MONITORING FUSARIUM, GIBBERELLA AND DIPLODIA EAR ROTS AND ASSOCIATED MYCOTOXINS IN MAIZE GROWN UNDER DIFFERENT CROPPING SYSTEMS

by

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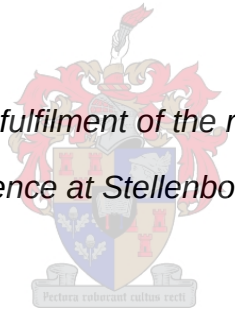

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SUMMARY

Maize ear rots represent a significant problem in most maize production areas resulting in reduced yield and quality due to visible fungal infection and mycotoxin contamination of maize grain. Mycotoxigenic fungi affecting cereal grains are particularly important for humans and animals as they pose food safety and security concerns. Increased maize productivity relies on integrated management strategies which include limiting soil erosion and water runoff. Therefore, agricultural practices that involve no-till and the retention of previous crop residues and/or cover crops are steadily increasing in maize production areas in South Africa. The relationship between no-till, the presence of crop residue in the field and maize ear rot disease severity and mycotoxin contamination is not well understood. The increase in the use of cropping systems that support the retention of crop residues in the field could have substantial impacts on maize production and food safety in South Africa. Adequate understanding of the role of agricultural practices in disease outbreaks can assist in enhancing management of maize ear rot pathogens.

In this study, the influence of different cropping systems on *F. verticillioides* and *F. graminearum* accumulation, Diplodia ear rot (DER) incidence as well as mycotoxin contamination in maize grain was determined. Cropping systems did not significantly affect *F. verticillioides* accumulation, zearalenone and nivalenol contamination in all the years of evaluation. *Fusarium graminearum* accumulation, DER incidence and deoxynivalenol contamination were, however, significantly affected in certain years when disease development was favoured. A survey to establish the effect of no-till and conventional tillage practices on Fusarium ear rot, Gibberella ear rot and DER in maize grain and resultant mycotoxin contamination in maize grain was also conducted in commercial farms in South Africa. Additionally, the survival of *F. graminearum* and *F. verticillioides* as well fumonisin contamination in crop residue samples collected from conservation and conventional tillage commercial farms in South Africa was also investigated. Tillage practices did not have an effect of fungal accumulation, disease incidence and mycotoxin contamination in maize grain. The results from this study indicate that under local conditions, conservational agricultural practices can be used without the potential risk of enhanced disease accumulation and mycotoxin contamination. *Fusarium graminearum* and *F. verticillioides* accumulation and traces of fumonisins were quantified from all analysed crop residues and did not differ between tillage practices. The recovery of these ear rot-causing fungi from crop residues is an indication of its potential to act as inoculum reservoirs for these fungi. Although the levels of fungal target DNA quantified from the crop residues was low, the fungi may reproduce, survive and infect subsequent hosts.

OPSOMMING

Mielie-kopvrotte verteenwoordig 'n groot probleem in die meeste mielie-produserende gebiede en lei tot verminderde opbrengs en kwaliteit as gevolg van sigbare swam-infeksie en mikotoksienbesmetting van mieliegraan. Mikotoksigeniese swamme wat kopvrot veroorsaak, is veral belangrik vir mense en diere omdat dit die veiligheid en voedselsekuriteit beïnvloed. Verhoogde produktiwiteit van mielies is moontlik met geïntegreerde bestuurstrategieë wat die beperking van grond-erosie en afloopwater insluit. Landboupraktyke wat geenbewerking en die behoud van vorige oesreste en/of dekgewasse behels, is stadig besig om in mielie-produserende gebiede in Suid-Afrika toe te neem. Die verhouding tussen grondbewerking, die teenwoordigheid van oesreste op die land, en mielie-kopvrotsiektes en mikotoksienbesmetting, word nie goed begryp nie. Die toename in die gebruik van verbouingsstelsels wat die behoud van oesreste op die land ondersteun, kan 'n aansienlike impak op mielieproduksie en voedselveiligheid in Suid-Afrika hê. Voldoende begrip vir die rol van landboupraktyke in die uitbreek van siektes kan help met verbeterde bestuur van mielie-kopvrotpatogene.

In hierdie studie is die invloed van verskillende verbouingsstelsels op *F. verticillioides* en *F. graminearum* opeenhoping, Diplodia kopvrot-voorkoms, asook mikotoksienbesmetting in mieliegraan vasgestel. Verbouingsstelsels het nie *F. verticillioides* opeenhoping en zearalenone en nivalenol besoedeling in al die jare van evaluering betekenisvol geaffekteer nie. *Fusarium graminearum* opeenhoping, DER voorkoms en deoxynivalenol besoedeling is egter betekenisvol beïnvloed in sekere jare wanneer siekte-ontwikkeling bevoordeel is. 'n Opname om die effek van geenbewerking en konvensionele bewerkingspraktyke op Fusarium kopvrot, Gibberella kopvrot en DER in mieliegraan, en die gevolglike mikotoksienbesmetting in mieliegraan vas te stel, is in kommersiële plase in Suid-Afrika uitgevoer. Daarbenewens is die voortbestaan van *F. graminearum* en *F. verticillioides*, sowel as fumonisien besoedeling in oesreste monsters wat vanaf bewaring en konvensionele bewerking kommersiële plase in Suid-Afrika versamel is, ook ondersoek. Bewerkingspraktyke het nie 'n effek op swam-opeenhoping, siekte-voorkoms en mikotoksienbesmetting in mieliegraan gehad nie. Die resultate van hierdie studie dui daarop dat onder plaaslike toestande, bewaringslandboupraktyke gebruik kan word sonder die potensiële risiko van verhoogde siekte-opeenhoping en mikotoksienbesmetting. *Fusarium graminearum* en *F. verticillioides* opeenhoping en spore van fumonisien is vanaf alle ontlede oesreste gekwantifiseer en het nie tussen bewerkingspraktyke verskil nie. Die terugkry van hierdie kopvrot-veroorsakende swamme vanaf oesreste is 'n aanduiding van hul potensiaal om as inokulumbron vir hierdie swamme op te tree. Hoewel die vlakke van swamteiken

DNA, gekwantifiseer vanaf die oesreste, laag was, kan die swamme oorleef, vermeeder en volgende gashere infekteer.

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CONTENTS

CHAPTER 1

Epidemiology and management of mycotoxigenic fungi associated with South African maize

INTRODUCTION

Maize is one of the most cultivated grain crops in the world and serves as a staple food commodity in many parts of South Africa (Fandohan *et al.*, 2003). It is also used for various purposes including animal feed and as an energy source (Shiferaw *et al.*, 2011). Maize production in South Africa is mainly threatened by excessive soil loss and poorly distributed, unreliable rainfall patterns (Lawrance *et al*., 1999). Maize is also prone to a large number of of fungal diseases including those caused by mycotoxigenic fungi. These are amongst the most important pathogens affecting maize due to their ability to produce toxic metabolites that threaten food and feed safety for both humans and animals and may result in serious economic repercussions (Munkvold, 2003a).

Ear rots are ranked as the third most important maize disease following maize streak virus and leaf blight (Mavhunga, 2013). Distribution of these ear rots is dependent on climatic and geographical conditions (Butron *et al.*, 2015). An estimated 15 different *Fusarium* species attack maize ears (Fandohan *et al.*, 2003) yet *Fusarium verticillioides* Saccardo Nirenberg [= *F. moniliforme* (Sheldon)] {teleomorph *G. fujikuroi* (Sawada) causing Fusarium ear rot (FER) and the *Fusarium graminearum* species complex (FGSC) (Schwabe*)* [Teleomorph *Gibberella zeae* (Schwein. Petch] causing Gibberella ear rot (GER) and *Stenocarpella maydis* (Berkeley) (Syn) (*Diplodia maydis*) (Berk.) (Sacc) causing Diplodia ear rot (DER) are the most economically important ear rot-causing fungi. Maize ear rots can significantly decrease yield, affect grain quality and limit the use of certain cultivars (Davis *et al.*, 1989). In addition to quantitative losses*, Fusarium* spp*.* can produce secondary metabolites known as mycotoxins upon infection. The most prevalent toxins produced by *F. verticillioides* upon maize infection are fumonisins whilst *F. graminearum* species complex produces zearalenone, deoxynivalenol and nivalenol (Wang *et al*., 2011). The production of mycotoxins by ear rot fungi has greater impacts than the disease alone would generally have (McMullen *et al.*, 1997).

Mycotoxins are potentially harmful to humans and animals when contaminated maize/maize-based products are consumed (Watson, 2007; Murillo-Williams and Munkvold, 2008; Popovski and Celar, 2012). Mycotoxins have been reported as the perpetrators for numerous health conditions in humans and livestock, resulting in liver and esophageal cancer amongst other numerous complications (Nedelnik *et al.,* 2012). Animals are unwarily exposed to mycotoxins through the contamination of feed and the mycotoxins are further

transferred to animal products, which in turn may expose humans who consume these contaminated food products (Njobeh *et al.*, 2012; Milani, 2013). Mycotoxin production can occur in the field, during harvesting, processing as well as during storage (Whitlow and Hagler, 2005). Furthermore, mycotoxins are a huge limiting factor in the global trade of food and feed (Steyn, 2011). The infection of maize by ear rot fungi and contamination by mycotoxins is governed by factors such as climate, plant stressors, geographical distribution as well as agricultural practices (Janse van Rensburg *et al.*, 2015b).

The severe lack of reliable resistant cultivars and other reliable control measures enhances the pressure in finding management strategies to prevent mycotoxin contamination of food commodities (Marocco *et al.*, 2008). Regulations for mycotoxin limits have been put into place in a number of countries, including South Africa. International agencies are striving for a standardised worldwide regulation (Anonymous, 2015). This is a difficult task, considering the commercial interests of different countries, economic and political effects which all play a crucial role.

Several strategies can be used to limit human and animal exposure to mycotoxins; this includes pre- and post-harvest methods. Most ear rot causing pathogens have the ability to survive on crop residues; elimination of crop residues through tillage and crop rotations is advisable (Munkvold, 2003b). Strict measures when handling and storing feed (Geraldo *et al.*, 2006), suspiciously mouldy products should not be fed to animals or consumed by humans (Oancea and Stoia, 2008). This review aims to look at the epidemiology of the fungal pathogens responsible for FER, GER and DER, their respective mycotoxins and current management practices. It will further look at conservation agricultural practices and how these cropping systems affect maize ear rot accumulation and mycotoxin contamination in maize.

MAIZE PRODUCTION IN SOUTH AFRICA

Maize (*Zea mays* L.) is the most commonly cultivated field crop in South Africa (Anonymous, 2013b) and is amongst the top three significant cereal crops in the world following wheat and rice (Verheye, 2010). South Africa is the second largest maize producing country in Africa, producing an estimated 10 - 12 million tons of maize annually (Anonymous, 2014). In South Africa, maize is produced under various agro-ecological conditions and is grown under commercial, small-scale and subsistence farming levels (Anonymous, 2014). Provinces in South Africa where maize is predominantly cultivated include the North West, Free State, KwaZulu-Natal and Mpumalanga. It serves as a staple food commodity (Flett, 2001), and forms an integral part of the diet of many Africans (Shabangu, 2009). Maize serves as a good source of carbohydrates, vitamin A and E, essential minerals and protein (Fandohan *et al.*, 2003) and functions as a multifunctional crop. About 60% of the maize produced in South

Africa is used for animal feed purposes with some for vegetable oil and vitamins (Shabangu, 2009). In Africa, 95% of maize serves mainly as a human food source (Fandohan *et al.*, 2003). Maize consumption has significantly increased in the past decade in Africa and demand is expected to increase with population growth (Anonymous, 2013b). Maize production is often threatened by both biotic and abiotic factors; biotic factors include rainfall, soil fertility and climate and abiotic factors are mainly pests and microorganisms such as bacteria, viruses and fungi (Fandohan *et al*., 2003). Maize ear rots, predominantly caused by mycotoxigenic fungi, are considered amongst the most important diseases affecting maize worldwide (Fandohan *et al*., 2003).

MAIZE EAR ROTS

Fusarium ear rot (FER)

The *Gibberella fujikuroi species* complex (GFSC) is made up of a number of organisms including these agriculturally important species, *Fusarium verticillioides (*Sacc.*)* Nirenberg (formerly *F. moniliforme Sheldon*) (*Gibberella moniliformis* Wineland), *Fusarium proliferatum* (Matsushima) Nirenberg (teleomorph *G. intermedia*), and *Fusarium subglutinans* (Wollenw. & Reink) Nelson *et al*. (Burlakoti and Burlakoti, 2015). Fusarium ear rot of maize is caused by *F. verticillioides*, *F. proliferatum* and *F. subglutinans* (Leslie and Summerell, 2006). However, *F. verticillioides* has been identified as the main causal organism of FER in maize in many parts of the world including Africa and is endemic to most maize producing areas (Boutigny *et al*., 2012; Balconi *et al.*, 2014). Within the four phylogenetically distinct lineages, *Fusarium verticillioides* forms part of the African clade (Kvas *et al.*, 2009). *Fusarium verticillioides* is widespread in tropical and subtropical regions, and is a major concern for maize growers (Nayaka *et al.*, 2009). Literature pertaining to *Fusarium verticillioides* suggests that it is the most prevalent fungus isolated from maize (Boutigny *et al.*, 2012). It is responsible for an estimated 60% loss in maize worldwide (Marocco *et al*., 2009). The amount of losses due to FER is highly dependent on environmental conditions (Dragich and Nelson, 2014). There have been reports of *F. verticillioides* potentially inflicting opportunistic infections in humans (Hennequin *et al.*, 1997). Furthermore, *F. verticillioides* is able to produce an array of toxic secondary metabolites known as fusaric acids, fusarins and fumonisins, of which fumonisins are the most widespread and well-studied (Glenn, 2007).

Taxonomy: Fusarium verticillioides was previously clustered with *F. proliferatum* due to the high degree of morphological similarities between the two species and these were later distinguished by the production of false conidia heads by *F. proliferatum* (Glenn, 2007). *Fusarium verticillioides* belongs to teleomorph *Gibberella moniliformis* and *Gibberella intermedia*, respectively (Leslie and Summerell, 2006). *Fusarium verticillioides* has

undergone a number of taxonomic reviews and Nirenberg allocated section *Liseola* (Rodriguez-Brljevich, 2008). *Fusarium verticillioides* is an ascomycetous fungus that produces microconidia in long chains from mono- or polyphialides which distinguishes it from other *Fusarium* species (Pitt and Hocking, 2009).

Disease cycle and Epidemiology: Fusarium verticillioides (Sacc) is a filamentous fungus that produces micro- and macro-conidia (Burnman, 2009). Microconidia are believed to serve as primary inoculum for infections. The most common source of inoculum is airborne conidia that infect silk or damaged kernels (Ono *et al.*, 2011). Insects and birds play a role in the occurrence of FER. This is a result of the said animals feeding and damaging the cob, making it easily accessible to *F. verticillioides* inoculum. The corn borer can also carry spores over from one plant to another (Czembor, 2010). *Fusarium verticillioides* produces hyphae that allow for prolonged survival between host species, it is able to infect and overwinter saprophytically on crop stalk residues from previous planting seasons (Munkvold, 2003b). Crop residues have been identified as a major source of primary inoculum for *F. verticillioides* maize grain infections (Ono *et al*., 2011). One of the major factors influencing ear rot infections is climate (Popovski and Celar, 2012). FER is normally associated with warm and dry weather and infect during the maize grain fill developmental stage (Munkvold, 2003a; Marocco *et al.*, 2008).

Symptoms: Characteristic symptoms associated with FER include white, pale pink to purple mould; infected kernels can also be identified by white streaking that appear as 'starbursts' on the surface (Das, 2014). White to pink mould growth can also be observed along stalk borer feeding channels (Flett *et al*., 1996). FER infection is randomly distributed amongst kernels (Munkvold, 2003a). *Fusarium verticillioides* can infect, cause disease and produce fumonisins without displaying symptoms (Marocco *et al.*, 2008). Infection incidence ranges from 50-100%, most of which are symptomless infections (Marocco *et al.*, 2009). These symptomless infections are of great concern as maize appearing to be of good quality is likely to be contaminated with fumonisins (Glenn, 2007).

Gibberella ear rot (GER)

The *Fusarium graminearum* species complex (FGSC) is responsible for blight, stalk and ear rots on small grain cereals such as wheat, barley, oats, triticale and maize (Turkington *et al.*, 2014). There have been many taxonomic classifications of the FGSC. It was initially grouped into two groups based on its ability to form homothallic perithecia in nature (Bowden and Leslie, 1999; Popovski and Celar, 2012). The first group (*F. graminearum* Group 1) comprised of soil borne pathogens responsible for causing crown and foot rot diseases, and

a second group (*F. graminearum* Group 2) comprised of ear rot causing fungi (Marasas *et al.*, 1977). There are currently 16 genetically and geographically different species belonging to the FGSC (O'Donnell *et al*., 2004, 2008; Starkey *et al*., 2007; Yli-Mattila *et al*., 2009; Sarver *et al*., 2011; Aoki *et al*., 2012). The phylogenetically distinct lineages were identified as: *F. austroamericanum* (lineage 1), *F. meridionale* (lineage 2), *F. boothii* (lineage 3), *F. mesoamericanum* (lineage 4), *F. acacia-mearnsii* (lineage 5), *F. asiaticum* (lineage 6), *F. graminearum* (lineage 7), *F. cortaderiae* (lineage 8), *F. brasilicum* (lineage 9), and *F. gerlachii, F. louisianense, F. ussurianum, F. nepalense, F. vorosii, F. austroamericanum* (no lineage numbers) (Goswami and Kistler, 2004; Glenn, 2007). The occurrence of some of these species is restricted to certain geographical areas. The common species occurring in South Africa are *F. boothii, F. acacia-mearnsii, F. meridionale, F. cortaderiae* and *F. graminearum sensu stricto (*Boutigny *et al*., 2011; Mavhunga, 2013*). Fusarium graminearum* has been labelled as the main causal agent for GER in many parts of the world (Sutton, 1982; Munkvold, 2003a), however; Boutigny *et al*. (2011) found *F. boothii* to be the most prominent causal organism for GER in South African maize samples. Although the FGSC results in major yield losses, the primary effects are contamination of grain with mycotoxins (Trail, 2009*).* The FGSC is associated with the synthesis of type B trichothecenes, deoxynivalenol and nivalenol as well as the mycotoxin zearalenone (Presello *et al.*, 2005; Trail, 2009). FGSC strains are usually associated with the production of one of the three trichothecene chemotypes, 3-acetyldeoxynivalenol chemotype (3-ADON), 15-acetyldeoxynivalenol chemotype (15-ADON) or 4-nivalenol chemotype (4-NIV) (Qui *et al*., 2016).

Taxonomy: The asexual stage (anamorph) conidia can be described morphologically as colourless, and curved (Das, 2014). Only macroconidia, which are multi-celled and have different shapes are produced during the asexual stage. The sexual stage (Teleomorph) comprises of *Gibberella zeae*, an ascomycete characterised by the production of ascospores (Dragich and Nelson, 2014). *Fusarium graminearum* is classified as: Superkingdom: Eukaryota, Kingdom: Fungi, Phylum: ascomycota, Subphylum: *Pezizomycotina*; Class: *Sordariomycetidae*; Subclass: *Hypocreomycetidae*; Order: *Hypocreales*; Family: *Nectriaceae*; Genus: *Gibberella* (Goswami and Kistler, 2004).

Disease cycle and epidemiology: The FGSC are homothallic fungi that contain both the sexual and asexual reproduction stages (Turkington *et al.*, 2014). During sexual reproduction in warm, wet conditions, *Gibberella zeae* produces ascospores that are later released as conidia from perithecia (Turkington *et al.,* 2014) to carry on the disease cycle. *G. zeae* spores are primarily dispersed by wind and rain (Dragich and Nelson, 2014).

The asexual stage produces macroconidia, which serve as secondary inoculum and is produced inside the infected plant (Agrios, 1994). Macroconidia can be described as hyaline and canoe-shaped spores (Agrios, 1994). Inoculum for further dispersal in the field is produced on crop residues or infected seed (Dragich and Nelson, 2014). FGSC sporulates on crop residues as a saprophyte, survival is favoured by crop residues that do not degrade rapidly (Champeil *et al*., 2004). Other possible sources of inoculum may be alternate plant hosts, grasses and weeds (Champeil *et al*., 2004). FGSC initially infects the silks of the maize ear and later progresses from the ear tip to the base of the ear. FGSC perithecial and ascospore production is favoured by warm wet conditions and optimal temperatures i.e. 29°C and 25°- 28°C respectively (Doohan *et al.*, 2003). GER dominate in cooler areas with high precipitation during the growing season (Munkvold, 2003a). Disease development is favoured by temperatures between 18°- 21°C (Woloshuk and Wise, 2010).

Symptoms: GER can be identified by the colour of the fungal mycelia produced on the diseased maize ear (Dragich and Nelson, 2014), which begins initially as white mould that turns pink/red with disease progression (Mesterhazy *et al.*, 2012) and normally infects from the tip of the ear and ramifies towards the base. FGSC rarely infects the entire ear, however, if infection occurs through a wound, the infection moves towards the tip of the ear before it covers the base (Mesterhazy *et al.*, 2012). The teleomorph (*G. zeae*) associated brown perithecia can also be visible on ear and kernel shanks of infected maize (Logrieco *et al.*, 2002). Severe or early infections can result in the entire ear being colonised by mycelia, kernel rotting and the husks become attached to kernels on the ears (Agrios, 1994; Das, 2014).

