# THE MAIZE TRUST

# FINAL REPORT

**Project no M141/27 (001000 - P05000051)**

**Date**

## 1. Personal details of the Project Leader *(Refer to application)*

| | |
|---|---|
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| Citizenship: | RSA |
| Highest qualification: | Ph.D. |
| Year obtained: | 1999 |

## 2. Contact details of the Project Leader *(Refer to application)*

| | |
|---|---|
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## 3. Project information *(Refer to application)*

| | | |
|---|---|---|
| Title: | **Monitoring Fusarium and Gibberella ear rots and the mycotoxins they produce on maize grown under different conservation tillage/rotation systems M141/27 (001000 - P05000051)** | |
| Duration of project: | **(April 2014 - March 2017)** | |
| Budget year: | **2016** | |
| Total Annual Budget of Project: | **R500 000** | |
| Amount contributed by the Maize Trust for the current year: | **R250 000** | |
| Other sources of funding during the current year: | Contributor | Amount contributed |
| | **ARC** | **R250 000** |

## 4. Co-workers (research team) and other support staff *(Refer to application)*

| **Name** | **Qualification** | **Institution** | **Role** |
|---|---|---|---|
| Prof Altus Viljoen | PhD | Stellenbosch University | Advise and assist with trial planning and mycotoxin analysis. |
| Dr Lindy Rose | PhD | Stellenbosch University | Assist with mycotoxin analysis, write up and data analysis. |
| Dr Andre Nel | PhD | ARC-GCI | Agronomical planning and advice as well as planting and maintenance of the two conservation tillage/rotation trials |

| | | | |
|---|---|---|---|
| Dr Marietjie Stander | PhD | Stellenbosch University | Multi-toxin analysis of milled samples annually |
| Dr Belinda Janse Van Rensburg | PhD | ARC-GCI | Milling and RT-qPCR of mycotoxin producing fungi in samples |
| Ms Londiwe Mabuza | BSc Honns | ARC-GCI | Masters student |

**5. Summary** (Description of the project, capturing the main findings; 250 words)

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 practices and crop rotations, however, these have not been sufficiently evaluated in South Africa. The increasing shift towards conservation agriculture 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 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 real-time quantitative polymerase chain reaction (qPCR) was used to quantify target DNA of *F. verticillioides* and *F. graminearum*. Furthermore, the mycotoxins fumonisins and zearalenone were quantified using high performance liquid chromatography 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 cropping system 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 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 cropping systems and Diplodia ear rot 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 cropping systems 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 Conservation Agriculture systems can be used without the potential increase of maize ear rots and mycotoxin production under local conditions.

## 6. Objectives (Refer to application)

### 6.1. Strategic objectives (Maize Trust objectives)

| Strategic objectives | Yes/No |
|---|---|
| To establish the magnitude of mycotoxin contamination of maize during the stages of its production, storage, and processing in South Africa | No |
| To regularly monitor the occurrence of fumonisins, aflatoxins, zearalenone, and trichothecenes (DON and NIV) in locally produced and imported maize | No |
| To determine the factors which contribute to mycotoxin contamination during the production (pre-harvest), storage (post-harvest) and processing of maize | Yes |
| To develop practical, affordable and environmentally sound methods to manage toxigenic fungi in maize, with particular emphasis on introducing resistance in local maize cultivars | Yes |
| To support the development of sound mycotoxin risk management practices in the maize supply chain to ensure the delivery of safe products to the consumer. | Yes |

### 6.2. Project objectives (List main objectives)

Conservation agriculture (CA), particularly zero tillage is practiced on more than 100 million ha worldwide, while the area in South Africa devoted to CA is minimal. The main benefit of CA is soil conservation and the sustainability that results from it. An additional benefit of CA is savings on fuel and machinery, an important aspect applicable to producers at all levels. It is important to take note that spectrum shifts in weed, pest and disease dynamics are potential dangers that might need to be assessed and novel control interventions may need to be developed

In previous studies (Flett, McLaren & Wehner (1998); Flett, McLaren & Wehner, 2001 and Flett & Wehner, 1991) where Fusarium ear rots, caused by *Fusarium verticillioides (F. moniliforme)*, *F. proliferatum* and *F. subglutinans* and Gibberella ear rots caused by the

*Fusarium graminearum* species complex, recently found to be caused by *F. boothii* (Boutigny *et al*, 2011), were found not to differ under various tillage and rotation systems. The quantification of ear rots and the concomitant mycotoxins under these systems, using modern technologies, will determine which systems will be preferred in reducing maize ear rots, improving grain quality and reducing mycotoxins. As there are already two CA/rotation trials being planted at two localities i.e. Buffelsvlei and Erfdeel, on different soil types and initial results indicate improved grain quality (using SA grading regulations)(Andre Nel, personal communication) with very little extra effort important information on ear rots and mycotoxins can be obtained.

