP-CP-SG	1	-		0.00	
	DB-DB-SG	1	-		0.00	
	FW-FW-SG	1	-		0.00	
	SB-SB-SG	1	-		0.00	
	SG-SG-SG	(1)	-		0.00	
LSD cultivar			-	*65.8*	-	-

Table 33: ZEA concentration (µg.kg^{-1}) in sorghum roots observed in three sorghum cultivars over a three-year legume/sorghum rotation system.

Cultivar	Rotation system[1]	Consecutive years of sorghum	2016/17		2017/18	
			Mycotoxin (ug.kg^{-1})	Cultivar Mean	Mycotoxin (ug.kg^{-1})	Cultivar Mean
NS5511	BA-SG-SG	2	0.05	0.21	0.178	0.2465b
	CP-SG-SG	2	0.02		0.000	
	DB-SG-SG	2	0.14		1.113	
	FW-SG-SG	2	0.03		0.000	
	SB-SG-SG	2	1.03		0.000	
	SG-SG-SG	(2)	0.01		0.970	
	BA-BA-SG	1	-		0.000	
	CP-CP-SG	1	-		0.000	
	DB-DB-SG	1	-		0.697	
	FW-FW-SG	1	-		0.000	
	SB-SB-SG	1	-		0.000	
	SG-SG-SG	(1)	-		0.000	
PAN8706W	BA-SG-SG	2	0.6	3.25	1.580	1.2665a
	CP-SG-SG	2	0.32		1.803	
	DB-SG-SG	2	4.54		0.987	
	FW-SG-SG	2	0.93		2.014	
	SB-SG-SG	2	13.11		1.309	
	SG-SG-SG	(2)	0.02		0.961	
	BA-BA-SG	1	-		1.115	
	CP-CP-SG	1	-		1.843	
	DB-DB-SG	1	-		0.667	
	FW-FW-SG	1	-		0.983	
	SB-SB-SG	1	-		1.253	
	SG-SG-SG	(1)	-		0.682	
PAN8816	BA-SG-SG	2	0.02	0.03	0.000	0.1519b
	CP-SG-SG	2	0.03		0.000	
	DB-SG-SG	2	0.05		1.113	
	FW-SG-SG	2	0.06		0.000	
	SB-SG-SG	2	0.02		0.090	
	SG-SG-SG	(2)	0.03		0.00	
	BA-BA-SG	1	-		0.620	
	CP-CP-SG	1	-		0.000	
	DB-DB-SG	1	-		0.000	
	FW-FW-SG	1	-		0.000	
	SB-SB-SG	1	-		0.000	
	SG-SG-SG	(1)	-		0.000	
LSD $_{cultivar}$			-	-	-	*0.4351*