Diplodia ear rot (DER)

DER is regarded as possibly the most destructive ear rot disease occurring on maize production areas worldwide (Rogers *et al.*, 2014). It is caused by the fungal species *Stenocarpella maydis* (Berkeley) (Syn) (*Diplodia maydis*) (Berk.) (Sacc) and *Stenocarpella macrospora* (Earle) B Sutton (Wicklow *et al.*, 2011) and maize has been identified as the only known commercial host for *S. maydis* (Masango *et al.*, 2015). *Stenocarpella maydis* is more prevalent than *S. macrospora* (Romero and Wise, 2015). Van Rensburg and Ferreira (1997) reported that in South Africa, diplodia epidemics have been observed during the 1986/87, 1987/1988 and 1988/1989 seasons. These epidemics are normally observed when early season drought and late season rainfall conditions prevail (Rossouw *et al.*, 2009). Losses are primarily due to grain yield and quality reductions due to fungal infection. Epidemics in the 1980s have resulted in 30-60% yield losses in South Africa (van Rensburg and Ferreira, 1997). *Stenocarpella maydis* causes between 5-37% reductions in germination

of infected maize seeds (Wicklow *et al.*, 2011). *Stenocarpella maydis* can also synthesise a number of mycotoxins of which diplodiotoxin is the most important (Flett *et al*., 1998). Diplodiotoxin allegedly causes diplodiosis, a disease that affects the nervous system observed in cattle and sheep (Odriozola *et al.,* 2005; Masango *et al.*, 2015). Symptoms of diplodiosis may include but are not limited to paralysis, ataxia and eventually; death (Rabie *et al.*, 1985). It has been reported that diplodiosis is considered the sixth most important mycotoxicoses of sheep and cattle in South Africa (Kellerman *et al.*, 1996). Other *S. maydis* metabolites have been isolated namely, diplonine, chaetoglobosins which were found to induce symptoms similar to diplodiosis when administered to animals (Rabie *et al*., 1985), and dipmatol, for which no reports on toxicity exist to date (Rabie *et al*., 1985; Masango *et al.*, 2015).

Taxonomy: Stenocarpella maydis previously known as *Diplodia maydis*, was classified as *Stenocarpella* based on its conidiogenesis. Conidiogenous cells are enteroblastic, phialidic, determinate, discrete, and cylindrical (Masango *et al*., 2015). Scolecospores are hyaline, aseptate cells formed in pycnidia on infected kernels and are extruded in cirrhi (Rossouw *et al.*, 2009). According to literature it possesses no sexual (teleomorph) state (Masango *et al.*, 2015).

Disease cycle and epidemiology: During winter seasons *S. maydis* overwinters as conidia in pycnidia on maize crop residues/debris (Masango, *et al.*, 2015), during warm/wet conditions in spring and summer, the fungus produces flask shaped asexual fruiting bodies called pycnidia which produce conidia. Conidia are formed from the pycnidia cell walls and are exuded in a cirrhus under warm wet weather conditions. Conidia are primarily dispersed by wind and rain. *Stenocarpella maydis* infects during the first three weeks of silking (Rossouw *et al.*, 2009). Conidia infect ears through ear tips and the ear leaf shank and symptoms may take 3 to 4 weeks before they can become visible. Seeds infected with *Stenocarpella maydis* often fail to germinate hence discouraging seed-borne disease infections.

Symptoms: Visible symptoms normally result from ramification of mycelia from the base to the tip of the ear. DER is characterised by a thick white to grey mould on the maize ear, with black pycnidia visible on a cross section of an infected ear at the base of the kernels. When early infection take place and conditions are favourable for infection and fungal ramification, the entire ear may be affected, in this case, a grey-brownish colour with a shrunken rotten maize ear is observed. Timing of the infection and favourable climatic conditions influence DER development, late infections show no severe symptoms but white mould can be visible when the ear is carefully inspected at the ear base where it makes contact with the stele or if

broken in cross section, this is also known as hidden diplodia (Masango *et al*., 2015). If infection occurs during the blistering stage, kernel formation can be completely prevented (Rossouw *et al*., 2009).

MYCOTOXINS

Mycotoxins can be described as low molecular weight compounds produced as secondary metabolites by toxigenic fungal strains (Njobeh *et al.*, 2012). Mycotoxins are difficult to classify as they possess diverse chemical structures and biosynthetic origins (Bennett and Klich, 2003). Naturally occurring mycotoxins have been found to be more toxic than pure chemically synthesised mycotoxins, this could be due to synergistic interactions in nature amongst these mycotoxins (Whitlow and Hagler, 2005). Both *F. verticillioides* and *F. graminearum* produce mycotoxins and can occur in one field at the same time (Dragich and Nelson, 2007).

Mycotoxins have been a major food safety problem for many years, an estimated 25% of the world's food crops are affected by mycotoxins (Logrieco *et al.*, 2002). More than 300 mycotoxins are known (Oancea and Stoia, 2008; Steyn, 2011) with more than 100 mycotoxins identified in South Africa (Mavhunga, 2013). Although many mycotoxins exist, only the potentially harmful and disease causing mycotoxins are of health and economic importance (Morgavi and Riley, 2007; Zain, 2011). Aflatoxins, trichothecenes and fumonisins, are of particular interest (Binder *et al.*, 2007). Maize serves as a good substrate for mycotoxin production due to its high carbohydrate content that provides the necessary carbon precursors for synthesis (Moturi, 2008).

Toxins are a devastating health hazard and this can be dated back to human ergotism in Europe, alimentary toxic aleukia in Russia, and acute aflatoxicoses in Africa (Steyn, 2011). Another example of acute animal disease outbreaks due to mycotoxicoses is the turkey X disease that resulted in the mortality of some 100 000 turkeys, 14000 ducklings in the early 1960s in England (Cole, 1986; Whitlow and Hagler, 2005). This was before the first mycotoxin was identified and sparked large interest in mycotoxin research. The adverse effects on humans and animals are usually chronic, i.e. low dose exposure for prolonged periods (WHO, 2006). As a result, diseases such as cancer, kidney failure, and lethargy immune system suppression may occur (Dragich and Nelson, 2014).

Apart from the apparent health factors, mycotoxin contamination also poses serious economic losses such as reduced exports and reduced animal feed quality which are crucial for developing countries (Degraeve *et al.*, 2016). The mycotoxins produced by ear rot fungi such as *F. verticillioides* and *F. graminearum* are considered amongst the most important due to the high levels of consumption of maize in especially African countries.

Fumonisins

Fumonisins are polyketide derived secondary metabolites synthesised by the *FUM* gene cluster (Sagaram *et al.*, 2006) and were fully described and characterised in 1988 in South Africa (Gelderblom *et al*., 1988). Fumonisins are synthesised by at least 13 different *Fusarium* spp. from the *Liseola* section (Rheeder *et al*., 2002; Bennett and Klich, 2003; Picot *et al.*, 2010). *Fusarium verticillioides* is the most prevalent and well documented fumonisin producer with almost all strains being able to synthesise fumonisins (Marocco *et al.*, 2009). Some of the highest fumonisin producers of *F. verticillioides* have been reported in South Africa (Rheeder *et al.*, 2002). Fumonisins are believed to be formed through the condensation of amino acid alanine into an acetate derived precursor (Bennett and Klich, 2003). There are currently 28 fumonisin analogues known to date (Bennett and Klich, 2003). As opposed to other mycotoxins, fumonisins have a longer structure, similar to that of sphinganine (Zain, 2011). Some of their effects include inhibition of sphingolipid synthesis. Sphingolipid forms part in a number of signalling pathways essential for cell membrane functions (Wang *et al.*, 1991). Fumonisins are classified according to toxicity as analogues A, B, C and G. Analogues B are the most prominent with fumonisin B_1 predominantly occurring in maize (Flett, 2001), and is considered to have cancer causing properties hence classified as a B_1 carcinogen.

Fumonisins have been shown to be phytotoxic to maize although this finding is not commonly accepted. It had a direct effect on root growth, root morphology and other aspects of maize seedling disease (Williams *et al*., 2007; Arias *et al*., 2012) as well as to maize callus in culture (van Asch *et al*., 1992). The production of fumonisin was shown to be essential for the development of foliar disease symptoms on maize seedlings (Glenn *et al*., 2008) while Desjardins *et al*. (1995) concluded that fumonisins may play a role in virulence but is not essential for pathogenicity to maize seedlings. Non-fumonisin producing mutants were, however, shown to be as virulent on maize ears as their wild-type, fumonisin-producing strains (Proctor *et al*., 2002). The conflicting literature indicates more research is required to clarify the role of this toxin in fungal infection.

Fumonisin production is dependent on warm dry weather and high humidity during the grain filling stage (Munkvold, 2003a; Marocco *et al.*, 2009) with the optimum production temperature ranging from 20-30°C (Munkvold, 2003a). Drought stress, insect damage, presence of other fungal diseases on the affected grain and host susceptibility all have an effect on FER infection and subsequent fumonisin production (Parsons and Munkvold, 2012). High oxygen tension and low pH in kernels can also enhance fumonisin production (Miller, 2001). Fumonisins have been associated with human oesophageal cancer in the rural Transkei region of the Eastern Cape in South Africa (Marasas *et al.*, 1981), China and Italy (Sopterean and Puia, 2012). Fumonisins have also been linked to equine

leukoencephalomalacia (ELEM) in horses, which is the softening of the brain white matter. This disease dates back to as early as 1891 (Haliburton and Buck, 1986) and has been reported in South Africa, Egypt, China, Greece, Germany, and South America (Haliburton and Buck, 1986). Pulmonary oedema in pigs has also been attributed to consumption of fumonisin contaminated feed (Richard, 2007; Dragich and Nelson, 2014). In terms of the amended foodstuffs, cosmetics and disinfectants Act 54 of 1972, fumonisin legislation guidelines have been set at 2 ppm for maize flour or maize meal ready for consumption and 4 ppm for maize products intended for further processing in South Africa.

Zearalenone

Zearalenone was one of the first mycotoxins to be discovered and was also referred to as a F-2 toxin (Whitlow and Hagler, 2005). It is a non-steroidal compound that occurs in a wide range of cereal and cereal derived food and feed (Qui *et al.*, 2016). Zearalenone is biosynthesized by a series of naturally occurring *Fusarium* spp. in maize. Amongst those are *F. graminearum*, *F. culmorum (*W.G. Smith*) Sacc*., *F. cerealis* (Cooke) Sacc. and *F. semitectum* (Zinedine *et al.,* 2007). Zearalenone is a phenolic resorcyclic acid lactone with the chemical structure, 6- [10-Hydroxy-6- oxo-*trans*-1-undecenyl]-B-resorcyclic acid lactone (Zinedine *et al*, 2007, Whitlow and Hagler, 2005). It consists of five different metabolites, α-Zearalenol (α-ZEA), β- Zearalenol (β- ZEA), α- Zearalenol (α –ZAL), β-Zearalenol (β-ZAL), which are produced from the biotransformation of zearalenone after ingestion by animals (Zinedine *et al.*, 2007; Hueza *et al*., 2014).

Zearalenone has been reported to have the least phytotoxic abilities when compared to other mycotoxins (Ismaiel and Papenbrock, 2015). Low levels (5 µg ml^{-1}) of zearalenone inhibited maize root and shoot elongation, this was however stimulated by high levels (10 and 25 µg ml^{-1}) of zearalenone (McLean, 1995; Ismaiel and Papenbrock, 2015). The chemical structure of zearalenone resembles that of the estrogen hormone structure and therefore contains estrogenic properties and can be linked to a number of reproduction problems such as breeding, hormonal imbalances and fecundity in animals (Whitlow and Hagler, 2005; Zinedine *et al*, 2007). Zearalenone interacts with estrogen receptors and initiates selective RNA transcription which results in the accumulation of excessive water and reduced lipid content in the muscles (Agag, 2004). Zearalenone also affects endocrine function (Uegaki *et al.*, 2015), it has also been linked to ‘scabby’ grain toxicoses in the USA, China, Japan and Australia with symptoms ranging from nausea to diarrhoea (Zinedine *et al.*, 2007). The European Union (EU) maximum tolerable limits for zearalenone in unprocessed maize have been set at 0.2 ppm and 0.05 ppm in maize based snacks and breakfast cereals (Hueza *et al*., 2014).

Zearalenone is normally produced during the growing phase of the grain when the fungus initially infects during periods of heavy rainfall (Geraldo *et al.*, 2006). Optimum temperature for production ranges between 12-14°C, production can occur at temperatures lower than 10°C (Agag, 2004). *Fusarium graminearum* is able to simultaneously synthesize zearalenone and other mycotoxins such as deoxynivalenol and nivalenol in the same host (Agag, 2004).

Deoxynivalenol

Deoxynivalenol in maize is produced by *F. graminearum* and *F. boothii* and is classified as a type B tricothecene (Sampietro *et al.*, 2013; Anonymous, 2013c). This mycotoxin occurs in almost all cereal crops including wheat, maize, barley, rye and sorghum. Deoxynivalenol chemotype strains of the FGSC are classified into two types, 3-DON and 15-DON (Popovski and Celar, 2012). Fungi that produce 3-DON have been identified as more toxic than 15-DON chemotype (Pestka, 2010). Boutigny *et al*. (2011) found GER in South Africa to be solely associated with the 15-DON chemotype. Deoxynivalenol has received very little attention in Africa (Milani, 2013) with only one report of deoxynivalenol in Cameroon documented in maize (Milani, 2013). Deoxynivalenol is not as toxic as the rest of the trichothecene mycotoxins (Wegulo, 2012). It is water soluble, heat stable and one of the most common mycotoxins found in feed worldwide (Willyerd *et al.*, 2010).

Deoxynivalenol has been shown to play a role in pathogenesis. *Fusarium graminearum* strains that lacked deoxynivalenol producing capabilities were unable to cause as much disease as the deoxynivalenol producing strains (Munkvold, 2003a). Demeke *et al.* (2010) found a positive correlation between fungal biomass and deoxynivalenol content in wheat grain. The phytotoxic effect of DON was shown in wheat seedlings, coleoptile segments, anther-derived callus and anther-derived embryos (Bruins *et al*., 1993). Adams and Hart (1989), in contrast, reported that DON was not a virulence or pathogenicity factor for *F. graminearum* on maize, following virulence trials with non-toxic protoplast fusion *F. graminearum* strains.

Deoxynivalenol is commonly known as vomitoxin because it induces vomiting, feed refusal and decreased weight in pigs (Reid *et al.*, 2001). Low dosage exposure to deoxynivalenol may cause skin irritations, lack of appetite and nausea (Sopterean and Puia, 2012). Long term exposure may result in weight gain suppression, necrosis of the digestive tract and altered nutritional efficiency and decreased performance (Anonymous, 2013c).

Deoxynivalenol has also been found to inhibit protein synthesis and suppress the immune system in eukaryotes (Dragich and Nelson, 2014), thus increasing vulnerability to other diseases affecting animals (Pestka and Bondy, 1990). In humans, symptoms varying from nausea, diarrhoea, dizziness, fever, and headaches have been reported resulting from

large consumptions of deoxynivalenol contaminated food (Anonymous, 2007). In 1987, deoxynivalenol was linked to food borne human mycotoxicoses in India from consumption of contaminated bread (Reddy and Raghavender, 2008). In terms of the amended foodstuffs, cosmetics and disinfectants Act 54 of 1972, recent restrictions have been set for deoxynivalenol allowable limits in South Africa, cereal grains intended for further processing must not exceed 2 ppm and flour, semolina and flakes ready for human consumption must contain levels below 1 ppm. Deoxynivalenol production is largely influenced by plant stress, temperature, moisture content and relative humidity (Wegulo, 2012).

Nivalenol

Nivalenol (2, 13-epoxy-3, 4, 7, 15-tetrahydroxytrichothec-9-en-8-one) is a type B trichothecene, with a high structural similarity to deoxynivalenol. A single oxygen atom is responsible for the slight structural difference (Scudamore *et al.*, 2008; Nagashima and Nakagawa, 2014). It is mainly produced by *Fusarium cerealis* and *Fusarium poae* whilst *Fusarium culmorum* and *F. graminearum* are also low scale producers. Nivalenol was initially isolated from *Fusarium nivale (*Anonymous, 2010*).* Nivalenol is at least ten times more toxic to humans and animals than deoxynivalenol (Sopterean and Puia, 2012; Sampietro *et al.*, 2013), but is produced on a much lower scale and hence poorly studied (Cheat *et al.*, 2016). Information on nivalenol phytotoxic abilities is scanty, as plant systems require genomic knowledge to prepare nivalenol sensitivity (Suzuki and Iwahashi, 2014). It has however been suggested that deoxynivalenol may be more phytotoxic than nivalenol (Suzuki and Iwahashi, 2014). Effects of nivalenol in animals include decreased appetite, weight gain suppression, and immune system defects (Anonymous, 2010). Nivalenol is soluble in a wide range of polar organic solvents such as acetonitrile, methanol, ethanol, chloroform and ethyl acetate (Malachova *et al.*, 2014).

MANAGEMENT STRATEGIES

Controlling maize ear rots and mycotoxins requires a comprehensive control strategy and control should be integrated at all production and storage practices (Munkvold, 2003b). Numerous pre-and postharvest management strategies have been evaluated for reducing ear rot pathogens and their associated mycotoxins with varying degrees of effectiveness.

Preharvest strategies

Cultural practices: Crop rotation and tillage practices are extremely crucial and the most feasible management practice for ear rots of maize (Flett *et al*., 2001; Romero and Wise, 2015). Repeated cultivation of maize increases ear rot outbreaks as the main source of inoculum is plant residues. Rotations with two different crops can be efficient in eliminating

disease incidence (Munkvold, 2003b). Crop rotation, crop residue management, appropriate planting and harvest dates may help mitigate fungal inoculum necessary for fungal colonisation and mycotoxin production (Champeil *et al*., 2004; Zain, 2011). Literature suggests that maize ear rot pathogens produce mycotoxins as a way to overcome stressful conditions (Picot *et al.*, 2010). Minimising plant stress by supplying efficient nutrients needed for plant growth is essential in the control of maize ear rots (Munkvold, 2003b). Other plant stressors that may enhance fungal infection and fumonisin production include water deficiencies and acidic conditions (Parsons and Munkvold, 2012). Burying of crop residues significantly reduces the risk of infection as maize ear rot pathogens are known to overwinter on crop debris. Tillage helps eradicate sources of inoculum but increases the risk of potential soil erosion therefore crop rotations are a much more appropriate alternative to tillage (Steckel, 2003).

Hybrid selection: Resistant varieties can greatly reduce ear rot incidences as well as mycotoxin production (Czembor, 2010). The use of resistant cultivars is both economically and environmentally safe and can ensure long term control of maize ear rots (Tembo *et al.*, 2014). Cultivars resistant to maize ear rots are currently not available in South Africa (Small *et al*., 2012a). The selection and breeding of resistant cultivars is the most effective and most promising control measure for ear rots of maize. Inbred lines with good levels of resistance to FER and fumonisin accumulation under South African conditions have been identified and could be used to develop resistant hybrids (Small *et al*., 2012b; Rose *et al*., 2016). Selection of less susceptible maize hybrids would help in limiting the disease severity. Morphological characteristics of hybrids are also an indication of the potential susceptibility of that hybrid to ear rots (Munkvold, 2003b). Hybrids with tight husks, squared tips are generally more susceptible to ear rots (Munkvold, 2003b).

Maize hybrids, genetically modified with genes from the bacterium *Bacillus thuringiensis* Berliner, known as *Bt*-maize are toxic to certain insects and nematodes, but harmless to animals and birds (Gonzalez-Cabrera *et al*., 2006). Reduced feeding by insects on these genetically modified maize hybrids has been shown to result in lower infection by *Fusarium* spp. such as *F. verticillioides* and *F. proliferatum* (Munkvold *et al*., 1999). Numerous international reports indicated that *Bt*-maize has significantly lower fumonisin levels compared to non-*Bt* isohybrids (Munkvold *et al.*, 1999; Abbas *et al.*, 2013; Agricultural Research Council, 2013). Fumonisin detoxification has also been achieved *in planta* through the expression of a degradative enzyme originating from *Exophiala spinifera* J.W.Carmich and *Rhinocladiella atrovirens* Nannf. in genetically modified maize plants (Duvick *et al*., 1998).

Chemical control: There are currently no fungicides registered for the control or prevention of maize ear rots, because it affects grain unlike foliar diseases that affect leaves and stems (Janse van Rensburg *et al.*, 2015a). Before application, fungicides should be carefully investigated as a study conducted by Miguel *et al.* (2015) and Janse van Rensburg *et al.* (2015a) suggest that some fungicides may enhance fumonisin production in infected maize plants. Chemical elicitors that induce resistance in plants failed to effectively reduce FER or fumonisin contamination in maize in South Africa (Small *et al*., 2012a).

Postharvest strategies

Earlier harvesting of maize grain has been found to result in reduced accumulation of fungal ear rot infections and mycotoxin contamination (Munkvold, 2003b). Maize grain should be stored in dry, clean and cool facilities free of insect pests, temperatures between 1 and 4°C (Munkvold, 2003b).

CONSERVATION AGRICULTURE (CA)

Conventional tillage practices have been widely used in maize cultivation as a method for disease control, provision of desirable conditions for seed germination, root growth and development (Marocco *et al.*, 2009). The continued use of conventional tillage has led to the deterioration in soil structure, fertility and water holding capacity (Pittelkow *et al*., 2014). This is due to soil erosion and a decline in organic matter attributed to conventional tillage practices that involve deep ploughing, repeated cultivation of the same crop and burning of crop residues that leave the soil exposed to wind and rain (Marocco *et al.*, 2009). Other effects include increased emission of greenhouse gases from the use of heavy ploughing machinery (Berger *et al*., 2009). Studies have identified agriculture as a significant contributing factor to climate change (Kabirigi *et al.*, 2015). These factors threaten farming and food security in South Africa (FAO, 2014). The major challenge in crop production is the need to increase crop yields and simultaneously limit environmental impacts (Pittelkow *et al.*, 2014).