## 7. Work plan (Refer to application)

### 7.1. Work plan as stated in the application (List main tasks)

The two field trials started in 2008. Treatments consist of conventionally cultivated, mono-cropped maize and three other treatments of progressive degrees of CA principles applied.

Crop rotation systems consist of:

- Mono-cropped maize under conventional tillage.
- Mono-cropped maize under minimum soil disturbance.
- A two-year system with maize in rotation with a legume and sunflower under minimum soil disturbance. Sunflower rotation will be applied on the Hutton soil only.
- A three-year legume/sunflower (in a split-plot arrangement) - maize - babala rotation system under minimum soil disturbance. The babala serves as cover crop and the sunflower rotation will be applied on the Hutton soil only.
- To distinguish between seasonal and rotational effects systems 3 and 4 consist of two and three sub-treatments, respectively, each representing an alternative phase of the rotation system during each season.
- The statistical layout is a randomised complete block with four replicates. Main plots are at least 15 x 20 m in size and commercial implements are used for agronomical activities.

Measurements:

- Crop yield, biomass yield, grain quality and weather variables (Dr Andre Nel).
- Soil nutrient status, soil strength (if possible) and soil cover (Dr Andre Nel).
- Quantification of Fusarium and Gibberella ear rots using RT-qPCR techniques (Dr Belinda Janse van Rensburg) and mycotoxins using LCMS multimycotoxin analysis (Prof Altus Viljoen and Dr Marietjie Stander).

- Quantification of *Stenocarpella maydis* using percentage infected ears (Prof Bradley Flett).

**7.2. Achieved tasks according to the stated work plan** (List measurable units as milestones and provide an indication of progress made)

Example:

| **Milestones** | **Achievements** |
|---|---|
| RT-qPCR | Task achieved: All samples analysed |
| LCMS | Task achieved: All samples analysed |
| Publication of research | Task not complete: Manuscript is being prepared for publication |

**8. Detailed report covering the research conducted during the full grant period** (Introduction, methods, results, discussion, tables, figures, etc.)

**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 of 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).

Multitoxin 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 practices 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 objectives of this study were 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

### Conservation Agriculture field 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 repared consisting of a total volume of 25 µL of 12.5 µL of SYBR® green, 0.625 µL (250mM) of FgramB379: CCA TTC CCT GGG CGT and 0.625 µL (250nM) 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 reaction plate was used for qPCR reactions and steps. For *F. verticillioides* the reaction consisted of a 5 min denaturation step at 95º C, 40 cycles at 95 º C for 10s and 65 º C for 10s, followed by cooling to 65 º 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 NaCI, 1.2 g $Na_2HPO_4$, 0.2 g $KH_2PO_4$, 0.2 g KCI, 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 of the method used 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 NaCI, 1.2 g $Na_2HPO_4$, 0.2 g $KH_2PO_4$, 0.2 g KCI, 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 for the used method was 0.03 ppm for deoxynivalenol and 0.04 ppm for nivalenol. 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, Stellenbosch, South Africa.

**Climatic data**

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

**Statistical analyses**

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 (data not shown). The mean FER visual disease severity ranged from 0.10-15.30% throughout the six-year study period (data not shown).

*Gibberella ear rot (GER) disease ratings:* Cropping systems appeared to not influence GER severity during the six-year study. Mean GER severity ranged from 0 to 3.65% during the six-year study (data not shown).

*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 (P=0.008, Table 4). 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 (data not shown). 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 (data not shown).

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 (P= 0.010, Table 4). 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 4) where the three-year maize/cowpea/babala (NT -CP-BA) rotation system had significantly higher *F. graminearum*

target DNA 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 (data not shown). The effect of cropping systems on the accumulation of *F. graminearum* target DNA was not significant for any other season evaluated.