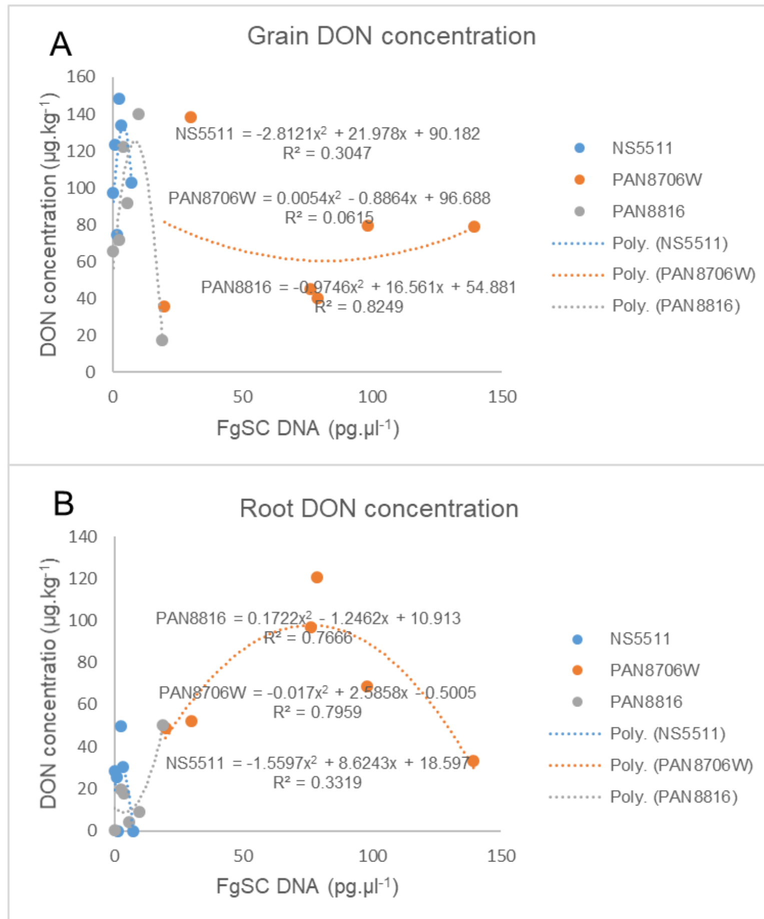

Figure 18: Relationship between *Fg*SC and DON concentration in grains (A) and roots (B) of sorghum cultivars during the 2016/17 season at Potchefstroom.

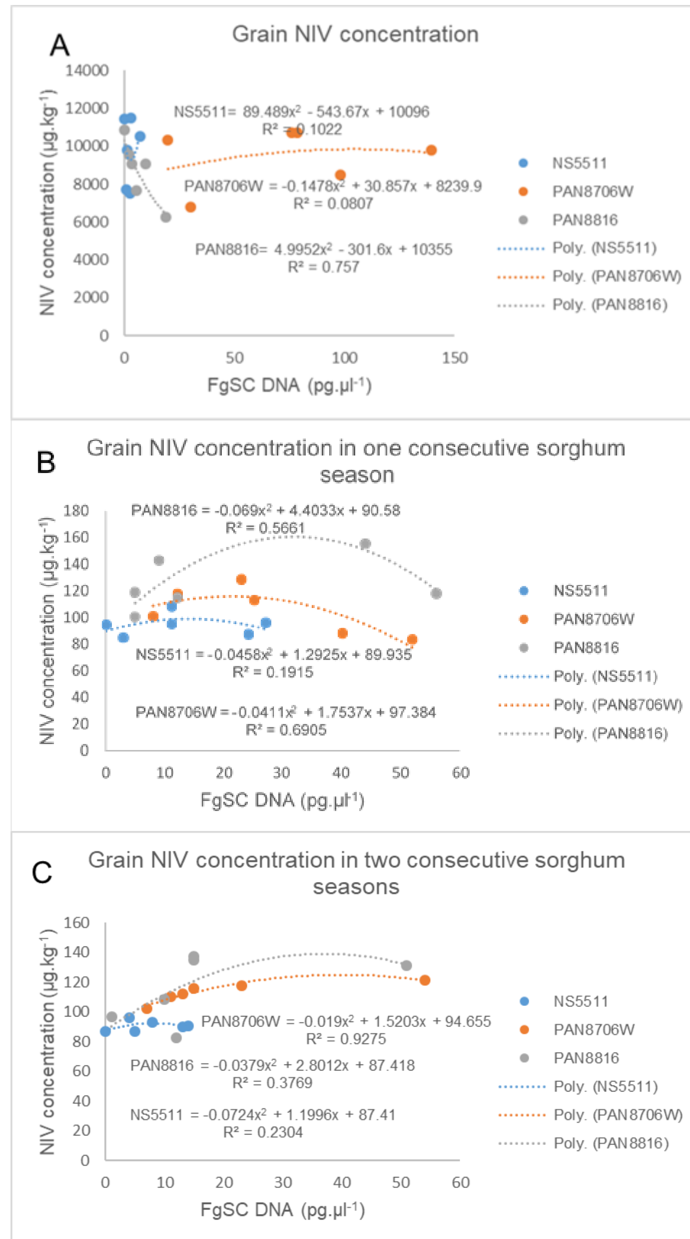

Figure 19: Relationships between *Fg*SC and NIV concentrations in grains (A) for 2016/17 and (B–C) of sorghum cultivars during the 2017/18 season at Potchefstroom.