To address soil erosion and water run-off issues that threaten productivity, agricultural practices that involve minimal soil disturbance and incorporation of previous crop residues on the soil surface are steadily increasing in maize production areas throughout the world (Marocco *et al*., 2008). CA is a systematic approach that discourages soil disturbance by integrating zero tillage, permanent soil cover and crop rotation to establish a balanced, sustainable agro-system (Berger *et al.*, 2009). This stepwise approach ensures the efficiency of this cropping system by enhancing the quality of the soil, providing cheaper, more productive and environmentally friendly crop production (Hossain, 2013). It further promotes soil fertility, microbial biodiversity, water conservation and profitability (Hossain, 2013).

Monneveux *et al*. (2006) observed an increase in organic carbon, soil bulk density and nitrogen and microbial diversity in zero tillage. CA has proven to have higher yields and outputs than conventional agriculture when practiced for a certain period of time (Hossain, 2013). Upon adoption of CA farming, an estimated increase of approximately 34.21% and 35.68% in average crop yields and net farm income respectively was reported after the first five years (Du Toit, 2007).

South Africa is the 12th country worldwide with the highest CA adoption and the highest in Africa (Derpsch and Friedrich, 2009). Conservation Agriculture is practiced on approximately 368000 ha of South Africa's arable land (Derpsch and Friedrich, 2009) (Table 1). It has been reported in literature that the simultaneous application of no till, crop rotation and stubble retention results in positive complimentary outcomes (Dumanski *et al.*, 2006; Du Toit, 2007; Berger *et al.*, 2009; Pittelkow *et al.,* 2014).

Conservation tillage

Conservation tillage also known as minimised or no-till, involves no seed bed preparation and weeds are controlled by herbicides (Du Toit, 2007; Lotter *et al.*, 2009). Seeds are planted using a hand hoe or a tractor with drawn implements (Mhlanga and Muoni, 2014). Conservation tillage also allows the soil ecosystem to return to its natural composition as well as increased nitrogen and soil microbial biomass (Monneveux *et al.*, 2006). Conservation tillage is practiced in 9% of the world's arable land (Pittelkow *et al.,* 2014). Despite the importance of conservation tillage on conservation agriculture, the process cannot achieve the desired results without being integrated with crop rotations, cover crops and pest management (Berger *et al.,* 2009).

Conservation tillage is believed to enhance the potential for disease by leaving inoculum on the soil surface while conventional tillage decreases inoculum by ploughing it into the soil (Champeil *et al*., 2004). There are a number of contradicting studies conducted on the effect of tillage practices on *Fusarium* spp. accumulation in wheat and maize. In South Africa, no-till increased levels of DER (Flett and Wehner, 1991), and had no effect on FER and GER in maize (Flett and Wehner, 1991). Marocco *et al.*, (2008) observed an increase in fumonisin contamination in monoculture maize under no-till fields when compared to conventional tillage fields during the first year of a three-year study, with no significant differences in the subsequent years in a study conducted in Italy. A study by Suproniene *et al*. (2012) found *Fusarium graminearum* to not be affected by tillage practices, however its resultant mycotoxins zearalenone and deoxynivalenol were significantly lower in the no-till systems during certain seasons in wheat. The effect of tillage practices on disease incidence is entirely dependent on biological composition of the pathogen, dispersal and survival mechanisms (Bailey, 1996). Changes in the microbial composition of soils under no-till may

mitigate disease incidence by antagonism (Bailey, 1996). Effects of no-till vary with the pathogen, crop and environment (Govaerts *et al*., 2006). No-till ensures less plant stress by maintaining high moisture levels throughout the growing season (Bailey and Duczek, 1996).

Cover crops or stubble retention

Crop residues are fragments of plants that remain on the surface of the soil after harvesting (Manstretta and Rossi, 2015). It has been previously reported that at least 30% crop residues should be present on the soil surface before planting (Monneveux *et al.*, 2006). This layer ensures protection of soils against environmental impacts such as wind and rain, hence preventing soil erosion. Cover crops are necessary for soil structure improvement by increasing carbon content and water content (Lotter *et al.*, 2009). Furthermore, cover crops also limit weed growth through competition and depriving weed seeds of sunlight needed for germination (Hobbs *et al.*, 2008) as well as reduce the need to use herbicides (Florentin *et al.*, 2010). Soil nutrition build up is achieved through the breakdown of the cover crops. Cover crops may also help in temperature extremes by preventing direct evaporation (Monneveux *et al.*, 2006). Cover crop retention also plays a role in infiltration as it enables soil to absorb more water (Kabirigi *et al.*, 2015). Crop residue retention paired with no-till have been adopted enthusiastically in a number of areas in the world (Hobbs *et al*., 2008).

Crop residues have been labelled as the principal source of inoculum for maize ear rot pathogens (Champeil *et al*., 2004; Govaerts *et al*., 2006). This is due to the fact that crop residues may include diseased plant parts. Crop residues left on the soil surface are believed to provide conditions that favour pathogen survival and growth (Manstretta and Rossi, 2015). Most maize ear rot causing fungi are able to persist on crop residues as saprophytes (Champeil *et al*., 2004). *Fusarium* spp. have been linked to minimal tillage practices that promote the retention of crop residues on the soil surface and crop residue mass has been seen to have a positive correlation to disease occurrence in wheat (Champeil *et al*., 2004). Maiorano *et al.* (2008) found a positive correlation between the presence of crop residues on the soil surface and the level of *F. graminearum* accumulation and deoxynivalenol contamination in wheat. It was suggested that residues store enough water on the surface to facilitate the release of spores by *Fusarium* spp. and the splash of inoculum is favoured by the presence of crop material on the soil surface (Maiorano *et al*., 2008). Other factors such as amount of crop residues, decomposition rate, and microbiological activity in the residue contribute to pathogen survival, inoculum production and dispersal (Manstretta and Rossi, 2015). Sutton (1982) reported that *F. graminearum* was able to survive much longer on residues with a slower decomposition rate. Crop residue management is suggested as a disease control measure for maize ear rots (Munkvold,

2003b). Crop residues are normally exposed to a range of environmental factors than buried residues which may result in an increase in disease incidence.

Crop rotation

Crop rotations are the most efficient way to reduce biological cycles of pests and disease associated with no till, making CA more feasible (Florentin *et al.,* 2010). Most diseases build up in the soil and crop residues when monoculture is practised (Kheyrodin, 2011) as most fungal pathogens survive on crop residues (Kheyrodin, 2011). Planting a non-susceptible/non-host crop helps break the cycle of disease infestation by reducing pathogen inoculum levels (Flett, and Wehner, 1991; Kheyrodin, 2011). Crop rotations also assist in limiting weed occurrence (Monneveux *et al.*, 2006), building soil structure, improving crop yield and plant vigour which assist in reduction of environmental stress impacts on maize and may help reduce susceptibility to toxigenic fungi (Hossain, 2013).

In wheat, crop rotations with soybean resulted in reduced levels of *Fusarium graminearum* when compared to rotations with corn regardless of tillage practice (Dill-Macky and Jones, 2000). Reports have also suggested an increase in *F. graminearum* and deoxynivalenol concentrations in rotation systems involving maize (Bernhoft *et al.*, 2012). The decomposition rate and residue quantities resulting from maize crops may be a contributing factor to disease incidence (Champeil *et al*., 2004). *Fusarium graminearum* is believed to persist longer in crop residues that take longer to decompose and are larger in quantity (Champeil *et al*., 2004). Frequent use of a susceptible host in a rotation system further increases chances of disease incidence (Champeil *et al*., 2004). Short cereal rotation systems, in combination with no-till, are potentially more likely to promote *Fusarium* infection than longer cereal rotations including legumes or catch crops (Bailey and Duckzek, 1996; Baliukoniene *et al*., 2011). Diseases normally target crops in the same family hence, this should be taken into account when considering whether a crop is appropriate in a rotation scheme (Kheyrodin, 2011). The period of the rotation is also a critical factor, as longer rotations limit disease occurrence as opposed to shorter rotations (Champeil et al., 2004). Maize ear rot causing fungi have a wide range of alternate host crops, and are able to colonize and survive on crops not necessarily classified as hosts (Munkvold, 2003b).

Integrated pest (disease) management

Integrated disease management (IPM) as defined by the FAO, is ‘’the careful consideration of all available pest control techniques and integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce risks to human health and the environment (FAO, 2014). IPM complements conservation agriculture because it functions

on similar principles as well as enhances biological processes (Leake, 2003). IPM has become a mandatory part of CA, since CA does not have specific pest control recommendations, and is highly beneficial when combined with IPM (Leake, 2003). After certain periods of application, CA and IPM may result in enhanced biological activity that limits the need for chemical use.

CONCLUSION

According to a number of reports, South Africa has the highest per capita soil loss in the world (Le Roux *et al.*, 2007; Anonymous, 2013a). This may be attributed to conventional farming practices that involve deep ploughing of soils, soil exposure to wind and rain as well as repeated planting of the same crop (Mhlanga and Muoni, 2014). In order to address the issue of soil loss, decline in soil fertility and water run-off, focus has shifted to conservation agriculture (CA) (Pittelkow *et al.,* 2014). This farming system involves minimal soil disturbance, permanent soil cover and crop rotations aimed at maximising soil quality and minimise erosion (Berger *et al*., 2009). Conservation tillage has been adopted on approximately 125 million hectares of land globally (Pittelkow *et al.*, 2014). In South Africa, CA has been adopted on a moderate but expanding scale (Lotter *et al.*, 2009) and holds promise in sustaining productivity, increasing profits as well as ensuring food security by managing agro-ecosystems (Pittelkow *et al.*, 2014).

Ear rots are one of the most economically important diseases in maize production. Maize infection by ear rots not only render the grain unsuitable for human consumption due to its unappetising appearance or reduced nutritional value but often leads to mycotoxin production (Boutigny *et al.*, 2012). The biggest challenge in crop production is the need to sustainably produce high yielding crops, with minimal diseases and pests (Pittelkow *et al.*, 2014). Ono *et al*. (2011) reported that although CA farming is a more sustainable and a less resource consuming alternative, it may enhance disease accumulation and mycotoxin contamination in maize. The increase in the use of CA has also been attributed to the re-emergence of a number of diseases because of the nature of the agricultural system that is based on crop residue retention which is believed to be a source of inoculum and provides necessary conditions for disease development (Bailey, 1996). According to Flett *et al*. (1998), alternating tillage practices have no effect on fusarium ear rot caused by *Fusarium* spp., but however increased *S. maydis* incidence.

The absence of effective fungicides and resistant cultivars for the control of ear rots is an indication that the most viable control measure of maize ear rot and mycotoxin contamination is through integrated control measures with an emphasis on agricultural practices (Dill-Macky, and Jones, 2000). The effect of agricultural practices on FER, GER and DER occurrence and mycotoxin contamination is still very controversial. More research

with advanced technological strategies is needed to investigate and help identify cropping systems that ensure the sustainable use of CA while mitigating the risk of disease incidence and mycotoxin contamination, this will in turn ensure the development of affordable, safe and sustainable maize production. As it is believed that healthy soils (CA) with enhanced microbial activity have the potential to suppress disease occurrence (Mahmood and Trethowan, 2015). Therefore, the aim of **Chapter 2** was to determine the impact of different tillage and rotation practices on the occurrence of maize ear rots and their resultant mycotoxins.

Due to the potential for GER, FER and DER to develop from inoculum that survive on maize crop residues, there is a strong possibility that tillage and rotation practices may influence not only ear rot infection levels but subsequent mycotoxin accumulation. It is therefore crucial to investigate and identify cropping/rotation systems that ensure the sustainable use of CA while reducing the risk of disease and mycotoxin occurrence. Therefore, the effects of different tillage practises on maize ear rot and mycotoxin contamination in commercial maize production systems were surveyed in **Chapter 3.**

To enhance conservation agriculture benefits, the gap between reduced tillage and disease incidence, taking interaction of crop residue retention and crop rotation systems into account needs to be filled. This is expected to aid in the development of practical, affordable and environmentally sound maize production systems to manage accumulation of toxigenic fungi in maize.

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Table 1. Adoption of conservation tillage practices worldwide in 2007/08 ranging from highest to lowest.

Country	Area under no-tillage (ha) 2007/2008
USA	26 593 000
Brazil	25 502 000
Argentina	19 719 000
Canada	13 481 000
Australia	12 000 000
Paraguay	2 400 000
China	1 330 000
Kazakhstan	1 200 000
Bolivia	706 000
Uruguay	672 000
Spain	650 000
South Africa	368 000
Venezuela	300 000
France	200 000
Finland	200 000
Chile	180 000
New Zealand	162 000
Colombia	100 000
Ukraine	100 000
Others	1 000 000
Total	**105 863 000**

Source (Derpsch and Friedrich, 2009)

CHAPTER 2

Accumulation of toxigenic *Fusarium* species and *Stenocarpella maydis* in maize grain grown under different cropping systems

ABSTRACT

Mycotoxigenic fungi such as *Fusarium graminearum*, *Fusarium verticillioides* and *Stenocarpella maydis* infect maize grain and can be detrimental to humans and animals due to the toxins they produce. Disease management strategies include tillage practises and crop rotations, however, these have not been sufficiently evaluated in South Africa. The increasing shift towards conservation agriculture (CA) in South Africa, may influence maize production, because of the ability of ear rot fungi to survive on crop residues. The effect of cropping systems (CS) on fungal ear rot accumulation and mycotoxin contamination in maize grain was investigated in two localities over a four to six-year period. Cropping systems evaluated were 1) monoculture maize conventional tillage, 2) monoculture maize no-till, 3) two and 4) three-year rotation systems consisting of maize/cowpea and maize/cowpea/babala (all no-till), respectively. In Buffelsvallei, two additional crop rotations, maize/sunflower and maize/sunflower/babala (all no-till) were included. All trials were naturally infected and disease severity and incidence were determined visually while quantitative PCR was used to quantify target DNA of *F. verticillioides* and *F. graminearum*. Furthermore, the mycotoxins fumonisins and zearalenone were quantified using HPLC while deoxynivalenol and nivalenol were quantified using liquid chromatography tandem mass spectrometry. Disease incidence and mycotoxin contamination were inconsistent throughout the study period. This was mostly associated with seasonal and geographical differences during the six-year study. In Buffelsvallei CS had a significant effect ($P < 0.05$) on the accumulation of fumonisins and *F. graminearum* for 2010/11, deoxynivalenol (2011/12) and Diplodia ear rot (DER) incidence (2013/14). *Fusarium graminearum* and fumonisin accumulation was significantly higher in the three-year maize/cowpea/babala rotation and two-year sunflower rotation in the 2010/11 season, respectively. The levels of deoxynivalenol in monoculture maize, using conventional tillage (2011/12) was significantly higher when compared to all other CS and DER incidence was significantly higher in maize conventionally tilled, no-till and two-year maize/cowpea and maize/sunflower cropping systems in the 2013/14 season. The various CS had no significant effects on fungal infection or mycotoxin accumulation in maize grain obtained from trials conducted at Erfdeel. The results of this study indicate that CA systems can be used without the potential increase of maize ear rots and mycotoxin production under local conditions.

INTRODUCTION

Maize (*Zea mays* L.) is an important staple food, feed and energy crop in South Africa. It is also prone to a multitude of root, stalk, leaf and ear rot diseases (Fandohan *et al.*, 2003). Predominant ear rots in most maize-producing areas include Fusarium ear rot (FER), Gibberella ear rot (GER) and Diplodia ear rot (DER) (Boutigny *et al.*, 2012). Fusarium ear rot is mainly caused by *Fusarium verticillioides* Sacc. Nirenberg (syn = *F. moniliforme* Sheldon), which is present in most maize-producing areas (Fandohan *et al.*, 2003). It is responsible for substantial losses in grain yield and quality due to its ability to produce mycotoxins known as fumonisins (Fandohan *et al*., 2003). Fumonisins are the most predominant group of mycotoxins and have been classified as potentially carcinogenic, neurotoxic, mutagenic, immunosuppressive, and hepatotoxic (Gelderblom *et al.*, 1992).

Fusarium graminearum (Schwabe) [Teleomorph *Gibberella zeae* (Schwein. Petch], which causes GER, produces pink to red mould that discolours infected maize kernels (Reid *et al*., 1999) and is responsible for the production of a wide range of toxic metabolites including zearalenone and trichothecenes such as deoxynivalenol and nivalenol. Deoxynivalenol and nivalenol are accountable for feed refusal, vomiting, gastric ulcers and decreased weight if ingested by animals (Youssef, 2009). Zearalenone, which has structural similarity to oestrogen, is attributed to several reproduction disorders such as fecundity and stillbirths in animal species (Zinedine *et al.*, 2007).

Stenocarpella maydis (Berkeley) (Syn) *(Diplodia maydis) (*Berk.) (Sacc) is the causal agent DER, a common maize ear rot found in most maize-producing areas. DER is responsible for massive yield losses and infected kernels are usually lighter and have decreased nutritional value (Flett and McLaren, 1994). It has also been linked to mycotoxicoses of cattle and sheep commonly known as diplodiosis (Rabie *et al.*, 1985). Symptoms include paralysis, ataxia and still births (Odriozola *et al.,* 2005). Apart from commonly being associated with southern African countries, there have been reports of occurrence in Brazil and Argentina (Odriozola *et al.,* 2005; Masango *et al*., 2015).

Multi-toxin contamination in agricultural commodities is of great significance due to impacts on productivity, the economy as well as human and animal health (Degraeve *et al.*, 2016). Mycotoxigenic fungi are either classified as field or storage fungi (Placinta *et al.*, 1999). In maize, the most important stage of ear rot infection and mycotoxin contamination is during pre-harvest production, where disease incidence and mycotoxin contamination is influenced by numerous factors ranging from climatic conditions, soil fertility, insect damage, susceptibility of plant variety and agricultural practices (Reid *et al.*, 2001).

The use of Conservation Agriculture (CA) ensures the efficiency of a cropping system by enhancing the quality of the soil, providing cheaper, more productive and environmentally friendly crop production (Lawrance *et al*., 1999). The principal challenge in crop production is

the need to sustainably produce high yielding crops, with minimal diseases and pests. Tillage influences both the physical and chemical properties of the soil, therefore a reduction in tillage practices may significantly influence pathogen species but this is entirely dependent on the pathogen's life cycle and survival mechanisms (Govaerts *et al*., 2006). The effect of tillage practises on disease incidence is vaguely understood and sometimes contradictory (Lawrance *et al*., 1999). One of the reported setbacks involved with reduced tillage practices is the potential for increased disease incidence (Sumner *et al*., 1981) although Flett *et al*. (1998) found tillage practices to not have an influence on FER and GER accumulation in maize grain. Changes in cropping systems can have effects on factors that correlate to disease development such as soil structure, plant growth, closeness of crop to pathogens, residue availability, soil temperature and water content (Watkins and Boosalis, 1994; Lawrance *et al.*,1999). Crop rotations have also been identified as a viable method for disease control in no till systems (Ward and Nowell, 1998).

With the lack of resistant cultivars and effective chemical control measures for maize ear rots, it is of fundamental importance that the effects of these agricultural practices be investigated to help limit disease incidence and mycotoxin contamination in maize grain. Therefore, the objective of this study was to 1) investigate the effect of cropping practices on maize ear rots and mycotoxins and 2) determine the potential role of crop rotations in CA systems with regards to maize ear rot infections and mycotoxin contamination. This knowledge will assist in identifying a suitable cropping system that improves grain quality by reducing ear rot infections and mycotoxin contamination while also providing more insight into the impact of CA on mycotoxigenic fungi and their metabolites.

MATERIALS AND METHODS

CA trials

Field trials were carried out for six (2009/10 - 2014/15) and four (2011/12-2014/15) seasons in two different localities. These localities were based in the North-West and Free State provinces at Buffelsvallei (latitude -26.495; longitude 26.602, sandy loam soil) and Erfdeel (latitude -26.982 longitude 27.027, sandy textured soil), respectively. A randomized, complete block design with four replicates was implemented, which consisted of six cropping systems in Buffelsvallei (sandy loam soil) and four cropping systems in Erfdeel (sandy textured soil). Maize cultivars in both localities were PAN 6Q- 521 R in 2009/10, PAN 5Q 563 R in 2010/11, PAN 5Q 649 R in 2011/12 and 2012/13, PAN 5Q 649 RR in 2013/14 and BG 5685 R in 2014/15 (Table 1). Treatments in Buffelsvallei included 1) maize monoculture, conventionally tilled (MM-CT), 2) maize monoculture, no-till (MM-NT), 3) no-till maize, two season rotation with sunflower (NT-SF), 4) no-till maize, two season rotation with cowpea (NT-CP), 5) no-till maize, three season rotation with babala and sunflower (NT-BA-SF) and

6) no-till maize three season rotation with babala and cowpea (NT-BA-CP) (Table 2). Treatments in Erfdeel included 1) maize monoculture, conventionally tilled (MM-CT), 2) maize monoculture, no-till (MM-NT), 3) no-till maize, two season rotation with cowpea (NT-CP) and 4) no-till maize, three season rotation with babala and cowpea (NT-BA-CP) (Table 3). The experiment was conducted for four years in Erfdeel due to highly acidic soil conditions, planting did not take place during the first two years of the study. Maize ears were naturally infected by ear rot causing fungi and each season maize ears were harvested from the two middle rows of each treatment.