*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 4). Fumonisin levels (8.87 ppm) were significantly (P= 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 (data not shown). No zearalenone was detected in grain samples in the remaining seasons. According to the ANOVA analyses (data not shown) 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 4) 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 (data not shown).

**Erfdeel**

*Fusarium ear rot (FER) disease ratings:* Cropping systems had no significant effects on recorded for FER disease severity throughout the study period. Mean FER severity ranged from 3.75 - 1.30% during the study period (data not shown).

*Gibberella ear rot (GER) disease ratings*: Mean GER severity ranged from 0 to 11.06% during the study period. GER severity in maize grain was not significantly affected by any of the cropping systems (data not shown).

*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%. No significant differences were recorded in DER disease incidence in relation to cropping systems across all seasons (data not shown).

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. *Fusarium verticillioides* target DNA accumulation ranged from low to moderate (2.30 - 427 pg/µL) throughout the study period (data not shown).

Fusarium graminearum *target DNA*: The *F. graminearum* target DNA accumulation was low in Erfdeel during the study, ranging from 5.90 - 214 pg/µL. There were no significant differences in *F. graminearum* accumulation in relation to cropping systems in Erfdeel between 2011/12 - 2014/15 (data not shown).

*Fumonisins*: Low to moderate levels of fumonisins ranging from non-detectable - 0.55 ppm were recorded during the study period in Erfdeel. No significant differences were found for fumonisin contamination in relation to cropping systems in Erfdeel between 2011/12 - 2014/15 (data not shown).

*Zearalenone*: Zearalenone was not detected during the study period in Erfdeel (data not shown).

*Deoxynivalenol and nivalenol*: Deoxynivalenol frequency was low ranging from non-detectable - 0.47 ppm (data not shown). Nivalenol was not detected during the study period in Erfdeel. No significant differences were recorded in deoxynivalenol and nivalenol accumulation in relation to cropping systems across all seasons (data not shown).

**Climatic data**

In Buffelsvallei, the weather was characterised by dry and warm conditions. Mean maximum monthly temperatures steadily increased from 25.1ºC to 27ºC 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 the other four 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* occurrence 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 less 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 soyabean 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 extended 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 4A-G** Analyses of variance of the effects of cropping systems on deoxynivalenol contamination in maize grain from 2011/12 to 2014/15 in Erfdeel.

**Fumonisins (2010/11)**

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Treatment | 5 | 21.805 | 4.361 | 2.80 | 0.056 |
| Residual | 15 | 23.368 | 1.558 | | |
| Total | 23 | 51.163 | | | |

***Fusarium graminearum* (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 | | | |

**Deoxynivalenol (ppm) (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 | | | |

**Diplodia ear rot incidence % (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 | | | |

**Table 5** Mean *Fusarium graminearum* quantified during the 2010/11 season in maize grown under different tillage/rotation systems in Buffelsvallei

| Treatment | *Fusarium graminearum* |
|---|---|
| **1-Monoculture Maize CT** | 4.63[a] |
| **2-Baba legume/cowpea maize (3 year)** | 59.07[b] |
| **3-Baba Sunf maize (3 year)** | 11.66[a] |
| **4-Monoculture Maize No till** | 20.03[a] |
| **5-Cowpea maize (2 year)** | 2.62[a] |
| **6-Sunflower maize (2 year)** | 14.86[a] |
| **% CV** | 69.3 % |

Table 6: Mean groupings of fumonisin contamination in maize grain samples measured between during 2010/11 in Buffelsvallei

| Treatment | Fumonisins (ppm) |
|---|---|
| **1-Monoculture Maize CT** | 0.08 (-1.91)[a] |
| **2-Baba legume/cowpea maize (3 year)** | 0.56 (-0.51)[a] |
| **3-Baba Sunf maize (3 year)** | 1.27 (-0.74)[a] |
| **4-Monoculture Maize No till** | 1.43 (-1.35)[a] |
| **5-Cowpea maize (2 year)** | 0.06 (-1.97)[a] |
| **6-Sunflower maize (2 year)** | 8.87 (0.81)[b] |
| **% CV** | 23.9 % |