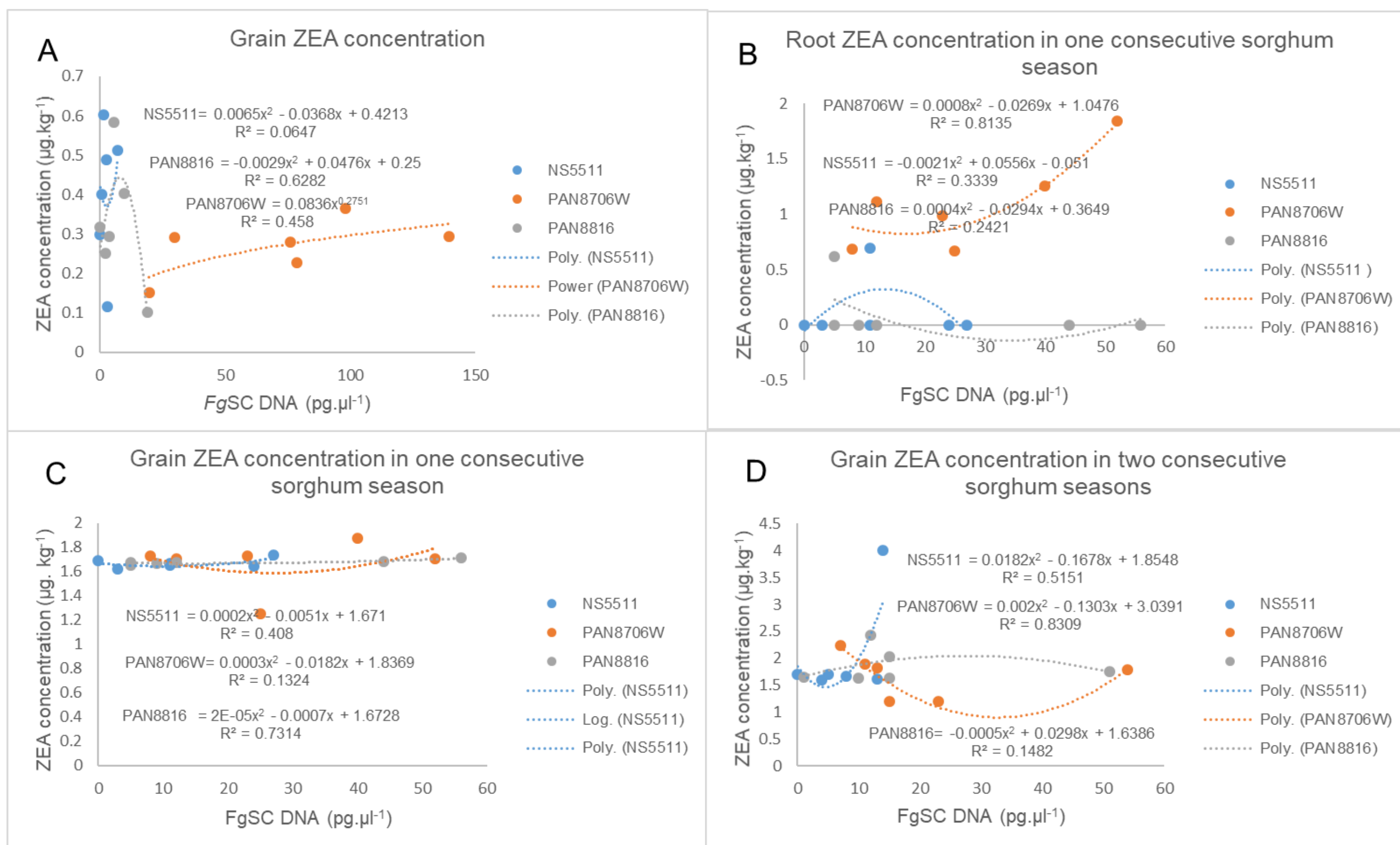

Figure 20: Relationships between *Fg*SC and ZEA concentrations in grains (A) and roots (B) and grains (C–D) of sorghum cultivars during the 2016/17 and 2017/18 season respectively at Potchefstroom.