Plots were fertilized with 600 mL/ha^{-1} N: P: K prior to planting (Table 1). Herbicides during planting included DUAL GOLD (960 g/L S-metalochlor, Syngenta, Basel, Switzerland) at a rate of 60 to 600 mL/ha, GRAMOXONE SL (250 g/L Paraquat, Syngenta, Basel, Switzerland) at a rate of 1 to 3L/ha, ROUNDUP (540 g/L glyphosate, Monsanto, Missouri, USA) at a rate of 2 to 4L/ha, KARATE (250 g/L Lambda-Cyhalothrin, Syngenta, Basel, Switzerland) at a rate of 70mL/ha. Stalk borers were controlled using KOMBAT (25 g/L Carbaryl, Kombat, Greytown, South Africa) and BULLDOCK (25 g/L Beta-cyfluthrin, Bayer Crop Science, Leverkusen, Germany).

Maize ear rot disease ratings

At the end of the season, maize ears were hand harvested. A grain disease rating was conducted according to Flett *et al.* (1998). DER incidence was determined based on discoloration, rot and mycelium were used to determine FER and GER. The percentage of visibly diseased grain samples was calculated by mass. To date, no method is available for the quantification of toxins produced by *S. maydis*.

Quantification of *F. verticillioides* and *F. graminearum* s.l.

DNA extraction: Maize ears were hand harvested at ≤ 12% moisture, and threshed per treatment. A 250-g sub-sample was taken from each threshed sample, milled and passed through a 1-mm mesh using a Cyclotech sample mill (Foss Tecator, Hoganas, Sweden). These samples were stored at -20°C for further analysis. DNA was extracted from 0.5-g milled flour using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) according to manufacturer's guidelines. The purity and the concentration of the DNA was measured using a Nanodrop® (2000c) Spectrophotometer (Thermo Scientific, Waltham, USA) at 260 nm (OD260). The DNA was diluted to 10 000 pg/µL and stored at -20ºC in 100 µL aliquots.

Standard curves: A high fumonisin producing *F. verticillioides* isolate (MRC826) and a *F. graminearum sensu lato* isolate (MRC 394) were obtained from the Medical Research Council to use in the standard curve set up. The respective fungi were plated out on potato dextrose agar (PDA) and DNA was extracted from mycelial growth after 1 week by using the

CTAB method adapted from Winnepenninckx *et al*. (1993). For *F. verticillioides*, a 10-fold dilution of the MRC826 DNA was used to generate a standard curve for quantification (Waalwijk *et al.*, 2008). The dilution range was 60 000, 6 000, 600, 60 and 6 pg/µL^{-1}. Two replicates per dilution were used to generate a standard curve. For *F. graminearum* s.l.*, a* 4-fold standard dilution was used to generate a standard curve for quantification (Nicolaisen *et al*., 2009). The dilution range was 7500, 1875, 468.8, 117.2 and 29.3 pg/µL. Two replicates per dilution were used to generate a standard curve.

Quantification of F. verticillioides *and* F. graminearum *s.l. target DNA:* For *F. verticillioides* quantification, the primers Taqfum-2F and Vpgen-3R in combination with the FUM-Probe 1 primer were used as tested by Waalwijk *et al*. (2008). The sensimix reagent kit (Sensimix™ no rox QT 505-05) from Celtic (Bioline, London, England) was used for qPCR. For each reaction, A 96- well plate containing 4 µL of DNA (10 000 pg/µL) sample was mixed with 12.5 µL sensimix, 2.125 µL Fum probe (1 µM), 0.875 µL (333 nM)Taqfum-2F: ATG CAA GAG GCG AGG CAA, 0.875 µL (333 nM) Vpgen-3R primer: GGC TCT CRG AGC TTG GCA T and 4.625 µL molecular grade water. Negative controls contained no template DNA but were treated like the reaction samples. For *F. graminearum* s.l. quantification, the primers FgramB379 and FgramB411 in combination with SYBRGreen as tested by Nicolaisen *et al*. (2009) were used. A 96- well reaction plate was prepared consisting of a total volume of 25 µL of 12.5 µL of SYBR® green, 0.625 µL of FgramB379: CCA TTC CCT GGG CGT and 0.625 µL FgramB411: CCT ATT GAC AGG TGG TTA GTG ACTGG, 9.25 µL of nuclease free water, 2 µL of the unknown 10 000 pg/µL target DNA. Negative controls contained no template DNA but were treated similar to the reaction samples. A CFX96™ Real-Time PCR detection system (Bio-Rad, Hercules, USA) with a 96 well plate was used for qPCR. For *F. verticillioides* the reaction consisted of a 5 min denaturation step at 95º C, 40 cycles at 95º C for 10s and 65º C for 10s, followed by cooling to 65º C; for *F. graminearum* s.l. consisted of 5 minutes denaturation at 95ºC, 40 cycles at 95ºC for 10s and 65ºC for 10s, followed by a melt curve step of 95ºC, and a cooling step at 65ºC. After runs were completed, data was generated from the amplification curves. Regression equations of standard curves from runs were highly correlated (R^2>0.99). Slopes were within the accepted criterion (between -3.1 and -3.6) and efficiencies were between 95 and 110%.

Fumonisin quantification

Fumonisins were analysed using the HPLC-VICAM method (Anonymous, 2002). A 50-g sub-sample was mixed with 5 g of sodium chloride (Merck, Darmstadt, Germany) prior to extraction. A methanol: water (80:20 v/v) extraction solvent (100 mL) was used to extract fumonisins for five minutes at high speed using a Waring laboratory blender (Waring

products division, Torrington, USA). The extract was then filtered through 24-cm fluted filter paper (VICAM). A 10-mL aliquot was diluted with 40 mL saline phosphate-buffer (1X PBS) (8.0 g NaCl, 1.2 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, dissolved in 990 mL purified water and the pH adjusted to 7.0). Diluted samples were extracted through microfiber filters (0.45 µm) and 10 mL of the filtrate was passed through VICAM FumoniTest affinity columns at a flow rate of 1 drop per second. Subsequently, 10mL of PBS was passed through the column at a rate of 1 drop per second. The column was then washed with 1.5 mL HPLC grade methanol at a rate of 1 drop per second and the eluate was collected in a glass cuvette. The methanol eluate was dried in a TurboVap LV (Caliper Sciences, Massachusetts, USA) with the aid of a slow stream of high purity nitrogen gas. Samples were dissolved in 200 µL methanol and purified water (50:50 v/v). Each sample (50 µL) was transferred to 250 µL conical inserts, placed into a 2.5 mL glass vial which was then placed into a carousel. The first position of the carousel had a 2.5 mL glass vial with *o*-phthaldialdehyde (OPA from Sigma-Aldrich, Missouri, USA) which is the derivatisation agent. The Waters 717 plus autosampler was set up to mix 100 µL of the OPA with the 50 µL of sample in the conical insert. This mixture (20 µL) was injected after a delay time of 1 minute.

Fumonisin standards were obtained from Sigma-Aldrich. To generate a standard curve, standards were evaporated and reconstituted with a calibration standard solution ranging from 2 ppm, 5 ppm, 10 ppm, 15 ppm and 20 ppm. Fluorescence was performed at excitation and emission wavelengths of 335 nm and 440 nm respectively using a Waters 2475 multi λ fluorescence detector equipped with a Symmetry C18 (5 µm 3.9 x 150 mm) analytical column (Waters, Milford, USA). The detection limit of the method used was 0.016 ppm and the recovery data were obtained in triplicate by spiking clean maize samples (VICAM) with 5 ppm fumonisin B_1 B_2 and B_3. The average recovery rates were 83% (FB_1), 81% (FB_2) and 83% (FB_3).

Zearalenone quantification

Zearalenone was analysed using the VICAM method adapted from Kruger *et al*. (1999). Milled sub samples (25 g) were mixed with sodium chloride (5 g) prior to extraction, and then blended (Waring products division, Torrington, USA) in 100 mL of methanol: water (80:20 v/v) at high speed for two minutes. The extract was filtered through 24-cm fluted filter paper from VICAM. The filtrate (4 mL) was mixed with 96 mL HPLC grade water (18 MΩ.cm) and filtered through a microfiber filter paper. The diluted extract (100 mL) was passed through ZearaTest affinity column from VICAM at a rate of approximately 3 drops per second. The column was washed with 25 mL HPLC grade water. Zearalenone was eluted by passing through 0.75 mL of methanol followed by 0.5 mL of water amounting to a total volume of

1.25 mL. The eluate (50 µL) was injected into the HPLC system. The mobile phase consisted of acetonitrile: methanol: water (46:46:8 v/v/v). The flow rate was set at 1 mL/min.

Zearalenone standards were obtained from Sigma-Aldrich. To generate a standard curve, standards were evaporated and reconstituted with a calibration standard solution ranging from 0.25 ppm, 0.5 ppm, 1.25 ppm, 2.5 ppm. Fluorescence was performed at excitation and emission wavelengths of 274 nm and 440 nm respectively using a Waters 474 multi λ scanning fluorescence detector and analytical column, Symmetry C18 3.9 x 150 mm (Waters, Massachusetts, USA). The detection limit was 0.0019 ppm and recovery data was obtained in triplicate by spiking clean maize samples (VICAM) with 5 ppm zearalenone. Average percentage recovery was 112%.

Deoxynivalenol and nivalenol quantification

Deoxynivalenol and nivalenol was extracted using the VICAM method (Anonymous, 2012). Milled maize sub samples (50 g) were placed on a blender jar (Waring products division, Torrington, USA) with 200 mL of purified water. The sample was blended at high speed for three minutes. The blended extract was then filtered through a 24-cm fluted filter paper from VICAM and filtrate was collected in a clean vessel. The filtrate (10 mL) was mixed with 40 mL phosphate-buffered saline (PBS) (8.0 g NaCl, 1.2 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, dissolved in 990 mL purified water with pH adjusted to 7.0) and poured into a folded filter (11 cm) inside a funnel in a clean vessel. The filtered extract (5 mL) was run through a glass syringe barrel on a pump and passed completely through deoxynivalenol-/nivalenol WB affinity column from VICAM at a rate of approximately 1 drop per 2 seconds. Deoxynivalenol/nivalenol WB affinity column was washed with 10 mL PBS followed by 10 mL purified water at a rate of about 1 drop/second. A glass cuvette was placed under deoxynivalenol/nivalenol WB columns and deoxynivalenol/nivalenol was eluted by 0.5 mL HPLC grade methanol and 1.5 mL HPLC acetonitrile, amounting to a total of 2 mL.

Standards for deoxynivalenol and nivalenol were obtained from Sigma-Aldrich. To generate a standard curve, standards were evaporated and reconstituted with a calibration standard solution ranging from 0.1 ppm, 0.5 ppm and 5 ppm for nivalenol and 0.1 ppm, 0.5 ppm and 5 ppm for deoxynivalenol. The level of dectection was 0.03 ppm for deoxynivalenol and 0.04 ppm for nivalenol. The average percentage recovery was 90% for both deoxynivalenol and nivalenol. Deoxynivalenol and nivalenol were separately quantified using LC-MS/MS at the Central analytical facility (Dr M. Stander), Stellenbosch University, and Stellenbosch, South Africa.

Climatic data

Climatic data between the 2009/10 – 2014/15 were collected in the Buffelsvallei and Erfdeel regions. Monthly maximum temperatures (^{0}C), rainfall (mm) and relative humidity (%) were recorded between July of the planting year and June of the harvesting year.

Statistical analysis

Maize ear rot incidence, fungal target DNA, disease incidence and mycotoxin accumulation of the two conservation agriculture trials were analysed separately by season. The data of the trials were analysed using ANOVA statistical models and skewed data were transformed using a log transformation. To compare treatment effects, Fischer's protected least significant difference (LSD) was calculated at a 5% significance level (Gen Stat, version 15).

RESULTS

Buffelsvallei

Fusarium ear rot (FER) disease ratings: There were no significant differences between the cropping systems evaluated in this study with regards to FER severity during the six-year period (Table 4). The mean FER visual disease severity ranged from 0.10-15.30% throughout the six-year study period (Table 5).

Gibberella ear rot (GER) disease ratings: Cropping systems appeared to not influence GER severity during the six-year study (Table 6). Mean GER severity ranged from undetectable to 3.65% during the six-year study (Table 5).

Diplodia ear rot (DER) disease ratings: Diplodia ear rot incidence was only affected by cropping systems in one of the six years of this study. In 2013/14, the monoculture maize conventionally tilled (MM-CT; 3.89%), maize monoculture no-till (MM-NT; 3.00%), two-year maize/sunflower (NT-SF; 4.18%) and two-year maize/cowpea (NT-CP; 2.05%) had significantly higher DER incidence when compared to the three-year maize/sunflower/babala (NT-BA-SF; 0.87) and three-year maize/babala/cowpea (NT-BA-CP; 0.94) systems (Table 5).

Fusarium verticillioides *target DNA*: The cropping systems evaluated did not have significant effects on *F. verticillioides* target DNA quantified during all seasons of the study (Table 8). Mean *Fusarium verticillioides* target DNA accumulation in maize grain was inconsistent during the six-year study period where it was moderate to high (311 – 2198 pg/µL) during the first two seasons (2009/10 and 2010/11) and lower (2.10 – 223 pg/µL) during the

(2011/12 and 2012/13) seasons and moderate (115 – 417 pg/µL) again during the (2013/14 and 2014/15) seasons (Table 5).

Fusarium graminearum *target DNA*: Variability in *F. graminearum* target DNA accumulation throughout the six-year study period in Buffelsvallei was observed (Table 5). A significant relationship between cropping systems and fungal target DNA accumulation could only be established for the 2010/11 maize-growing season (Table 9). During this season, the mean *Fusarium graminearum* target DNA accumulation (59.07 pg/µL; Table 5) was significantly influenced by the cropping systems evaluated (Table 9) in the three-year maize/cowpea/babala (NT-BA-CP) rotational system as opposed to the other cropping systems. The lowest target DNA concentration was recorded in the two-year maize/sunflower rotation (NT-SF; 2.62 pg/µL) (Table 5). *Fusarium graminearum* accumulation was generally lower in the first three seasons of the study but increased as the study progressed (Table 5). The effect of cropping systems on the accumulation of *F. graminearum* target DNA was not significant for any other season evaluated (Table 9).

Fumonisins: Fumonisin accumulation in maize grain was below the allowable 4 ppm (Anonymous, 2016) in five of the six treatment years of the study (Table 5). Fumonisin contamination in maize grain was found to be significantly affected by cropping systems in only one (2010/11) of the six seasons (Table 10). Fumonisin levels (8.87 ppm) were significantly ($P \leq 0.05$) higher in the two-year maize/sunflower (NT-SF) rotation for the 2010/11 season when compared to the other treatments (Table 5). The fumonisin accumulation in this crop rotation system, however, did not differ significantly from that of the maize monoculture no-till (MM-NT) treatment that had a mean fumonisin value of 1.43 ppm (Table 5).

Zearalenone: Trace amounts of zearalenone were quantified from some of the grain samples in two of the six seasons (2009/10 and 2010/11) in Buffelsvallei (Table 5). No zearalenone was detected in grain samples in the remaining seasons. According to the ANOVA analyses (Table 11) zearalenone accumulation in maize grain was not significantly influenced by any of the cropping systems in all seasons evaluated.

Deoxynivalenol and nivalenol: The mean level of deoxynivalenol quantified in samples representing the maize monoculture conventionally tilled system (MM-CT; 0.52 ppm) (Table 5) was significantly higher in comparison to the other treatments except when compared to maize monoculture no-till system (MM-NT; 0.18 ppm) (Table 5). Cropping systems had a significant effect ($P=0.03$, Table 12) on deoxynivalenol accumulation in maize grain in the

2011/12 season compared to the other seasons. Deoxynivalenol levels ranged from not detectable (ND) to 0.5 ppm throughout the six seasons in Buffelsvallei while no nivalenol was detected across all years in Buffelsvallei (Table 5).

Erfdeel

Fusarium ear rot (FER) disease ratings: Cropping systems had no significant effects on recorded for FER disease severity throughout the study period (Table 13). Mean FER severity ranged from 3.75 - 1.30% during the study period (Table 14).

Gibberella ear rot (GER) disease ratings: Mean GER severity ranged from non-detectable to 11.06% during the study period (Table 14). GER severity in maize grain was not significantly affected by any of the evaluated cropping systems (Table 15).

Diplodia ear rot disease ratings: Diplodia ear rot incidence was low in Erfdeel throughout the study with the incidence percentages ranging from 0.40 - 5.90% (Table 14). No significant differences were recorded in DER disease incidence in relation to cropping systems across all seasons (Table 16).

Fusarium verticillioides *target DNA*: ANOVA indicated that cropping systems at Erfdeel had no significant effect on *F. verticillioides* target DNA accumulation in maize grain between 2011/12-2014/15 (Table 17). *Fusarium verticillioides* target DNA accumulation ranged from low to moderate (2.30 - 427 pg/µL) throughout the study period (Table 14).

Fusarium graminearum *target DNA*: The *F. graminearum* target DNA accumulation was low in Erfdeel during the study, ranging from 5.90 - 214 pg/µL (Table 14). There were no significant differences in *F. graminearum* accumulation in relation to cropping systems in Erfdeel between 2011/12 - 2014/15 (Table 18).

Fumonisins: Low to moderate levels of fumonisins ranging from non-detectable - 0.55 ppm were recorded during the study period in Erfdeel (Table 14). No significant differences were found for fumonisin contamination in relation to cropping systems in Erfdeel between 2011/12 - 2014/15 (Table 19).

Zearalenone: Zearalenone was not detected during the study period in Erfdeel (Table 14).

Deoxynivalenol and nivalenol: Deoxynivalenol frequency was low ranging from non-detectable - 0.47 ppm (Table 14). Nivalenol was not detected during the study period in

Erfdeel (Table 14). No significant differences were recorded in deoxynivalenol and nivalenol accumulation in relation to cropping systems across all seasons (Table 20).

Climatic data

In Buffelsvallei, the weather was characterised by dry and warm conditions. Mean maximum monthly temperatures steadily increased from 25.1^{0}C to 27^{0}C from the 2009/10 season to the 2014/15 season (Table 21). The observed rainfall pattern was generally higher during the planting and silking stages (November-March) and lower towards harvesting periods (April-August) (Table 21). Seasons 2011/12 and 2012/13 recorded the lowest rainfall when compared to all the other four studied seasons.

In Erfdeel, a similar pattern in the mean maximum monthly temperatures was observed compared to Buffelsvallei where the mean monthly temperatures increased from 26.1ºC to 27.1ºC from season 2009/10 to 2014/15 (Table 21). Mean maximum temperatures were slightly higher in Erfdeel when compared to Buffelsvallei during the six-year study period. Rainfall was generally higher during the planting and silking stages (November-March) and lower towards harvesting periods (April - August, Table 21), rainfall was however slightly lower in Erfdeel when compared to Buffelsvallei.

DISCUSSION

Conservation cropping systems are based on three principles which are no-till, cover crop retention and crop rotations (Marocco *et al*., 2009). In this study, the effect of these cropping systems on ear rot diseases and mycotoxins were determined from 2009/10 to 2014/15. The cropping system did not have a significant effect on *F. verticillioides* target DNA accumulation in both Buffelsvallei and Erfdeel in all evaluated seasons. The major setback of crop residue retention is the build-up of disease inoculum (Govaerts *et al*., 2006). Crop residue retention is suspected to influence disease accumulation through the provision of suitable disease development conditions and harbouring inoculum for further infection (Watkins and Boosalis, 1994). Almeida *et al*. (2000) found that *Fusarium* spp. isolated from buried soybean residues was higher than *Fusarium* spp. isolated from surface residues. The lack of significant effects in cropping systems involving crop residue surface retention over six and four years, respectively, in this study indicates that surface retention of crop residues did not lead to *F. verticillioides* inoculum build up. The results in this study are in agreement with findings by Flett and Wehner (1991) and Flett *et al*. (1998) where it was reported that cropping systems had no significant effects regarding *F. verticillioides* occurence in maize grain. This is a noteworthy finding, indicating that conservation agricultural production systems can be used in the studied localities without the potential increase of *F. verticillioides* in maize grain.

Fusarium graminearum accumulation was significantly elevated in the three-year maize/cowpea/babala rotation system only for the 2010/11 season in Buffelsvallei. This result suggests that *F. graminearum* contamination is largely unaffected by cropping systems employing different tillage and cover crops. However, during years with high or average disease levels, cropping systems may affect disease severity. Crop rotations have been reported as effective in the control of *F. graminearum* in wheat but not as effective on maize (Flett *et al.*, 2001). In wheat, crop rotations with soybean resulted in reduced levels of *F. graminearum* when compared to rotations with maize regardless of tillage practice (Dill-Macky and Jones, 2000). This may be due to the fact that both soybean and wheat produce much lesser crop residues when compared to maize (Champeil *et al*., 2004). Maize residues take longer to decompose when compared to other crops and are more likely to harbour *F. graminearum* inoculum much longer (Hooker and Schaafsma, 2005). Cowpea and babala also produce minimal crop residues therefore the increase in *F. graminearum* target DNA may be due to its persistence in maize residues (Marburger *et al*., 2015)

Fumonisin contamination was observed to be above the 4 ppm allowable limit as per South African legislation in the two year maize/sunflower rotation during the 2010/11 growing season and corresponded with high *F. verticillioides* target DNA accumulation for the same period and cropping system. This may be attributed to changes in soil management and stresses on plants (Marocco *et al.*, 2009). The high rainfall towards the harvesting period during this season could have caused the high fumonisin contamination (Ono *et al*., 1999). Fluctuations in rainfall patterns and relative humidity may influence fumonisin contamination in maize grain by inflicting physiological stresses on plants (Fandohan *et al*., 2003). Several other factors such as drought, presence of other diseases, high oxygen tension and low pH may have played a role in enhancing fumonisin contamination in maize grain (Parsons and Munkvold, 2012). These conditions contribute to plant stress and predispose plants to infection by *F. verticillioides* and resultant mycotoxin contamination. It is evident from this six-year study that fumonisin contamination of maize grain is not a threat under local climatic and geographic conditions and it is not greatly influenced by cropping systems. The effect of crop rotations combined with tillage effects on fumonisin contamination in maize grain is not well documented in literature and requires more extensive research.