**Table 7** Mean groupings of deoxynivalenol contamination in maize grain samples measured between during 2011/12 in Buffelsvallei

| Treatment | Deoxynivalenol (DON) |
|---|---|
| **1-Monoculture Maize CT** | 0.516 (-0.73)[b] |
| **2-Baba legume/cowpea maize (3 year)** | 0.019(-2.25)[a] |
| **3-Baba Sunf maize (3 year)** | 0.015(-2.56)[a] |
| **4-Monoculture Maize No till** | 0.175(-1.92)[ab] |
| **5-Cowpea maize (2 year)** | 0.025(-2.50)[a] |
| **6-Sunflower maize (2 year)** | 0.001(-3.00)[a] |
| **% CV** | 20.6% |

**Table 8** Mean groupings of fumonisin contamination in maize grain samples measured between during 2010/11 in Buffelsvallei

| Treatment | Diplodia |
|---|---|
| **1-Monoculture Maize CT** | 0.4706[b] |
| **2-Baba legume/cowpea maize (3 year)** | -2.1065[a] |
| **3-Baba Sunf maize (3 year)** | -1.3904[a] |
| **4-Monoculture Maize No till** | 0.3368[b] |
| **5-Cowpea maize (2 year)** | 0.5093[b] |
| **6-Sunflower maize (2 year)** | 0.2971[b] |
| **% CV** | |

## 9. Scientific outputs

### 9.1. Expected outputs of project as stated in the application

| Outputs | Date of completion | Nature of output e.g. number and title of papers |
|---|---|---|
| Scientific papers: | 2017 | 1 scientific article |
| Technical reports: | 2017 | 6 biannual reports |
| Databases: | | |
| Procedures/methods: | | |
| Human capacity development: | 2017 | 1 MSc |
| Technology transfer: | 2017 | 4 popular articles |
| Other outputs: | | |

### 9.2. Actual outputs of project

| Outputs | Date of completion | Nature of output e.g. number and title of papers |
|---|---|---|
| Scientific papers: | 2017 | Paper still in preparation for submittance. |
| Technical reports: | 2017 | 6 biannual reports and 3 annual reports |
| Databases: | | |
| Procedures/methods: | | |
| Human capacity development: | 2017 | 1 MSc |
| Technology transfer: | 2017 | Popular articles will be published on acceptance of publication |
| Other outputs | | |

### 9.3. Outputs not achieved (give reasons)

Manuscript for publication still in process of being prepared.

**10. Successful institutional and inter-institutional collaboration**

| Researcher | Institution | Role |
|---|---|---|
| Dr Lindy Rose | Stellenbosch University | Supervisor of MSc student |
| Dr Belinda Janse van Rensburg | ARC-GCI | Co-supervisor of MSc student |
| Dr Marietjie Stander | Stellenbosch University | LCMS analysis of samples |
| Dr Andre Nel | ARC-GCI | Maintenance of field trials, planning and data analysis |

**11. Benefit of the outputs to the Maize Industry**

With the attempts of the Maize Trust to research, develop and encourage the use of conservation tillage systems which include both reduced soil disturbance and crop rotation in South Africa there is a need to determine the potential threats that such systems may or may not impose. The recorded increase of *Stenocarpella maydis* ear rot under reduced and no-till systems is cause for concern and there is an urgent need to develop alternate control interventions if we are to pursue the positive effect of CA in maize production. To date data and reports on the effect of CA systems on Fusarium ear rots and Gibberella ear rots on maize are scarce and inconclusive. These experiments have been running a number of seasons and have stabilized in terms of the system as a whole. This is an ideal opportunity to utilize present studies to obtain further reliable information on the effect these systems have on the Fusarium and Gibberella ear rots and their mycotoxins using recent up-to-date technology.

**12. Progress with regards to human resource development**

(e.g. Training of post-graduate students in mycotoxin research)

1 MSc student trained - Ms Londiwe Mabuza

**13. Funds available to complete the execution of the proposed tasks according to the expenditure statement of the project** (Were the funds adequate?)

Yes.

**14. Comments** (Discuss anything you wish to share with The Maize Trust)

None.

## 16. Signature of the Project Leader

| Potchefstroom | 12/07/2017 |
|---|---|
| **Project Leader** | **Place** | **Date** |