Summary

Agricultural sustainability is increasingly becoming a challenge with the growing population and climate change. Contributing to this unsustainability are effects of drought, storms with intense rainfall and flooding leading to crops being washed away, increases in pests and diseases and mono-cropping. Modern ploughing, fertilizers and pesticides have also resulted in depletion of topsoil, especially organic content and associated bioactivity. Hence, there is an urgent need to investigate how sorghum root rot, grain mold pathogens and accumulation of mycotoxins can be managed using crop rotation, as it is a staple food in South Africa and many developing countries. Annual sorghum yield losses are estimated at 30% due to pests and diseases. Several species of soilborne fungi are known to cause root rot of sorghum, the most common being *Macrophomina phaseolina*, *Fusarium moniliforme (sensu lato)*, *Periconia circinata*, *Pythium* spp. and *Colletotrichum graminicola*. Sorghum grain mold (SGM) pathogens are also associated with mycotoxin production and include fungi such as *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Cladosporium* spp., *Colletotrichum* spp., *Curvularia* spp., *Phoma* spp. and *Fusarium* spp., including *F. moniliforme* (*sensu lato*) (*F. thapsinum* and *F. verticillioides*) and *F. graminearum.*

Maintenance of soil fertility is essential if sorghum production, nutrient value and market value are to be sustained. This study aimed at establishing 1) the effect of crop rotation systems with legumes (as they have the ability to fix nitrogen) on the nutrient status of soil and the relationship between soil health and root rot severity, 2) determining the colonization by *F. graminearum* Species Complex (*Fg*SC) on three sorghum cultivars and their response to grain mold pathogens and 3) determining whether legume based rotation systems assist in reducing deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) concentrations in sorghum grain and to establish whether the mycotoxin concentration in sorghum grain might be due to translocation of mycotoxins from the root system to sorghum grain.

Generally, this study showed that benefits from crop rotation systems were limited under the conditions of this study although a tendency of increased availability of nutrients and disease decline were achieved. Soil fertility improved with the successive seasons of legume based crop rotation, which emphasized the effect that crop rotation has on soil nutrients. Legumes are the best alternating crops with cereal because of their nitrogen fixing ability. In the current study only P and K were, however, detected in higher levels under bambara, cowpea and sorghum monoculture. The importance of cultivar choice was, in addition, emphasised as NS5511 gave the best yield in the absence of N nutrition and high visual root rots.

The highest visual root rot severity was recorded in NS5511, despite limited colonisation detected in the roots. This observation again emphasised the need to include more measures of disease evaluation, and not to rely on visual evaluation only, as this does not necessarily reflect internal infection. High ergosterol was detected in both roots and grains in 2017/18 season although a decline in common pathogens targeted using molecular techniques was detected. This suggest that there were other "unknown" pathogens colonising sorghum roots and could mean that these pathogens survived in the preceding season and multiplied with the favourable conditions. It is therefore imperative that a wider range of soil borne pathogens be investigated in future studies. Higher ergosterol detected in the grain under monoculture rotation system was expected due to the depletion of nutrients. Lower occurrence of ergosterol was detected under FW/SG/SG, BA/BA/SG and SB/SB/SG which showed the effect that crop rotation systems have in reducing inoculum in the soil. Three prominent soil borne root pathogens, that were detected in 2016/17, were also absent in the subsequent season, showing the impact of rotating crops. PAN8706W resulted in lower yield under low visual root rot rating conditions and high pathogen colonisation in both roots and grain, suggesting that it could be less tolerant to soil borne and grain mold pathogens.

Low levels of mycotoxins were detected in sorghum grain and roots in the current study suggesting that these do not pose a threat to consumers or the environment as levels detected were far below the permitted levels stipulated by EU regulations. Their accumulation should however be monitored in the future to avoid heath risks and deaths. PAN8706W showed a greater tendency towards susceptibility based on NIV, DON and ZEA concentrations observed in the grain and roots. Nivalenol occurred in greater quantities in PAN8816, but the decline in mycotoxins observed despite increasing *Fg*SC could suggest that this cultivar is less favourable for mycotoxin production in both grains and roots. NS5511 was not affected by the occurrence of mycotoxins suggesting it to be resistant to their production. The choice of legume remains challenging, as no single legume was connected to enhanced reduction in the disease parameters applied in the current study and effectiveness was dependent on the specific disease parameter being applied. PAN8816 could potentially be an alternative sorghum cultivar to use in rotation systems to reduce mycotoxin production as in this study these did not seem to be affected by the presence of *Fg*SC. Translocation of mycotoxins from the roots to the head has been regarded as a major challenge but this study indicated that very few mycotoxins were recorded in roots as opposed to the grains, relative to the level of *Fg*SC observed.