Deoxynivalenol was significantly lower in rotation systems as opposed to monoculture systems during the 2011/12 season, thus supporting findings by Bernhoft *et al.* (2012) where it was reported that a lack of crop rotation increased levels of deoxynivalenol in cereals. In wheat, rotation systems involving soya bean reduced deoxynivalenol concentration by 49% when compared to rotation systems involving maize (Champeil *et al*., 2004). This emphasises the importance of choosing the correct preceding crop to be used in a rotation system. The frequency of the rotation is also an important factor, as the longer the rotation,

the higher the chance of reducing disease accumulation and potential mycotoxin contamination (Champeil *et al*., 2004). Results from this study suggest that when environmental conditions are favourable, a lack of crop rotations under no-till may have an impact on deoxynivalenol contamination (Lori *et al*., 2009). The absence of significant effect in zearalenone and nivalenol contamination can be attributed to their general low contamination throughout all seasons in both localities. Previous studies in South Africa have found nivalenol to be scarce in maize (Rheeder *et al*., 1995).

In this study the three-year rotations resulted in reduced levels of DER incidences when compared to other treatments only in the 2013/14 season. These results do support findings by Flett (1991), Baliukoniene *et al*. (2011) and Kheyrodin (2011) that maize monoculture (till and no-till) over a period of years leads to a build-up of *S. maydis* inoculum. This may well be due to the elongated periods of *S. maydis* inoculum persistence on maize residues as they take longer to decompose (Glenn, 2007). Maize is the only known commercial host for *S. maydis* and this would explain its persistence when maize is grown under monoculture (Masango *et al*., 2015). Flett *et al*. (2001) reported that wheat, soybean and peanut are better suited in reducing DER incidences as opposed to sunflower. It is therefore recommended to optimize tillage systems to control fungal infection in crop production by introducing efficient rotation systems (Oldenburg *et al.*, 2015).

Previous reports in South Africa have indicated that tillage practices have no effect on *F. verticillioides* and *F. graminearum* accumulation in maize grain (Flett and Wehner, 1991; Flett *et al.*, 1998). It was evident from this study that *F. graminearum* target DNA accumulation, DER incidence, fumonisin and deoxynivalenol contamination in maize grain may be affected by tillage and rotation systems in seasons with average or high disease. This difference can be attributed to their use of outdated plating out methods for fungal biomass quantification. Morphological characteristics are not enough to correctly identify fungal isolates at species level (Gong *et al*., 2014). The real-time polymerase chain reaction (qPCR), method of quantification used in this study offers rapid, accurate, specific and sensitive target DNA detection and quantification (Nicolaisen *et al.*, 2009).

Primary inoculum and weather conditions are suspected to play a critical role in the inconsistency observed between seasons in this study. Rotation systems restricted to cereal crops, in combination with no-till, are more probable to enhance Fusarium infection than longer rotations including legumes or catch crops (Baliukoniene *et al.*, 2011). This study simultaneously examined the combined effects of tillage and rotation practices while previous studies only focused on tillage systems. No tillage paired with rotations and residue retention enhance plant growth and generally decrease disease incidence (Govaerts *et al*., 2006) while crop residue retention in no till systems increase microbial diversity in the soil and further enhance biological control potential. Balanced crop rotations in this system

further assist in the regulation of pathogenic species (Govaerts *et al.*, 2006), however, crop rotations have also been found to be less effective to control diseases caused by *Fusarium* spp. due to their wide host range and long term survival abilities (Krupinsky et *al*., 2002). *Fusarium* spp. are able to colonise and survive on tissue of plants not necessarily considered as hosts (Munkvold, 2003) and this may limit the effectiveness of crop rotation systems in conservation agriculture.

The absence of significant effects during most of the study period indicate that conservation agriculture can be used without the possibility of drastically increasing disease and mycotoxin contamination in South African maize grain. However, the effect of CA on disease and mycotoxin contamination should be periodically surveyed especially during years where prevailing environmental conditions differ significantly to previous years. It is evident that many factors individually play a critical role in disease development and mycotoxin production. Disease accumulation, incidence and mycotoxin contamination varied between seasons as well as geographical location. It is therefore important to consider factors such as environmental conditions and geographical location that might play a role in disease development and mycotoxin contamination. Predictive models may assist farmers in making informed decisions regarding the potential of disease accumulation and mycotoxin infection as cropping systems were observed to not have a major effect in this study.

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Table 1. Cultivars, inputs and plant density of trials planted in Buffelsvallei and Erfdeel from 2009/10 to 2014/15.

Season	**Buffelsvallei (loam soil)**		
	Cultivar	Fertiliser (N:P:K)	Plant density (seeds ha^{-1})
2009-10	PAN 6Q-521 R	51:11:6	20000
2010-11	PAN 6P-563 R	97:18:9	24500
2011-12	PAN 5Q 649 R	101:17:8	24000
2012-13	PAN 5Q 649 R	104:8:4	24000
2013-14	PAN 5Q 649 R	75:12.5:6.3	27000
2014-15	BG 5685 R	100:23:11.5	25000
Season	**Erfdeel (Sandy soil)**		
	Cultivar	Fertiliser (N:P:K)	Plant density (seeds ha^{-1})
2011-12	PAN 5Q 649 R	100:16:8	24000
2012-13	PAN 5Q 649 R	100:17:22	24000
2013-14	PAN 5Q 649 R	99.2:16:20	25000
2014-15	BG 5685 R	99:18:9	22000

Table 2. Cropping systems evaluated from 2009/10 to 2014/15 in Buffelsvallei.

Crop system		Season		
	Cultivation	1	2	3
1. Maize monoculture	CT*	Maize	Maize	Maize
2. Maize monoculture	NT#	Maize	maize	Maize
3. Maize - cowpea	NT#	Maize	Cowp	Maize
4.Maize - sunflower	NT#	Sunflower	Maize	sunf
5.Maize-babala cowpea	NT#	Maize	Babala	Cowpea
6.Maize babala sunflower	NT#	Sunflower	Maize	Babala

*CT= conventional till.

NT = No-till.

Table 3. Cropping systems evaluated from 2011/12 to 2014/15 trials in Erfdeel.

Crop system		**Season**		
	Cultivation	**1**	**2**	**3**
1. Maize monoculture	CT*	Maize	Maize	Maize
2. Maize monoculture	NT#	Maize	maize	Maize
3. Maize – cowpea	NT#	Maize	Cowpea	Maize
4.1 Maize babala cowpea	NT#	Maize	Babala	Cowpea

* CT = conventional till.

NT = No-till.

Table 4. Analysis of variance of the effects of cropping systems on Fusarium ear rot (%) severity in maize grain from 2010/11 to 2014/15 in Buffelsvallei.

2010/11					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	55.606	11.121	4.23	0.130
Residual	15	39.432	2.629		
Total	23	112.301			
2011/12					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1373.5	274.7	1.13	0.393
Residual	13	3161.0	243.2		
Total	21	5299.8			
2012/13					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	379.38	75.88	2.07	0.153
Residual	10	365.98	36.60		
Total	17	829.84			
2013/14					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	63.37	12.67	1.14	0.382
Residual	15	166.84	11.12		
Total	23	300.20			
2014/15					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	125.44	25.09	0.97	0.467
Residual	15	388.41	25.89		
Total	23	521.39			

Values are significant at $P \leq 0.05$

Table 5A-G. Mean groupings of Fusarium ear rot, Gibberella ear rot, Diplodia ear rot severity, *Fusarium verticillioides*, *Fusarium graminearum* target DNA, fumonisin, zearalenone, deoxynivalenol and nivalenol contamination in maize grain samples measured between 2009/10 - 2014/15 in Buffelsvallei.

Treatment		Season				
	2009/10	2010/11	2011/12	2012/13	2013/14	2014/15
A. Fusarium ear rot (%)						
1-MM-CT	*	2.96[a]	0.70[a]	15.00[a]	12.89[a]	13.00[a]
2-MM-NT	*	4.27[a]	2.00[a]	15.30[a]	14.07[a]	6.10[a]
3-NT-SF	*	4.30[a]	2.03[a]	8.20[a]	15.28[a]	8.80[a]
4-NT-CP	*	3.06[a]	2.30[a]	5.20[a]	14.23[a]	8.30[a]
5-NT-BA-SF	*	5.87[a]	0.20[a]	3.80[a]	13.22[a]	7.90[a]
6-NT-BA-CP	*	7.22[a]	0.10[a]	6.20[a]	10.09[a]	6.30[a]
B. Gibberella ear rot (%)						
1-MM-CT	*	0.21[a]	0.21[a]	ND	0.41[a]	0.65[a]
2-MM-NT	*	0.00[a]	ND	3.51[a]	3.65[a]	0.25[a]
3-NT-SF	*	0.64[a]	0.64[a]	ND	1.02[a]	ND
4-NT-CP	*	0.37[a]	0.37[a]	ND	0.62[a]	ND
5-NT-BA-SF	*	0.67[a]	0.67[a]	ND	0.14[a]	ND
6-NT-BA-CP	*	0.00[a]	ND	ND	2.63[a]	ND
C. Diplodia ear rot (%)						
1-MM-CT	*	2.69[a]	ND	12.40[a]	3.89 (0.47)[b]	0.96[a]
2-MM-NT	*	5.02[a]	3.78[a]	11.60[a]	3 .00(0.34)[b]	1.75[a]
3-NT-SF	*	2.19[a]	1.50[a]	5.80[a]	4.18 (0.50)[b]	1.50[a]
4-NT-CP	*	3.94[a]	3.46[a]	0.30[a]	2.05 (0.30)[b]	5.43[a]
5-NT-BA-SF	*	2.42[a]	1.68[a]	1.10[a]	0.87 (1.39)[a]	0.75[a]
6-NT-BA-CP	*	3.10[a]	3.87[a]	0.60[a]	0.94(-2.11)[a]	0.80[a]
D. *F. verticillioides* (pg/µL)						
1-MM-CT	311[a]	909[a]	11.60[a]	29[a]	281[a]	241[a]
2-MM-NT	606[a]	2023[a]	20.10[a]	223[a]	257[a]	160[a]
3-NT-SF	667[a]	1895[a]	3.70[a]	30[a]	334[a]	209[a]
4-NT-CP	742[a]	809[a]	6.80[a]	141[a]	115[a]	251[a]
5-NT-BA-SF	410[a]	1115[a]	6.30[a]	40[a]	417[a]	260[a]
6-NT-BA-CP	583[a]	2198[a]	2.10[a]	48[a]	305[a]	210[a]
E. *F. graminearum* (pg/µL)						
1-MM-CT	58[a]	4.63[a]	15.70[a]	157[a]	133[a]	234[a]
2-MM-NT	59[a]	20.03[a]	7.20[a]	946[a]	285[a]	430[a]
3-NT-SF	77[a]	2.62[a]	5.90[a]	282[a]	126[a]	115[a]
4-NT-CP	56[a]	14.86[a]	38.60[a]	339[a]	139[a]	147[a]
5-NT-BA-SF	35[a]	11.66[a]	18.20[a]	249[a]	268[a]	385[a]
6-NT-BA-CP	113[a]	59.07[b]	10.40[a]	84[a]	49[a]	251[a]

Treatment	Season					
	2009/10	2010/11	2011/12	2012/13	2013/14	2014/15
F. Fumonisins (ppm)						
1-MM-CT	1.78[a]	0.08(-1.91)[a]	ND	0.05[a]	0.16[a]	0.67[a]
2-MM-NT	0.45[a]	1.43(1.35)[ab]	ND	0.15[a]	0.04[a]	0.17[a]
3-NT-SF	0.30[a]	8.87(0.91)[b]	ND	0.01[a]	0.06[a]	0.15[a]
4-NT-CP	0.15[a]	0.06(-1.97)[a]	0.01[a]	0.20[a]	0.02[a]	0.11[a]
5-NT-BA-SF	0.83[a]	1.27(-2.56)[a]	0.01[a]	0.20[a]	0.08[a]	0.31[a]
6-NT-BA-CP	0.90[a]	0.56(0.51)[a]	ND	0.18[a]	0.16[a]	0.17[a]
G. Zearalenone (ppm)						
1-MM-CT	0.02[a]	0.11[a]	ND	ND	ND	ND
2-MM-NT	0.12[a]	0.26[a]	ND	ND	ND	ND
3-NT-SF	ND	ND	ND	ND	ND	ND
4-NT-CP	0.22[a]	0.13[a]	ND	ND	ND	ND
5-NT-BA-SF	0.11[a]	ND	ND	ND	ND	ND
6-NT-BA-CP	ND	0.13[a]	ND	ND	ND	ND
H. Deoxynivalenol (ppm)						
1-MM-CT	ND	0.04[a]	0.52(-0.73)[b]	0.18[a]	0.60[a]	ND
2-MM-NT	ND	0.08[a]	0.18(1.92)[ab]	0.02[a]	1.12[a]	ND
3-NT-SF	ND	0.11[a]	0.03 (2.50)[a]	ND	0.39[a]	ND
4-NT-CP	ND	0.14[a]	0.0(-3.00)[a]	ND	0.64[a]	0.01
5-NT-BA-SF	ND	0.04[a]	0.02 (2.56)[a]	0.01[a]	1.07[a]	0.07
6-NT-BA-CP	ND	0.38[a]	0.02 (2.25)[a]	ND	0.48[a]	0.01
Nivalenol (ppm)						
1-MM-CT	ND	ND	ND	ND	ND	ND
2-MM-NT	ND	ND	ND	ND	ND	ND
3-NT-SF	ND	ND	ND	ND	ND	ND
4-NT-CP	ND	ND	ND	ND	ND	ND
5-NT-BA-SF	ND	ND	ND	ND	ND	ND
6-NT-BA-CP	ND	ND	ND	ND	ND	ND

[1]MM-CT = Maize monoculture conventionally tilled
[2]MM-NT= Maize monoculture no-till
[3]NT-SF = No-till maize/sunflower 2-year rotation
[4]NT-CP = No-till maize/cowpea 2-year rotation
[5]NT-BA-SF = No-till maize/babala/sunflower 3-year rotation
[6]NT-BA-CP = No-till maize/babala/cowpea 3-year rotation
[#]Values in brackets are log base 10 transformed
[#]Different letters indicate significant differences within a column ($P \leq 0.05$)
*= Data not available
ND = Not detected

Table 6. Analysis of variance on the effects of cropping systems on Gibberella ear rot (%) severity in maize grain from 2009/10 to 2014/15 in Buffelsvallei.

2010/11

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1.7464	0.3493	1.35	0.297
Residual	15	3.8799	0.2587		
Total	23	5.8193			

2011/12

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	2.0033	0.4007	0.85	0.537
Residual	13	6.1078	0.4698		
Total	21	8.8738			

2012/13

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	30.742	6.148	1.00	0.465
Residual	10	61.484	6.148		
Total	17	104.522			

2013/14

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	39.560	7.912	1.97	0.142
Residual	15	60.198	4.013		
Total	23	114.342			

2014/15

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1.3408	0.2682	2.25	0.103
Residual	15	1.7889	0.1193		
Total	23	3.8495			

Values are significant at $P \leq 0.05$

Table 7. Analysis of variance on the effects of cropping systems on Diplodia ear rot (%) severity in maize grain from 2009/10 to 2014/15 in Buffelsvallei.

2010/11

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.32857	0.06571	2.16	0.113
Residual	15	0.45575	0.03038		
Total	23	0.83981			

2011/12

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	27.267	5.453	2.18	0.111
Residual	15	37.545	2.503		
Total	23	73.476			

2012/13

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	469.11	93.82	1.08	0.425
Residual	10	865.25	86.53		
Total	17	1423.93			

2013/14

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	25.848	5.170	4.84	0.008
Residual	15	16.019	1.068		
Total	23	47.205			

2014/15

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	64.11	12.82	0.79	0.576
Residual	15	244.81	16.32		
Total	23	328.12			

Values are significant at $P \leq 0.05$

Table 8. Analysis of variance on the effects of cropping systems on *Fusarium verticillioides* target DNA in maize grain from 2009/10 to 2014/15 in Buffelsvallei.

2009/10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	4010122.	802024.	0.52	0.761
Residual	15	23336857.	1555790.		
Total	23	30944649.			

2010/11

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	7565176.	1513035.	0.79	0.572
Residual	15	28647330.	1909822.		
Total	23	56746573.			

2011/12

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	863.5	172.7	1.36	0.295
Residual	15	1911.7	127.4		
Total	23	3780.2			

2012/13

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	95721.	19144.	1.40	0.304
Residual	10	136765.	13676.		
Total	17	245947.			

2013/14

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	199154.	39831.	0.54	0.740
Residual	15	1097405.	73160.		
Total	23	1627657.			

2014/15

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	27201.	5440.	0.42	0.830
Residual	15	196246.	13083.		
Total	23	236158.			

Values are significant at $P \leq 0.05$

Table 9. Analysis of variance on the effects of cropping systems on *Fusarium graminearum* target DNA in maize grain from 2009/10 to 2014/15 in Buffelsvallei.

2009/10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	87108.	17422.	0.51	0.761
Residual	15	507692.	33846.		
Total	23	628190.			

2010/11

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	8610.0	1722.0	4.60	0.010
Residual	15	5617.0	374.5		
Total	23	17289.4			

2011/12

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	4197.5	839.5	1.62	0.214
Residual	15	7757.9	517.2		
Total	23	13056.6			

2012/13

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1434578.	286916.	0.91	0.510
Residual	10	3143972.	314397.		
Total	17	4774948.			

2013/14

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	166310.	33262.	1.79	0.176
Residual	15	279014.	18601.		
Total	23	538304.			

2014/15

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	317027.	63405.	0.98	0.463
Residual	15	972786.	64852.		
Total	23	1523391.			

Values are significant at $P \leq 0.05$

Table 10. Analysis of variance on the effects of cropping systems on fumonisin contamination in maize grain from 2009/10 to 2014/15 in Buffelsvallei.

2009/10					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	9.4644	1.8929	2.51	0.077
Residual	15	11.3105	0.7540		
Total	23	26.0376			
2010/11					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	229.989	45.998	5.25	0.006
Residual	15	131.520	8.768		
Total	23	365.807			
2011/12					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.00025922	0.00005184	0.75	0.597
Residual	15	0.00103266	0.00006884		
Total	23	0.00142191			
2012/13					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.10129	0.02026	0.49	0.774
Residual	10	0.40956	0.04096		
Total	17	0.61556			
2013/14					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.07518	0.01504	0.73	0.609
Residual	15	0.30716	0.02048		
Total	23	0.45459			
2014/15					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	3.1601	0.6320	0.76	0.593
Residual	15	12.4921	0.8328		
Total	23	17.9160			

Values are significant at $P \leq 0.05$

Table 11. Analysis of variance on the effects of cropping systems on zearalenone contamination in maize grain from 2009/10 to 2014/15 in Buffelsvallei.

2009/10					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.09514	0.01903	0.41	0.836
Residual	15	0.69985	0.04666		
Total	23	0.90365			
2010/11					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.18741	0.03748	1.08	0.411
Residual	15	0.52091	0.03473		
Total	23	1.00070			

Values are significant at $P \leq 0.05$

Table 12. Analysis of variance on the effects of cropping systems on deoxynivalenol contamination in maize grain from 2009/10 to 2014/15 in Buffelsvallei.

2010/11					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.32857	0.06571	2.16	0.113
Residual	15	0.45575	0.03038		
Total	23	0.83981			

2011/12					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	12.3420	2.4684	3.37	0.031
Residual	15	10.9916	0.7328		
Total	23	26.9117			

2012/13					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	0.08045	0.01609	0.93	0.501
Residual	10	0.17297	0.01730		
Total	17	0.28337			

2013/14					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	1.8667	0.3733	0.74	0.607
Residual	15	7.5979	0.5065		
Total	23	9.6662			

2014/15					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	5	4.1402	0.8280	1.79	0.175
Residual	15	6.9223	0.4615		
Total	23	11.3154			

Values are significant at $P \leq 0.05$

Table 13. Analysis of variance on the effects of cropping systems on Fusarium ear rot (%) severity in maize grain from 2011/12 to 2014/15 in Erfdeel.

2011/12					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	568.7	189.6	0.39	0.765
Residual	9	4411.7	490.2		
Total	15	5910.3			

2012/13					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	73.606	24.535	4.13	0.062
Residual	9	53.422	5.936		
Total	15	137.209			

2013/14					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	23.319	7.773	1.31	0.329
Residual	9	53.287	5.921		
Total	15	221.249			

2014/15					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	12.939	4.313	0.51	0.685
Residual	9	76.136	8.460		
Total	15	124.714			

Values are significant at $P \leq 0.05$

Table 14A-G. Mean groupings of Fusarium ear rot, Gibberella ear rot, Diplodia ear rot severity, *Fusarium verticillioides*, *Fusarium graminearum* target DNA, fumonisin, zearalenone, deoxynivalenol and nivalenol contamination in maize grain samples measured between 2011/12 - 2014/15 in Erfdeel.

Treatment	2011/12	2012/13	2013/14	2014/15
A. Fusarium ear rot (%)				
1-MM-CT	15.5[a]	3.25[a]	3.80[a]	10.31[a]
2-MM-NT	31.3[a]	8.72[a]	5.20[a]	12.50[a]
3-NT-CP	23.4	3.73[a]	2.20[a]	10.79[a]
4-NT-BA-CP	18.6[a]	5.50[a]	5.20[a]	10.31[a]
B.Gibberella ear rot (%)				
1-MM-CT	ND	ND	8.47[a]	0.31[a]
2-MM-NT	ND	ND	8.40[a]	ND
3-NT-CP	ND	ND	10.59[a]	ND
4-NT-BA-CP	ND	ND	11.06[a]	ND
C. Diplodia ear rot (%)				
1-MM-CT	0.86[a]	3.80[a]	1.37[a]	2.82[a]
2-MM-NT	2.13[a]	4.50[a]	1.00[a]	2.90[a]
3-NT-CP	0.40[a]	5.90[a]	1.00[a]	1.33[a]
4-NT-BA-CP	0.42[a]	2.40[a]	0.45[a]	1.63[a]
D. *F. verticillioides* (pg/µL)				
1-MM-CT	3.20[a]	271[a]	93[a]	195[a]
2-MM-NT	15.40[a]	39[a]	179[a]	264[a]
3-NT-CP	2.30[a]	25[a]	427[a]	263[a]
4-NT-BA-CP	7.30[a]	175[a]	93[a]	235[a]
E. *F. graminearum* (pg/µL)				
1-MM-CT	7.20[a]	214[a]	74[a]	163[a]
2-MM-NT	33.70[a]	10[a]	26[a]	110[a]
3-NT-CP	5.90[a]	151[a]	136[a]	134[a]
4-NT-BA-CP	7.90[a]	71[a]	47[a]	149[a]
F. Fumonisins (ppm)				
1-MM-CT	ND	0.19[a]	0.55[a]	0.01[a]
2-MM-NT	ND	0.45[a]	0.25[a]	0.01[a]
3-NT-CP	0.01[a]	0.19[a]	0.14[a]	0.01[a]
4-NT-BA-CP	0.01[a]	0.07[a]	0.08[a]	0.04[a]
G. Zearalenone(ppm)				
1-MM-CT	ND	ND	ND	ND
2-MM-NT	ND	ND	ND	ND
3-NT-CP	ND	ND	ND	ND
4-NT-BA-CP	ND	ND	ND	ND
H. Deoxynivalenol (ppm)				
1-MM-CT	0.01[a]	0.30[a]	0.12[a]	ND
2-MM-NT	0.15[a]	0.17[a]	0.47[a]	ND
3-NT-CP	0.01[a]	0.09[a]	0.25[a]	ND
4-NT-BA-CP	ND	0.51[a]	0.23[a]	ND

Treatment	2011/12	2012/13	2013/14	2014/15
I. Nivalenol (ppm)				
1-MM-CT	ND	ND	ND	ND
2-MM-NT	ND	ND	ND	ND
3-NT-CP	ND	ND	ND	ND
4-NT-BA-CP	ND	ND	ND	ND

[1]MM-CT = Maize monoculture conventionally tilled

[2]MM-NT= Maize monoculture no-till

[3]NT-CP=No till maize/cowpea 2-year rotation

[4]NT-BA-CP=No-till maize/babala/cowpea 3-year rotation

[#]Values in brackets are log base 10 transformed

[#]Different letters indicate significant differences within a column ($P \leq 0.05$)

*= No planting due to highly acidic soil/ data not available

ND = Not detected

Table 15. Analysis of variance of the effects of cropping systems on Gibberella ear rot (%) severity in maize grain from 2011/12 to 2014/15 in Erfdeel.

2011/12					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.	0.		
Residual	9	0.	0.		
Total	15	0.			
2012/13					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.	0.		
Residual	9	0.	0.		
Total	15	0.			
2013/14					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	24.74	8.25	0.50	0.694
Residual	9	149.57	16.62		
Total	15	184.80			
2014/15					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.27908	0.09303	1.00	0.436
Residual	9	0.83723	0.09303		
Total	15	1.39538			

Values are significant at $P \leq 0.05$

Table 16. Analysis of variance of the effects of cropping systems on Diplodia ear rot severity in maize grain from 2011/12 to 2014/15 in Erfdeel.

2011/12					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	7.964	2.655	1.85	0.208
Residual	9	12.916	1.435		
Total	15	31.878			
2012/13					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	25.70	8.57	0.52	0.680
Residual	9	148.60	16.51		
Total	15	184.79			
2013/14					
	15	1.8324			
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	1.707	0.569	0.29	0.833
Residual	9	17.772	1.975		
Total	15	20.250			
2014/15					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	7.836	2.612	1.17	0.373
Residual	9	20.021	2.225		
Total	15	54.429			

Values are significant at $P \leq 0.05$

Table 17. Analysis of variance of the effects of cropping systems on *Fusarium verticillioides* target DNA accumulation in maize grain from 2011/12 – 2014/15 in Erfdeel.

2011/12

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	402.76	134.25	1.99	0.186
Residual	9	607.37	67.49		
Total	15	1119.95			

2012/13

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	166301.	55434.	2.57	0.119
Residual	9	194372.	21597.		
Total	15	571775.			

2013/14

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	300199.	100066.	1.61	0.254
Residual	9	558119.	62013.		
Total	15	939102.			

2014/15

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	12778.	4259.	0.35	0.792
Residual	9	110549.	12283.		
Total	15	124286.			

Values are significant at $P \leq 0.05$

Table 18. Analysis of variance of the effects of cropping systems on *Fusarium graminearum* target DNA accumulation in maize grain from 2011/12 to 2014/15 in Erfdeel.

2011/12					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	2149.9	716.6	2.82	0.099
Residual	9	2283.5	253.7		
Total	15	5461.1			
2012/13					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	96205.	32068.	1.56	0.265
Residual	9	184722.	20525.		
Total	15	316374.			
2013/14					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	27428.	9143.	0.63	0.612
Residual	9	130040.	14449.		
Total	15	183968.			
2014/15					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	6011.	2004.	0.13	0.941
Residual	9	140052.	15561.		
Total	15	160844.			

Values are significant at $P \leq 0.05$

Table 19. Analysis of variance of the effects of cropping systems on fumonisin contamination in maize grain from 2011/12 to 2014/15 in Erfdeel.

2011/12					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.09573	0.03191	1.08	0.407
Residual	9	0.26675	0.02964		
Total	15	0.44685			
2012/13					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	1.2446	0.4149	0.46	0.717
Residual	9	8.1292	0.9032		
Total	15	9.6582			
2013/14					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.5201	0.1734	1.27	0.342
Residual	9	1.2284	0.1365		
Total	15	1.8881			
2014/15					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.003888	0.001296	1.00	0.436
Residual	9	0.011664	0.001296		
Total	15	0.019440			

Values are significant at $P \leq 0.05$

Table 20. Analysis of variance of the effects of cropping systems on deoxynivalenol contamination in maize grain from 2011/12 to 2014/15 in Erfdeel.

2011/12					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.06260	0.02087	1.07	0.409
Residual	9	0.17521	0.01947		
Total	15	0.28978			
2012/13					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.4105	0.1368	1.11	0.396
Residual	9	1.1141	0.1238		
Total	15	1.8319			
2013/14					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.2647	0.0882	0.63	0.614
Residual	9	1.2601	0.1400		
Total	15	1.8324			
2014/15					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.0008619	0.0002873	0.90	0.476
Residual	9	0.0028605	0.0003178		
Total	15	0.0048184			

Values are significant at $P \leq 0.05$

Table 21. Monthly accumulated rainfall, mean maximum temperatures and accumulated reference potential evapotranspiration at Buffelsvallei and Erfdeel.

Buffelsvallei

Month	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total/ Mean
						Rainfall (mm)							
2008/09								70	-	-	-	28	
2009/10	6	23	20	36	60	109	109	88	122	76	14	0	663
2010/11	0	0	0	10	73	126	98	77	90	81	11	34	600
2011/12	0	0	0	46	37	141	47	26	87	4	0	13	401
2012/13	0	0	25	39	46	164	101	32	88	45	1	0	541
2013/14	0	0	0	27	41	120	101	209*	103	4	2	3	609
2014/15	0	21	3	9	115	128	174	55	121	60	1	6	693
						Mean maximum temperature (ºC)							
2008/09								26.9	27.1	26.7	22.5	19.2	
2009/10	17.3	21.3	27.3	27.4	27.1	30.6	26.9	29.6	28.3	25.5	20.5	19.2	25.1
2010/11	13.8	23.7	28.5	29.8	28.8	28.6	27.2	28.2	27.9	23.2	21.9	19.1	25.1
2011/12	17.8	22.2	27.5	28.9	30.0	29.0	31.0	29.7	28.8	25.0	24.9	20.0	26.2
2012/13	20.8	22.7	24.7	29.4	30.3	27.2	30.2	31.6	28.9	25.6	23.7	21.8	26.4
2013/14	21.4	22.1	27.6	29.0	31.3	27.0	30.8	28.7	25.9	24.7	24.7	21.0	26.2
2014/15	20.0	23.1	28.4	30.8	27.6	29.4	30.1	31.0	28.5	26.9	27.8	20.3	27.0

Erfdeel

Month	Jul	Aug	Sept	Oct	Nov	Des	Jan	Feb	Mar	Apr	May	Jun	Total/ Mean
						Rainfall (mm)							
2008/09								65	53	3	4	31	
2009/10	2	0	28	49	74	156	161	74	87	26	33	0	690
2010/11	0	0	0	13	74	155	50*	55*	60	53	51	40	551
2011/12	8	0	4	24	24	135	71	84	30	9	1	13	403
2012/13	2	2	58	56	61	136	94	41	14	55	1	0	542
2013/14	0	0	0	42	63	150	111	62	139	8	13	4	594
2014/15	0	24	0	20	87	85	85	45	69	53	1	6	475
						Mean maximum temperature (ºC)							
2008/09								27.7	27.6	27.0	22.7	19.6	
2009/10	18.2	22.5	28.2	28.0	27.4	31.1	27.7	30.4	29.2	25.7	24.2	20.9	26.1
2010/11	21.0	24.5	29.6	30.3	29.8	29.3	28.4	29.2	29.8	24.4	22.5	19.8	26.6
2011/12	18.6	22.8	27.7	29.0	30.2	29.1	31.6	29.8	29.8	26.2	26.2	20.0	26.8
2012/13	21.4	23.7	24.9	29.4	30.5	28.6	30.8	32.0	29.5	25.7	24.2	21.7	26.9
2013/14	21.8	22.4	27.6	29.5	30.8	27.9	31.2	29.6	26.6	25.5	24.9	21.2	26.6
2014/15	20.1	23.3	28.7	30.8	27.2	30.2	31.3	31.4	28.6	26.8	27.0	19.9	27.1

CHAPTER 3

Prevalence and persistence of maize ear rot causing fungi and mycotoxin contamination in grain and crop residues of commercial maize grown under different tillage systems in South Africa

ABSTRACT

Maize is the largest field crop in South Africa and the third most important cereal crop in the world following wheat and rice. Maize ear rots may limit maize production by resulting in yield loss, quality reduction and safety concerns due to mycotoxin contamination. Mycotoxins have been associated with oesophageal cancer in humans and various mycotoxicoses in animals. Furthermore, mycotoxin contamination poses serious economic losses such as reduced exports, which can heavily impact on developing countries. The sustainable production of maize has driven the adoption of minimised tillage systems, which have the potential to preserve soil resources and increase profits. This study focused on investigating the influence of conventional tillage and no-till practices on the natural accumulation of *Fusarium verticillioides* and *F. graminearum* and the mycotoxins they produce in maize grain. The survival of these ear rot-causing fungi on maize crop residues was also investigated before and after harvest over a two-year period. Furthermore, the incidence of Diplodia ear rot (DER) in commercial maize production areas in South Africa was also assessed. Grain and crop residue samples were collected from eight no-till and six conventional tillage farms in 2013/14 as well as from nine no-till and seven conventional tillage farms in the 2014/15 season. Ear rot target DNA content in grain and crop residues were determined by quantitative PCR and Fusarium and Gibberella ear rot severity as well as DER incidence was determined visually. Fumonisins and zearalenone were quantified by high performance liquid chromatography while deoxynivalenol and nivalenol was quantified using liquid chromatography tandem mass spectrometry. Tillage practices did not markedly affect ear rot and mycotoxin contamination in grain and crop residues during both seasons of this study. Seasonal variation had a significant ($P < 0.005$) effect on *F. verticillioides* DNA accumulation in maize grain during the 2013/14 season. Environmental factors played a role in the occurrence of maize ear rots and subsequent mycotoxin production in this study. The presence of maize ear rot fungal inoculum prior to planting should be considered and further studies should explore the influence this has on infection of maize grain and mycotoxin accumulation.

INTRODUCTION

South Africa loses approximately 400 million tons of soil per year due to soil erosion, making it the country with the highest soil loss worldwide (Berger *et al*., 2009; Anonymous, 2013). Soil loss, paired with unreliable and poorly distributed rainfall patterns serve as a threat to agricultural crop production in the country (Smit, 1998). Therefore, the need for crop production systems that ensure the preservation of top soil and soil moisture while preventing run-off is of great significance (Smit, 1998). Conservation agriculture (CA) has therefore been established as an alternative farming system that aims to achieve high yields in a more sustainable way through the application of minimal soil disturbance, crop rotation and crop residue retention (Du Toit, 2007). This system is used to enhance soil fertility and water infiltration while reducing soil erosion and input costs (Lawrance *et al.*, 1999; Lori *et al*., 2009). South Africa practices CA on approximately 368000 ha of South Africa's arable land and contributes to the majority of the 0.4% CA practiced in Africa (Derpsch and Friedrich, 2009).

Upon adoption of CA farming, an estimated increase of up to 36% in crop yields and nett farm income was reported after the first five years (Du Toit, 2007) due to increased crop yield and less inputs required over time (Berger *et al*., 2009). Crop residue retention has been identified as a crucial part of CA and possible benefits include minimal soil erosion, soil moisture and organic carbon preservation, increased water infiltration and less herbicide usage (Florentin *et al*., 2010). It is also assumed that crop residues will return to the soil resulting in higher yielding crops (Monneveux *et al*., 2006). Difficulties associated with CA may include the inability to manage weeds and the potential for increased disease incidence as crop residues may promote a build-up of primary inoculum (Cotten and Munkvold, 1998; Dill-Macky and Jones, 2000).

Maize diseases caused by mycotoxigenic fungi pose a particular risk not only to humans but animals as well. Predominant maize ear rots in most maize-producing areas include Fusarium, Gibberella and Diplodia ear rot (Boutigny *et al.*, 2012). Fusarium ear rot (FER) results from infection by *Fusarium verticillioides* Saccardo Nirenberg [= *F. moniliforme* (Sheldon)], *Fusarium proliferatum* (Matsushima) Nirenberg and *Fusarium subglutinans* (Wollenw. & Reink) Nelson *et al*. (Leslie and Summerell, 2006; Boutigny *et al.*, 2012). *Fusarium verticillioides* is the most common Fusarium species isolated from maize (Boutigny *et al.*, 2012), particularly from the warmer maize production areas of South Africa (Janse van Rensburg *et al*., 2015). Gibberella ear rot (GER) caused by *Fusarium graminearum* (Schwabe*)* [Teleomorph *Gibberella zeae* (Schwein. Petch] is also widely distributed in most production areas and the fungus has become the predominant species associated with GER in South Africa (Boutigny *et al*., 2012). DER caused by *Stenocarpella maydis* (Berkeley)

(Syn) (*Diplodia maydis*) (Berk.) (Sacc) is associated with lodging of plants and discolouration of infected maize grain (Masango *et al*., 2015). DER was responsible for severe epidemics during the mid-1980s in South Africa with losses of up to R200 million (van Rensburg and Ferreira, 1997). This disease was previously restricted to southern African countries but has since been reported in various South American countries (Odriozola., 2005; Masango *et al*., 2015).

Following fungal infection, ear rot causing fungi are likely to contaminate grain with mycotoxins (Luongo *et al.*, 2005). The subsequent contamination of maize grain by mycotoxins is a cause for concern as it can lead to economic, yield and quality reductions (Boutigny *et al*., 2012). Furthermore, mycotoxins are associated with a number of human and animal diseases (Bondy and Pestka, 2000; Whitlow and Hagler, 2005; Zinedine *et al.*, 2007). Management strategies for the control of mycotoxigenic fungi and their toxins include pre- and post- harvest intergrated approaches (Munkvold, 2003). Conservation agriculture and tillage practises may also play a significant role in an integrated management approach.

According to Flett *et al.* (1998), alternating tillage practices have no effect on FER caused by *Fusarium spp*. but can enhance *S. maydis* in maize grain. An increase in GER has been reported in fields employing reduced tillage and is thought to be due to increased inoculum levels associated with crop residue retention (Kommendahl and Windels, 1981; Sutton, 1982). The increased use of cropping systems that support the retention of crop residues in the field could have substantial impacts on maize production and its safety for consumption by humans and animals. Adequate understanding of the role of crop residues and tillage practices in disease outbreaks can assist in enhancing management for maize ear rot pathogens (Bailey, 1996). Therefore, the effect of tillage practices on the accumulation of *F. verticillioides*, *F. graminearum* and their associated mycotoxins as well as the incidence of DER in maize grain under natural infection was investigated. Additionally, the accumulation of *F. verticillioides* and *F. graminearum* in crop residues was quantified to determine its ability to serve as principal inoculum for disease outbreaks and mycotoxin contamination.

MATERIALS AND METHODS

Field survey

Maize cob collection: Maize cobs were collected from eight different localities in the North-West, Free State and KwaZulu-Natal provinces over two seasons (2013/14; 2014/15) between April and June (Table 1). A total of 13 commercial farms employing no-till or conventional till were randomly selected for sampling in the 2013/14 season while 15 farms were selected for sampling in the 2014/15 season. The maize was allowed to dry in the field and sampling was done when the maize crops were at ≤ 12% grain moisture. Maize ears

(n=60) were randomly selected at each farm per season with a total of 1800 maize cobs evaluated for the two seasons. Cultivars sampled included 32Y85, 6Q354B, 30Y79, SC719, DKLP7374, 2653BR, Monsanto 7845, 6Q445B, 30B97VR, 3878BT, 3270BR and Monsanto 3573 (Table 1).

Crop residue collection: Pieces of residues that included approximately 1 cm to 50 cm length of plant material were collected on 5 x 5 m^2 areas in five random positions in the same conventional till and no-till farms where maize grain was sampled. Sampling was conducted just prior to harvest (May/June) and following harvest just before planting (September/October) from all fields surveyed in the 2013/14 and 2014/15 seasons.

Maize ear rot ratings

Maize ears were visually assessed for symptoms of FER and GER severity while DER incidence was determined according to Flett *et al.* (1998). The ear rot severity and incidence was determined based on discoloration and the percentage of visibly diseased grain samples was calculated by mass. To date, no method is available for the quantification of mycotoxins produced by *S. maydis*.

Quantification of *F. verticillioides* and *F. graminearum* s.l.

DNA extraction from grain: After threshing, a 250 g grain sub-sample was taken from each treatment and ground to fine powder through a 1 mm mesh using a Cyclotech sample mill (Foss Tecator, Hoganas, Sweden). These samples were stored at -20°C for further analysis. The DNeasy Plant Mini kit (Qiagen, Hilden, Germany) was used to extract DNA from the 0.5 g of milled flour according to the manufacturer's guidelines.

DNA extraction from crop residues: Crop residues were ground to fine powder using a Fritsch pulvensette corn mill (Germany). The CTAB method adapted from Winnepenninckx *et al*. (1993) was used to isolate DNA. Ground crop residue material (250 µg) was transferred to 2-mL tubes and 900 µL of the extraction buffer (CTAB 2% [1 M Tris (pH 8.0), 0.5 M EDTA, 5 M NaCl, 2% (w/v) CTAB]) was added. The tubes were briefly frozen in liquid nitrogen and subsequently placed in boiling water for 5 min. RNAse (2 µL) was added to each tube and the samples were then incubated in a water bath at 37ºC for 30 min. The samples were treated with 800 µL chloroform: isoamyl alcohol (24:1 v/v) and mixed by vortexing. The samples were centrifuged for 10 min at 12 000 rpm, and the supernatant (approximately 600 µL) was transferred to new tubes containing 500 µL iso-propanol. The contents were centrifuged for 20 min at 12 000 rpm (4ºC) and the supernatant was discarded. The DNA pellet was washed twice with 500 µL ice-cold 70% ethanol by

centrifugation at 12 000 rpm for 5 min. The supernatant was carefully discarded and the samples were allowed to air dry completely before adding 120 µL TE buffer [10 mM Tris (pH 8.0), 1 mM EDTA (pH 8.0)]. The samples were placed in a refrigerator (4ºC) overnight following which the purity and the concentration of both the grain and crop residue DNA were measured using a Nanodrop® (2000c) Spectrophotometer (Thermo Scientific, Waltham, USA) at 260 nm (OD260). The DNA was diluted to 10 000 pg/µL and stored at -20ºC in 100 µL aliquots.

Standard curves: Isolates obtained from the Medical Research Council including a high fumonisin-producing isolate (MRC826) and a *F. graminearum sensu lato* isolate (MRC 394) were used to generate standard curves. These isolates were plated out on potato dextrose agar (PDA) for one week and the CTAB method as adapted Winnepenninckx *et al*. (1993) was used to isolate DNA from the mycelial growth. A standard curve for *F. verticillioides* quantification (Waalwijk *et al.*, 2008) was generated from a 10-fold dilution of the MRC826 DNA. The dilution range was 60 000; 6 000; 600; 60 and 6 pg/µL. For *F. graminearum* s.l, a 4-fold standard dilution was used to generate a standard curve for quantification (Nicolaisen *et al*., 2009). The dilution range was 7500; 1875; 468.8; 117.2 and 29.3 pg/µL. Two replicates per dilution were used to generate the standard curves for both fungi.

Quantification of F. verticillioides *and* F. graminearum s.l. *target DNA*: Primers used for the detection of *F. verticillioides* were Taqfum-2F (ATG CAA GAG GCG AGG CAA) and Vpgen-3R (GGC TCT CRG AGC TTG GCA T) in combination with the FUM-Probe 1 primer as described by Waalwijk *et al*. (2008). A total reaction mixture of 25 µL containing 4 µL of sample DNA (10 000 pg/µL) mixed with 12.5 µL sensimix, 2.125 µL Fum probe (1 µM), 0.875 µL Taqfum-2F: (333 nM), 0.875 µL Vpgen-3R primer: (333 nM) and 4.625 µL molecular grade water was used for the real time quantitative PCR reactions. Negative controls contained no template DNA but were treated similar to the reaction samples. *Fusarium graminearum* s.l. was quantified according to a method described by Nicolaisen *et al*. (2009). The primers FgramB379 (CCA TTC CCT GGG CGT) and FgramB411 (CCT ATT GAC AGG TGG TTA GTG ACTGG) in combination with SYBRGreen were used. A total volume of 25 µL consisting of 12.5 µL of SYBR® green, 0.625 µL of FgramB379: and 0.625 µL FgramB411:, 9.25 µL of nuclease free water and 2 µL of the unknown target DNA was used for the real time quantitative PCR reactions. Negative controls contained no template DNA but were treated similar to the reaction samples. A CFX96™ Real-Time PCR detection system (Bio-Rad, Hercules, USA) was used for the real time qPCR reactions and cycling conditions for both *F. verticillioides* and *F. graminearum* s.l. consisted of 5 min denaturation at 95ºC, 40 cycles at 95ºC for 10s and 65ºC for 10s, followed by a melt curve step of 95ºC,

and a cooling step at 65ºC. Amplication curves were used to generate data after each reaction was completed. Regression equations of standard curves from quantification cycles were highly significant (R^2>0.99). Slopes were within the accepted criterion (between -3.1 and -3.6) and efficiencies were between 95 and 110%.

Fumonisin quantification

A 50-g sub-sample of maize grain was mixed with 5 g of sodium chloride (Merck, USA) prior to extraction. One hundred millilitres of the extraction solvent (methanol: water (80:20 v/v)) was added to each sample and mixed for 5 min at high speed using a Waring laboratory blender (Waring products division, Torrington, USA). The extract was then filtered through 24-cm fluted filter paper (VICAM) and 10-mL of the eluted liquid was diluted with 40 mL saline phosphate-buffer (1X PBS) (8.0 g NaCl, 1.2 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, dissolved in 990 ml purified water, pH adjusted to 7.0). Microfiber filters (0.45 µm) were used to filter diluted samples and 10 ml of the filtrate was passed through VICAM FumoniTest affinity columns at a flow rate of 1 drop per second. Subsequently, 10ml of PBS was passed through the column at a rate of 1 drop per second. The column was then washed with 1.5 mL HPLC grade methanol at a rate of 1 drop per second and the eluate was collected in a glass cuvette. The methanol eluate was dried in a TurboVap LV (Caliper Sciences) with the aid of a slow stream of high purity nitrogen gas. Samples were dissolved in 200 µL methanol and purified water (50:50 v/v). Each sample (50 µL) was transferred to 250 µL conical inserts which were placed in 2.5 mL glass vials for analyses. The derivatisation agent (*o*-phthaldialdehyde (OPA from Sigma-Aldrich) was placed in the first position of the carousel and set to mix 100 µL of the OPA with the 50 µL of sample by means of a autosampler (Waters 717 plus). This mixture (20 µL) was injected after a delay time of 1 minute.

Fumonisin standards (Sigma Aldrich) were used to generate a standard curve for quantification. The standards were evaporated and reconstituted with a calibration standard solution ranging from 20 ppm, 15 ppm, 10 ppm and 5 ppm. Fluorescence was performed at excitation and emission wavelengths of 335 nm and 440 nm, respectively, using a Waters 2475 multi λ fluorescence detector equipped with a Symmetry C18 (5 µm 3.9 x 150 mm) analytical column (Waters, Milford, USA). The detection limit of the method used was 0.016 ppm and the recovery data were obtained in triplicate by spiking clean maize samples (VICAM) with 5 ppm fumonisin B_1 B_2 and B_3. The average recovery rates were 83% (FB_1), 81% (FB_2) and 83% (FB_3).

Zearalenone quantification

The VICAM method adapted from Kruger *et al*. (1999) was used for zearalenone extraction and quantification. Sodium chloride (5 g) was mixed with 25 g finely ground maize sample

before extraction. In a blender jar (Waring products division, Torrington, USA), a 100 mL methanol: water (80:20 v/v) solvent was added to the sample and the mixture was blended at high speed for two minutes. A 24-cm fluted filter paper (VICAM, Milford, USA) was used to filter the blended extract. A 4 mL volume of the filtered extract was diluted with 96 mL HPLC grade water (18 MΩ.cm) and sieved through a microfiber filter paper (0.45 µm). One hundred millilitre of the extract was passed through a ZearaTest affinity column from VICAM at a rate of approximately 3 drops per second, HPLC grade water (25 mL) was used to subsequently rinse the column. Methanol (0.75 mL) and HPLC grade water (0.5 mL) were passed through the affinity column and the elute (1.25 mL) was collected in a glass cuvette. Fifty microliters of the mixture was injected to the HPLC system for analysis.

A standard curve was generated using zearalenone standards from Sigma-Aldrich. Standards were evaporated and reconstituted with a calibration standard solution ranging from 0.25 ppm, 0.5 ppm, 1.25 ppm, 2.5 ppm. The mobile phase consisted of acetonitrile: methanol: water (46:46:8 v/v/v). The detection limit of the method used was 0.0019 ppm. Fluorescence was performed at excitation and emission wavelengths of 274 nm and 440 nm respectively using a waters 474 multi λ scanning fluorescence detector. The analytical column, Symmetry C18 3.9 x 150 mm (Waters, Milford, USA) was used at a flow rate of 1mL/min. Recovery data was obtained in triplicate by spiking clean maize samples (VICAM) with 5 ppm zearalenone. Average percentage recovery was 112%.

Deoxynivalenol and nivalenol quantification

Fifty grams of milled maize sub samples was blended at high speed (Waring products division, Torrington, USA) for 3 min in 200 mL of HPLC grade purified water (18 MΩ.cm). Ten millilitres of supernatant which was filtred through fluted filter paper (24 cm) from VICAM was mixed with 40 mL phosphate buffered saline (PBS) 8.0 g NaCl, 1.2 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, dissolved in 990 mL purified water with pH adjusted to 7.0). This mixture was poured into a 11 cm folded filter paper and the 5 mL extract was passed through a glass syringe barrel on a pump and passed through deoxynivalenol/nivalenol WB affinity columns from VICAM at a 1 drop per 2 seconds rate. Ten millilitres of PBS, followed by HPLC grade purified water (10 mL) were used to rinse the column at a 1 drop/ second rate. Acetonitrile (1.5 mL) and methanol (0.5 mL) were passed through the affinity column and the elute (2 mL) was collected in a glass cuvette.

A standard curve was generated where deoxynivalenol and nivalenol standards obtained from Sigma Aldrich were evaporated and reconstituted with a calibration standard solution ranging from 0.1 ppm, 0.5 ppm and 5 ppm for nivalenol and 0.1 ppm, 0.5 ppm and 5 ppm for deoxynivalenol. Recovery limits were 0.03 ppm for deoxynivalenol and 0.04 ppm for nivalenol.Average percentage recovery was 90% for both deoxynivalenol and nivalenol. The

LCMS/MS system at the Stellenbosch University, Central analytical facilities (Dr M. Stander), Stellenbosch, South Africa was used to quantify deoxynivalenol and nivalenol.

Climatic data

Climatic data for the 2013/14 and 2014/15 seasons were collected from eight different localities by the Agricultural Research Council's Soil, Water and Climate Institute. Monthly minimum and maximum temperatures (^{0}C) and rainfall (mm) were recorded between November of the planting year and May of the corresponding harvesting year.

Statistical analysis

Data analysis was performed by the Agricultural research council's Biometry unit, using SAS statistical software (SAS 9.3). The data of all the variables was analysed using analysis of variance (ANOVA) statistical models and means of significant differences were compared using Fischer's protected t-test least significant differences (LSD) at a 5% significance level.

RESULTS

Fifteen farms, representing three maize-producing provinces in South Africa were surveyed (Table 1). Of these, 47% employed conventional tillage practises and 60% employed no-till as one farm employed both tillage practises (Table 1; Fig. 1). Sixty percent of the total number of farms surveyed practised crop rotation and 40% planted monoculture maize. Farmers, that utilised conventional tillage practises, more actively (57%) committed to a crop rotation system using wheat, sunflower or soybean (Table 1; Fig. 2) when compared to farmers who employed no-till (44%) (Fig. 3). Furthermore, monoculture maize was more often planted by farmers employing no-till (55%) (Fig. 3) as compared to farmers that use conventional till (43%) (Fig. 2).

Maize grain

Fusarium ear rot disease ratings: No significant relationship between tillage practice and FER severity was established during both study seasons (Table 2). Mean FER severity during 2013/14 no-till and conventionally tilled farms was 9.80% and 9.50% and no significant differences were observed from FER severity recorded during 2014/15 where FER severity under no-till was 7.88% and 10.36% under conventionally tilled fields (Table 3).

Gibberella ear rot disease ratings: GER severity was not significantly affected by tillage practice during both seasons of the study (Table 4). During 2013/14, mean GER severity recorded in conventionally tilled and no-till farms was 0.83% and 1.67% but did not

significantly differ from GER severity recorded in no-till (1.19%) and conventionally tilled (0.19%) farms during the 2014/15 season (Table 3).

Diplodia ear rot ratings: According to ANOVA tillage practice did not have an effect on DER severity in both study seasons (Table 5). Mean DER incidence recorded during 2013/14 in conventionally tilled and no-till farms was 0.83% and 1.33% respectively and did not differ significantly from DER incidence recorded in 2014/15 for conventional till (0.48%) and no-till (1.11%) farms (Table 3).

Fusarium verticillioides *target DNA*: Tillage practices did not have a significant effect on *F. verticillioides* accumulation during both seasons of the study (data not shown). A combined ANOVA for the two seasons indicated a significant seasonal effect on *F. verticillioides* fungal target DNA accumulation ($P \leq 0.005$; Table 6). The mean fungal target DNA concentration in samples from conventional tillage and no-till was 303.65 and 305.03 pg/uL, respectively, in the 2013/14 season and did not differ significantly (Table 3). It was significantly more than the fungal target DNA measured in grain obtained in 2014/15 conventional till (96.54 pg/uL) farms but did not differ significantly from grain sampled from no-till (144. 70 pg/uL) farms (Table 3).

Fusarium graminearum *target DNA:* According to the ANOVA, tillage practices did not have a significant effect on *F. graminearum* accumulation during both seasons of the study (Table 7). During the 2013/14 season, the target DNA content in grain from conventionally tilled and no-till farms was 95.43 and 37.93 pg/uL, respectively, and did not differ significantly from the *F. graminearum* content measured in 2014/15 for conventional till (39.42 pg/uL) or no-till (43.51 pg/uL) samples (Table 3).

Fumonisins: According to the ANOVA (Table 8), tillage practices did not have a significant effect on fumonisin contamination in maize grain during both study years. Low levels of fumonisins were recorded during both seasons of the study (Table 3). During the 2013/14, mean fumonisin levels measured in samples from conventionally tilled farms and no-till were 0.22 ppm and 0.11 ppm while in 2014/15 no fumonisins were detected in the conventionally tilled fields and the no-till farms contained a mean fumonisin content of 0.06 ppm (Table 3). ANOVA could not establish a significant seasonal effect on fumonisin accumulation (Table 8).

Zearalenone: Zearalenone was not detected in both years during which the survey (2013/14 and 2014/15) (Table 3) was conducted.

Deoxynivalenol and nivalenol: The ANOVA of deoxynivalenol and nivalenol content in maize grain (Table 9; Table 10) indicated that tillage practices did not have a significant effect on the accumulation of these mycotoxins during both study years. During the 2013/14 season, deoxynivalenol recorded in grain from no-till and conventionally tilled farms was 0.13 ppm and 0.36 ppm, respectively. Trace amounts of deoxynivalenol (0.03 ppm) were recorded in grain obtained from conventional tillage farms while no deoxynivalenol was recorded in grain from no-till farms during the 2014/15 season (Table 3). Nivalenol was only detected in grain from the conventionally tilled fields during both seasons of the study with 0.21 ppm and 0.01 ppm recorded for 2013/14 and 2014/15 seasons, respectively (Table 3).

Crop residues

Fusarium verticillioides target DNA: The ANOVA indicated that *F. verticillioides* target DNA accumulation in crop residues did not differ significantly between conventional tillage and no-till farms during the study period (Table 11). *Fusarium verticillioides* target DNA was recovered from all collected residues regardless of tillage system or sampling time (Table 12). During 2013/14, mean *F. verticillioides* target DNA recorded in pre-harvest crop residues under no-till was 168.10 pg/µL while 157.20 pg/µL was measured in grain from conventionally tilled fields (Table 12). This was, however, not significantly different to *F. verticillioides* target DNA recovered from crop residues sampled pre-harvest in no-till farms (44.52 pg/µL) and recovered from conventionally tilled farms (87.54 pg/µL). Conventionally tilled fields, sampled post-harvest had a mean *F. verticillioides* target DNA of 253.10 pg/µL and no-till fields 179.70 pg/µL in 2013/14 and did not significantly differ from post-harvest crop residues collected from no-till fields (348.65 pg/µL) and conventionally tilled farms (147.28 pg/µL) during 2014/15 (Table 12).

Fusarium graminearum target DNA: According to the ANOVA, *F. graminearum* target DNA accumulation in crop residues did not differ between fields under conventional and no-till during the study period (Table 13). *Fusarium graminearum* target DNA was recovered from all collected residues regardless of tillage system or sampling time (Table 12). During 2013/14, mean *F. graminearum* target DNA measured in no-till and conventionally tilled pre-harvest fields was 58.59 pg/µL and 48.46 pg/µL and did not significantly differ.Mean *F. graminearum* target DNA recorded in pre- harvest under conventional tillage and no-till was 66.04 pg/µL and 47.87 pg/µL during 2014/15 (Table 12). In the post-harvest crop residue samples, mean *F. graminearum* target DNA measured in conventionally tilled and no-till farms was 80.71 and 52.57 pg/µL and did not significantly differ from target DNA measured

in crop residues measured in conventionally tilled (73.23 pg/µL) and no till (35.38 pg/µL) farms during the same season (Table 12).

Fumonisins: Fumonisin contamination in crop residues was not significantly affected by tillage practice during both study seasons (Table 14). Trace amounts of fumonisins were quantified from all collected crop residues regardless of tillage system or sampling time (Table 12). During 2013/14 fumonisin content measured in the no-till and conventionally tilled pre-harvest crop residues was 0.05 ppm and 0.01 ppm, respectively, and did not significantly differ from no-till (0.07 ppm) and conventionally tilled (0.01 ppm) pre-harvest crop residues collected in 2014/15 (Table 12). Crop residue samples collected post-harvest in 2013/14 from no-till and conventionally tilled farms contained 0.06 ppm and 0.04 ppm total fumonisins, respectively. This, however, did not differ significantly from the fumonisin content measured in crop residues obtained from no-till farms (0.01 ppm) and conventionally tilled farms (0.00 ppm) during the 2014/15 season (Table 12).

Climatic data

Mean maximum temperature (Table 15), minimum temperature (Table 16) and mean rainfall (Table 17) data were recorded from November to May during 2013/14 and 2014/15 in the eight different localities surveyed. Mean and maximum temperatures did not vary greatly between the two seasons. Mean total rainfall was higher during the 2013/14 season when compared to the 2014/15 season.

DISCUSSION

Agricultural practices such as tillage, crop rotation and crop residue management may have significant effects on pests and pathogens. With no-till practices, crop residues provide pathogens the chance to persist in the soil, reproduce and spread as opposed to conventional tillage methods that plough crop residues into the soil (Steinkellner and Langer, 2004). *Fusarium verticillioides* can overwinter on crop residues and therefore acts as an inoculum reservoir resulting in increased disease severity (Marocco *et al*., 2008). In this study *F. verticillioides* accumulation in maize grain was not affected by tillage practices. These results are in agreement with findings by Flett and Wehner, (1991), Steinkellner and Langer (2004), Marocco *et al.* (2008), Ariño *et al.* (2009) and Ono *et al.* (2011) who all found that *F. verticillioides* accumulation was not affected by tillage practices. A seasonal effect was observed for *F. verticillioides* target DNA accumulation where it was higher during the 2013/14 and lower during the 2014/15 season. *Fusarium verticillioides* proliferation is normally associated with high temperatures and high rainfall towards the harvesting period (Cao *et al*., 2014). The mean rainfall between March and May was lower during 2014/15

season when compared to the 2013/14 season resulting in low *F. verticillioides* accumulation. Furthermore, a trend was observed during the 2014/15 season where *F. verticillioides* accumulation was higher in grain obtained from conventionally tilled fields when compared to no-till fields. Grain from no-till farms contains higher and more consistent soil moisture throughout the growing season than conventional tillage, resulting in less plant stress and a reduction in *Fusarium* spp. infection (Bailey and Duczek, 1996; Marocco *et al*., 2008). This study demonstrated that climatic conditions may be more influential than tillage systems in *F. verticillioides* accumulation under local conditions.

Fusarium graminearum accumulation in maize grain was not affected by tillage practices during both seasons of this study. This result is supported by findings of Maiorano *et al*. (2008) and Lori *et al*. (2009) who observed similar results in wheat. *Fusarium graminearum* accumulation under alternating tillage systems has been found to be inconsistent in studies on wheat (Suproniene *et al.*, 2012) because climate has been found to play a major role on disease development (Champeil *et al*., 2004; Pereyra and Dill-Macky, 2008). *Fusarium graminearum* has been reported to survive on both surface and buried crop residues. However it is able to reproduce and remain active in the first 5cm of the soil making surface crop residues more preferable (Champeil *et al*., 2004). For certain species, crop residues may only be to ensure pathogen survival and may not serve as an inoculum source (Lori *et al*., 2009) which could explain that the presence of *F. graminearum* on crop residues is not a direct indication of enhanced disease severity. This finding is of major significance to maize farmers using no-till practices as it indicates that *F. graminearum* accumulation is not a major threat to maize production under different tillage systems in South Africa.

Although not significant, a consistent trend was observed whereby DER severity percentage was greater over both seasons under no-till maize production systems than on conventional tillage maize production systems. The DER results in the survey follow a similar trend to what was observed in previous studies where cropping systems functioning on surface stubble retention favour the survival of the *S. maydis* pathogen (Flett and Wehner, 1991). Exposed crop residues favour survival and inoculum production by *S. maydis* more than buried crop residues. A positive relationship between DER and stubble mass has been determined and this would explain the prevalence of *S. maydis* under no-till fields (Flett and Wehner, 1991). DER disease severity has been said to be dependent on the amount of infection *S. maydis* stalk and ear infection in the previous growing season and the quantity of crop residues left on the soil surface (Kieh, 2014). Therefore, continued surveillance of DER incidence under no-till conditions is warranted to determine the impact of *S. maydis* prevalence in future disease epidemics, particularly during years when prevailing conditions are favourable for disease development.

Even though the target DNA frequency was generally low during the two seasons, the recovery of *F. verticillioides* and *F. graminearum* target DNA from naturally infected crop debris both before and after harvest in this study is an indication of the persistence of ear rot causing fungi on maize plant residues. The presence of these fungi might allow FER and GER inoculum to be carried over from one season to another (Pereyra and Dill-Macky, 2008). *Fusarium* spp. present in crop residues are however not a direct indication of infection and mycotoxin contamination in grain (Steinkellner and Langer, 2004). However, their presence guarantees survival and possible reproduction and could result in infection of available hosts especially under conducive environmental conditions (Fernandez *et al*., 1993). These results correspond to a report by Whitehair *et al*. (2014) who found no significant differences in *F. graminearum* composition in crop residues from various tillage practices. Crop residue colonisation by fungi may be dependent on several factors including temperature, relative humidity and disease severity in the crop during the previous season and competitive saprophytic ability of the fungi (Fernandez *et al*., 1993). The absence of significant differences may be attributed to some of these factors (Leplat *et al*., 2013).

Tillage practices did not have a significant effect on all mycotoxin accumulation in maize grain for both years surveyed. Fumonisin levels recorded in this study were low. Ariño *et al.* (2009) suggested that surface residues infected by *Fusarium* do not necessarily indicate an increase in fumonisin contamination in maize grain. Fumonisins are reported to have hepatoxic and nephrotoxic effects on a wide range of farm animals (Rossi *et al*., 2009). Alarming fumonisin levels in crop residues were not recorded in this study, however, seasonal variations combined with conducive environmental conditions can have considerable impacts on farmers that use crop residues as pasturage for domestic animals. Deoxynivalenol and nivalenol recorded in maize grain were also relatively low throughout the study and the lack of significant tillage effects can be attributed to the low occurrence of these toxins. Scala *et al.* (2016) reported climatic conditions to consistently affect deoxynivalenol contamination in wheat more than tillage practices.

Agrometeorological parameters significantly influence fungal infection and mycotoxin production and are likely to be more important than tillage practice in *F. verticillioides* target DNA accumulation in maize grain. It was expected that no-till systems would result in high fungal accumulation, disease severity and mycotoxin contamination due to the nature of the agricultural practice that encourages cover crop retention (Lori *et al.*, 2009), it appears from this study that tillage practices did not enhance maize ear rot accumulation and mycotoxin contamination in grain. Environmental conditions and low disease occurrence could have possibly overridden these effects in this study. Results from this study indicate that under local conditions, no-till production systems can be used without the potential increase of maize ear rots and mycotoxin contamination. Furthermore, due to the overall low occurrence

of ear rot diseases and therefore ear rot causing fungi and their mycotoxins in the duration of this study, it is difficult to obtain a clear relationship between the tested parameters. A survey for a longer period would possibly explore the effect of tillage on maize ear rots and mycotoxin production in depth. Reports on tillage practices and maize ear rot and mycotoxin contamination in maize grain are insubstantial (Marocco *et al*., 2008) and this study has attempted to address this issue. This study combined with other future studies will assist farmers in choosing suitable maize cropping systems with low disease and mycotoxin risks.

This study confirmed that previous crop residues may act as inoculum reservoirs for *F. graminearum* and *F. verticillioides* fungi and that agricultural practices that branch from residue retention are more likely to have increased potential for disease severity due to inoculum presence in crop residues in the field. The presence of crop residues on the soil surface is an indication of the potential for maize ear rot fungi to actively proliferate and infect hosts (Cotten and Munkvold, 1998). The dispersal of inoculum from crop residues to host plants is a critical event that would assist in clarifying the direct influence of crop residue management practices (Dill-Macky and Jones, 2000) on the occurrence of *F. verticillioides* and *F. graminearum* and the production of fumonisins. This information would be invaluable in the establishment of effective disease management strategies. Furthermore, the incorporation of supplementary non-host crop residues may help decrease initial inoculum levels in conservation agricultural fields (Cotten and Munkvold, 1998).

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Table 1. Provinces, farms, cultivars planted and rotation systems surveyed during the 2013/14 and 2014/15 maize growing seasons.

Province	Farm	Cultivar	Rotation crops
KwaZulu-Natal	Winterton[2]	White 32Y85	Wheat, Soya bean
KwaZulu-Natal	Cedara[1,2]	*	Monoculture
North-West	Ottosdal[2]	6Q354B	Monoculture
North-West	Ottosdal[2]	30Y70	Sunflower
North-West	Ottosdal[1]	SC719	Sunflower
North-West	Sannieshof[2]	Monsanto 7845	Monoculture
North-West	Sannieshof[1]	6Q445B	Sunflower
North- West	Hartebeesfontein[1]	DKLP7374	Monoculture
North-West	Hartebeesfontein[2]	Monsanto 3573	Sunflower
North- West	Coligny[2]	3270Br	Soya bean
Free state	Vredefort[1]	3878Bt	Monoculture
Free state	Vredefort[1]	*	Soya bean
Free state	Vredefort[2]	*	Monoculture
Free state	Kroonstad[2]	2653BR	Monoculture
Free state	Kroonstad[1]	30B97VR	Sunflower

[1] Conventional till

[2] No- till

*Information not available

Table 2. Analysis of variance on the effect of tillage practices on Fusarium ear rot severity in maize grain collected from no-till and conventional till fields during the 2013/14 and 2014/15 seasons.

Source	DF	Mean Square	F Value	Pr > F
Season	1	0.02	1.00	0.28
Tillage practice[1]	1	0.24	0.01	0.94
Tillage practice[2]	1	24.20	1.79	0.20
Season*Tillage practice	1	0.19	0.85	0.50

[1]Tillage practice= 2013/14
[2]Tillage practice= 2014/15

Table 3. Mean groupings of fungal target DNA and mycotoxin concentrations in maize grain grown under conventional and no-till fields during the 2013/14 and 2014/15 seasons.

	2013/14		2014/15	
	Conventional till	No-till	Conventional till	No-till
FER %	9.50	9.80	10.36	7.88
GER %	0.83	1.67	0.19	1.19
DER (%)	0.83	1.33	0.48	1.11
F. verticillioides (pg/µL)	303.65[a]	305.03[a]	144.70[ab]	96.54[b]
F. graminearum (pg/µL)	95.43	37.93	39.32	43.51
Fumonisin (ppm)	0.22	0.11	ND	0.06
Deoxynivalenol(ppm)	0.36	0.13	0.03	ND
Nivalenol (ppm)	0.21	ND	0.01	ND

ND = Not detected
Different letters indicate significant differences within the column ($P < 0.05$)

Table 4. Analysis of variance on the effect of tillage practices on Gibberella ear rot severity in maize grain collected from during the 2013/14 and 2014/15 seasons.

Source	DF	Mean Square	F Value	Pr > F
Season	1	0.14	1.0	0.89
Tillage practice[1]	1	2.38	0.40	0.54
Tillage practice[2]	1	3.97	1.40	0.26
Season*Tillage practice	1	2.03	1.53	0.10

[1]Tillage practice= 2013/14
[2]Tillage practice= 2014/15

Table 5. Analysis of variance on the effect of tillage practices on Diplodia ear rot severity in maize grain collected during the 2013/14 and 2014/15 seasons.

Source	DF	Mean Square	F Value	Pr > F
Season	1	0.61	0.22	0.64
Tillage practice[1]	1	0.87	0.27	0.61
Tillage practice[2]	1	1.58	0.70	0.42
Season*Tillage practice	1	0.03	0.01	0.91

[1]Tillage practice= 2013/14
[2]Tillage practice= 2014/15

Table 6. Analysis of variance on the effect of tillage practices on *Fusarium verticillioides* target DNA accumulation in maize grain collected during the 2013/14 and 2014/15 seasons.

Source	DF	Mean Square	F Value	Pr > F
Season	1	244098.32	9.32	0.005
Tillage practice[1]	1	6.56	0.00	0.99
Tillage practice[2]	1	9133.54	0.43	0.52
Season*Tillage practice	1	401062	0.15	0.70

[1]Tillage practice= 2013/14
[2]Tillage practice= 2014/15

Table 7. Analysis of variance on the effect of tillage practices on *Fusarium graminearum* target DNA accumulation in maize grain collected during the 2013/14 and 2014/15 seasons.

Source	DF	Mean Square	F Value	Pr > F
Season	1	2360.34	0.56	0.46
Tillage practice[1]	1	11336.50	1.19	0.29
Tillage practice[2]	1	69.91	0.03	0.87
Season*Tillage practice	1	6975.10	0.19	0.28

*[1]Tillage practice = 2013/14
*[2]Tillage practice = 2014/15

Table 8. Analysis of variance on the effect of tillage practices on fumonisin contamination in maize grain collected from during the 2013/14 and 2014/15 seasons.

Source	DF	Mean Square	F Value	Pr > F
Season	1	0.12	2.57	0.12
Tillage practice[1]	1	0.045	0.57	0.47
Tillage practice[2]	1	0.013	0.89	0.36
Season*Tillage practice	1	0.054	1.22	0.28

[1]Tillage practice= 2013/14
[2]Tillage practice= 2014/15

Table 9. Analysis of variance on the effect of tillage practices on deoxynivalenol contamination in maize grain collected during the 2013/14 and 2014/15 seasons.

Source	DF	Mean Square	F Value	Pr > F
Season	1	0.34	3.87	0.06
Tillage practice[1]	1	0.19	1.03	0.33
Tillage practice[2]	1	0.003	1.40	0.25
Season*Tillage practice	1	0.008	0.95	0.33

[1]Tillage practice= 2013/14
[2]Tillage practice= 2014/15

Table 10. Analysis of variance on the effect of tillage practices on nivalenol contamination in maize grain collected from fields under conventional tillage and no-till during the 2013/14 and 2014/15 seasons.

Source	DF	Mean Square	F Value	Pr > F
Season	1	0.062	1.18	0.28
Tillage practice[1]	1	0.15	1.33	0.27
Tillage practice[2]	1	0.00	1.31	0.27
Season*Tillage practice	1	0.07	1.46	0.24

[1]Tillage practice= 2013/14
[2]Tillage practice= 2014/15

Table 11. Analysis of variance on the effect of tillage practices on *Fusarium verticillioides* target DNA accumulation in crop residues collected during the 2013/14 and 2014/15 seasons.

Source	DF	Mean square	F value	Pr > F
Season	1	92945.99	1.10	0.30
Tillage practice[1]	1	14407.15	0.16	0.69
Tillage practice[2]	1	79626.28	0.94	0.48
Harvest Period	1	41773.28	0.40	0.53
Season*Treat	1	0.00	0.49	0.48
Harv*Treat	1	26165.23	0.30	0.59
Season*Harv	1	229589.64	2.71	0.11

[1]Tillage practice= 2013/14
[2]Tillage practice= 2014/15

Table 12. Maize ear rot causing target DNA quantified from maize crop residues collected pre-harvest and post-harvest on no-till and conventional till fields during the 2013/14 and 2014/15 seasons.

	2013/14		**2014/15**	
Pre-harvest	**Conventional till**	**No-till**	**Conventional till**	**No-till**
Fusarium verticillioides (pg/µL)	157.20	168.10	44.52	87.54
Fusarium graminearum (pg/µL)	48.46	58.59	66.04	47.87
Fumonisin (ppm)	0.01	0.05	0.01	0.07
Post-harvest				
Fusarium verticillioides (pg/µL)	253.10	179.70	348.65	147.28
Fusarium graminearum (pg/µL)	80.71	52.57	73.23	35.38
Fumonisin (ppm)	0.04	0.06	0.00	0.01

No significant diffences between treatments for any of the measured parameters ($P > 0.05$)

Table 13. Analysis of variance on the effect of tillage practices on *Fusarium graminearum* target DNA accumulation in crop residues collected from fields under conventional tillage and no-till during the 2013/14 and 2014/15 seasons.

Source	DF	Mean square	F value	Pr > F
Season	1	2149.15	0.43	0.52
Tillage practice[1]	1	1194.49	0.24	0.62
Tillage practice[2]	1	3091.48	0.62	0.74
Harvest Period	1	1675.13	0.34	0.57
Season*Treat	1	6040.40	1.20	0.27
Harv*Treat	1	5391.44	1.10	0.30
Season*Harv	1	3575.22	0.71	0.40

[1]Tillage practice= 2013/14
[2]Tillage practice= 2014/15

Table 14. Analysis of variance on the effect of tillage practices on fumonisin contamination in crop residues collected from fields under conventional tillage and no-till during the 2013/14 and 2014/15 seasons.

Source	DF	Mean square	F value	Pr > F
Season	1	0.019	1.17	0.28
Tillage practice[1]	1	0.015	0.86	0.35
Tillage practice[2]	1	0.016	0.93	0.49
Harvest Period	1	0.006	0.33	0.57
Season*Treat	1	0.00	0.00	0.99
Harv*Treat	1	0.002	0.10	0.75
Season*Harv	1	0.06	3.81	0.06

[1]Tillage practice= 2013/14
[2]Tillage practice= 2014/15

Table 15. Mean maximum temperatures (ºC) recorded from November to May during the 2013/14 and 2014/15 seasons.

Locality	**2013/14**								**2014/15**							
	Nov	Dec	Jan	Feb	Mar	Apr	May	**Mean**	Nov	Dec	Jan	Feb	Mar	Apr	May	**Mean**
Winterton	29.66	28.24	31.41	30.86	28.89	26.86	26.33	**28.89**	27.56	30.72	32.49	32.29	29.27	27.75	27.68	**29.68**
Cedara	25.49	23.96	27.70	28.21	26.27	24.05	24.02	**25.67**	22.79	25.14	27.37	25.44	25.69	22.67	24.19	**24.76**
Ottosdal	30.82	26.86	30.18	26.77	24.86	23.57	23.05	**26.59**	27.06	29.08	31.21	31.18	28.27	26.11	25.97	**28.41**
Sannieshof	32.64	28.57	32.29	29.41	27.95	27.39	26.29	**29.22**	29.70	31.84	33.67	33.72	30.51	28.21	27.69	**30.76**
Hartebeesfontein	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Coligny	30.29	26.29	29.42	25.79	*	*	*	**27.95**	*	27.99	29.34	30.59	27.45	25.27	25.34	**27.66**
Vredefort	30.50	28.02	*	*	*	*	*	**29.26**	*	30.79	31.53	32.20	28.58	26.45	26.61	**29.36**
Kroonstad	30.34	28.48	32.79	29.44	27.86	26.29	24.94	**28.59**	27.22	31.43	33.19	33.12	28.73	26.54	26.07	**29.47**
Mean total	**29.96**	**27.20**	**30.63**	**28.41**	**27.17**	**25.63**	**24.93**	**28.02**	**26.87**	**29.57**	**31.25**	**31.22**	**28.25**	**26.14**	**26.22**	**28.58**

*No meteorological data

Table 16. Mean minimum temperatures (ºC) recorded from November to May during the 2013/14 and 2014/15 seasons.

Locality	**2013/14**								**2014/15**							
	Nov	Dec	Jan	Feb	Mar	Apr	May	**Mean**	Nov	Dec	Jan	Feb	Mar	Apr	May	**Mean**
Winterton	13.59	15.03	16.46	16.34	14.43	8.28	5.14	**12.75**	13.41	15.65	16.26	15.42	14.49	10.58	5.77	**13.08**
Cedara	12.46	13.61	15.63	15.66	13.98	9.29	7.05	**12.53**	12.02	14.24	15.22	14.70	14.32	10.04	7.44	**12.57**
Ottosdal	13.34	15.26	16.35	16.45	13.42	6.07	2.55	**11.92**	12.64	15.99	15.27	13.10	13.09	8.10	3.05	**11.61**
Sannieshof	13.69	15.27	16.17	16.21	13.06	5.93	3.46	**11.97**	12.86	15.94	15.52	13.54	13.69	9.09	4.38	**12.15**
Hartebeesfontein	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Coligny	13.88	14.91	16.03	16.57	*	*	*	**15.35**	*	16.36	15.71	14.28	14.10	10.55	7.76	**13.13**
Vredefort	12.41	14.46	*	*	*	*	*	**13.44**	*	16.96	15.26	13.45	12.79	8.83	4.87	**12.03**
Kroonstad	13.06	14.90	16.83	15.89	13.33	7.49	4.88	**12.34**	12.29	15.62	15.96	14.15	13.78	9.34	6.55	**12.53**
Mean total	**13.20**	**14.78**	**16.25**	**16.19**	**13.64**	**7.41**	**4.62**	**12.9**	**12.64**	**15.82**	**15.60**	**14.09**	**13.75**	**9.50**	**5.69**	**12.44**

*No meteorological data

Table 17. Total rainfall (mm) recorded from November to May during the 2013/14 and 2014/15 seasons.

Locality	**2013/14**								**2014/15**							
	Nov	Dec	Jan	Feb	Mar	Apr	May	**Mean**	Nov	Dec	Jan	Feb	Mar	Apr	May	**Mean**
Winterton	2.98	5.17	4.73	4.36	8.63	1.21	0.01	**3.87**	3.11	4.72	1.52	2.10	3.72	1.13	0.00	**2.32**
Cedara	3.51	4.47	3.15	3.43	7.73	0.56	0.05	**3.27**	4.42	4.01	3.83	2.59	2.69	1.94	0.17	**2.81**
Ottosdal	0.86	6.19	3.75	5.67	6.64	0.39	0.06	**3.37**	2.79	5.02	1.56	1.71	1.51	0.16	0.00	**1.82**
Sannieshof	0.48	6.74	2.80	3.83	1.90	0.34	0.10	**2.31**	2.12	4.33	2.15	1.81	1.57	0.44	0.02	**1.78**
Hartebeesfontein	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Coligny	0.15	0.82	2.95	3.25	*	*	*	**1.79**	*	5.10	4.31	2.04	2.08	0.25	0.01	**2.30**
Vredefort	3.51	7.87	*	*	*	*	*	**5.69**	*	3.32	4.32	1.94	3.27	1.50	0.00	**2.39**
Kroonstad	1.95	4.08	2.54	4.89	3.02	0.30	0.16	**2.42**	3.61	2.93	1.55	1.82	2.47	1.25	0.04	**1.95**
Mean total	**1.92**	**5.04**	**3.32**	**4.24**	**5.58**	**0.56**	**0.076**	**3.24**	**3.21**	**4.20**	**2.74**	**2.00**	**2.47**	**0.95**	**0.034**	**2.20**

*No meteorological data

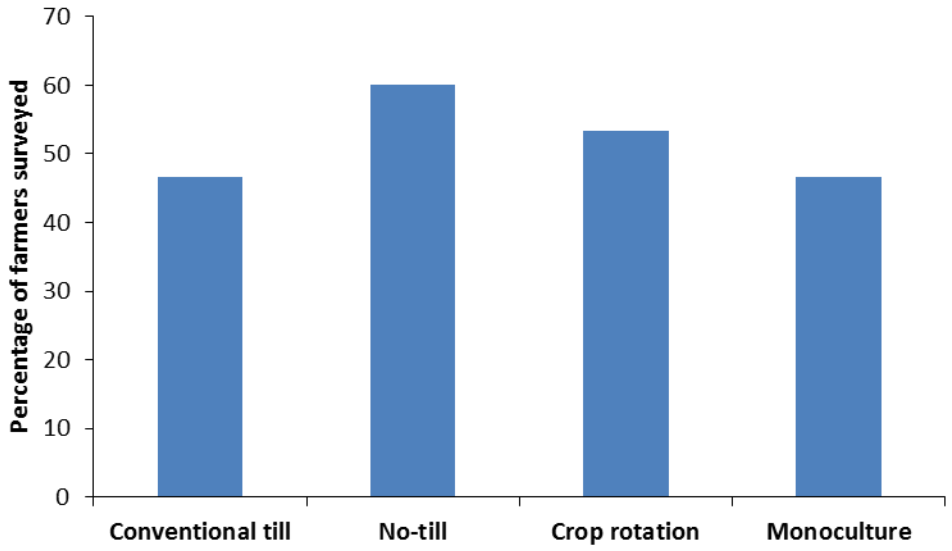

Figure 1. Bar graph representing the total percentage of surveyed farms under conventional till, no-till and farmers employing crop rotation and monoculture.

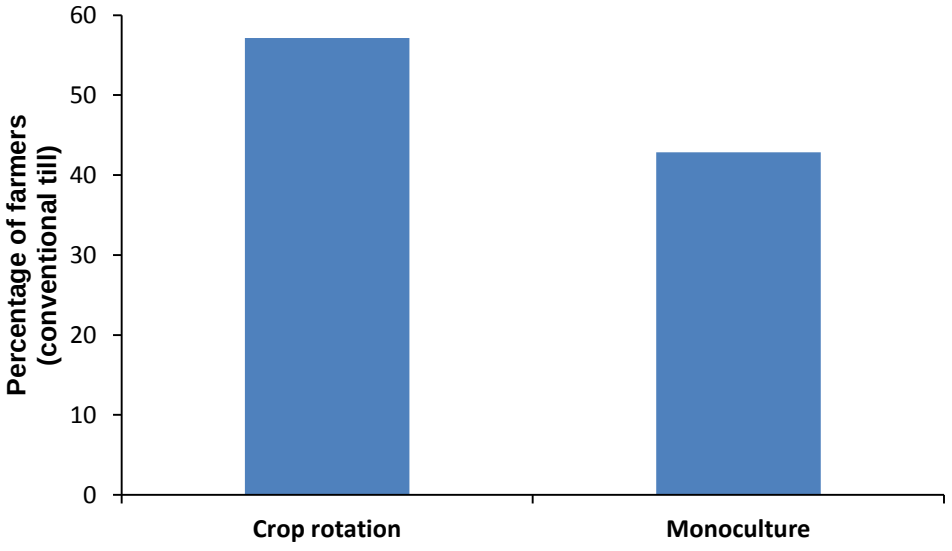

Figure 2. Bar graph representing the percentage of surveyed conventional till farmers using crop rotations and monoculture.

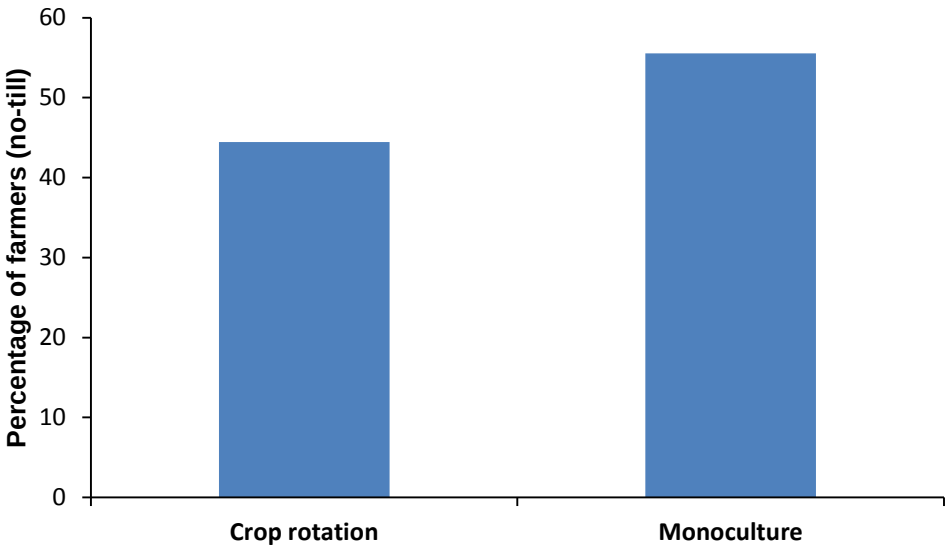

Figure 3. Bar graph representing the percentage of surveyed no-till farmers using crop rotations and monoculture.