THE MAIZE TRUST

FINAL REPORT FOR COMPLETED MYCOTOXIN RESEARCH PROJECTS

Project no P05000012

Closing date :	31 March 2020

1. Title of research project

The effect of prophylactic fungicide spray regimes to control maize leaf diseases on *F. verticillioides*, *F. boothii* and mycotoxin production in grain.

2. Personal details *(refer to application)*

Title of applicant:	Dr.
Name:	Belinda
Initials:	B
Surname:	Janse van Rensburg
National identification number:	7404270181081
Citizenship:	SA

3. Contact details of applicant *(refer to application)*

Name of institution/employer:	Agricultural Research Council – Grain Crops
Faculty, Department, Institute:	Plant Protection – Plant Pathology
Work telephone number:	018 299 6357
Mobile number:	082 775 2545
Website address:	www.arc.agric.za
E-mail address:	BelindaJ@arc.agric.za
Institution/employer postal address:	Private Bag X1251, Potchefstroom
Institution/employer physical address:	Chris Hani street 114, Potchefstroom

4. Project duration and funding overview *(refer to application)*

Duration of project:	Starting date (month, year) 1 April 2017 End date (month, year) 31 March 2020.	
Total budget of project:	**2017/18** R700 000	
Amount received from Maize Trust:	R350 000	
Other sources of funding for this project:	**Contributor**	**Amount received**
	ARC	R350 000
Total budget of project:	**2018/19** R700 000	
Amount received from Maize Trust:	R350 000	

Other sources of funding for this project:	Contributor	Amount received
	ARC	R350 000
Total budget of project:	2019/20 R700 000	
Amount received from Maize Trust:	R350 000	
Other sources of funding for this project:	**Contributor**	**Amount received**
	ARC	R350 000

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

The aim of this study is to determine whether prophylactic fungicide regimes for foliar diseases could reduce the risk of colonization of grains by *F. verticillioides* and *F. boothii* and their resultant mycotoxin production. Active ingredients from the Triazole-, Strobilurin- and Benzimidazole fungicide classes were tested in various combinations (currently being used by producers as prophylactic fungicides for the prevention and control of foliar diseases).

Although the effect of spray combinations, application date of fungicides and interactions between the two were recorded on the reduction of maize ear rot and associated mycotoxins, results were not consistent. It seems that spray applications at flowering or during soft dough stage can decrease either DON levels or *F. boothii* infections of maize grain at Cedara and Vaalharts, while earlier applications (pre-flowering) show significantly higher levels of *F. boothii* or DON. The same trend was observed in Potchefstroom where pre-flowering fungicide applications showed higher levels of *F. verticillioides* target DNA in maize grain. Similar results were obtained in Vaalharts in the 2017/18 season where cultivar BG3292 x the application at the 8 leaf stage and then again 28 (pre-flowering) days later, had the highest level of ZEA compared to the other interactions. BG3292 x the application at the 10 leaf stage and then again 56 days later (soft dough stage), had the lowest level of ZEA compared to the other interactions.

In the 2017/18 season (Cedara), fungicide combinations with Amistar Top resulted in higher levels of *F. boothii* when compared to the other spray combinations without Amistar Top. Similar results were observed in Vaalharts in the 2018/19 season, where Amistar Top / Artea x 5/28 days of application, Amistar Top / PunchXtra x 8/28 days of application, Amistar Top / Artea x 8/42 days of application and Amistar Top / PunchXtra x 10/28 days of application all had the highest DON levels. Similar to this, the Amistar Top / Artea interaction with the spray at 10 leaf stage followed by a second spray after 28 days also had the highest fumonisin level at Vaalharts (2018/10 season). In a previous study by Janse van Rensburg

et al. (2016), a prophylactic spray regime using Amistar Top showed no effect on the infection and colonization of *F. verticillioides* in maize grain and described the lack efficacy due to timing of the fungicide application relative to the plant growth stages. This study confirmed that Amistar Top should not be considered for the possible additional control of maize ear rots and mycotoxin contamination. Amistar Top is the only fungicide used in this study with the active ingredient strobilurin, which could explain the higher infection levels of *F. boothii* as well as DON and FUM synthesis in maize grain.

The inconsistent reactions of fungicide combinations and/or date of application against *F. verticillioides*, *F. boothii* as well as FUM, DON and ZEA synthesis may be due to the fungal infection process and plant morphology (Luna and Wise, 2015). Foliar fungicides are commonly applied over the top of the maize plant with ground or areal equipment and it is possible that this limit the amount of fungicide to penetrate the plant to come into contact with the fungus.

Prophylactic foliar fungicides used in this study can be effective as management tools for leaf diseases such as *Cercospora zea maydis* and *Escerohilum turcicum,* but because they do not consistently reduce ear rots and mycotoxins tested in this study, it is not economically beneficial to change spray dates to co-incide with the flowering or soft dough plant stages. It is recommended that producers continue to manage maize ear rots using methods such as cultivar selectin, crop rotation and residue management (where applicable). In conservation agriculture systems, crop rotation will be greatly beneficial to break the disease cycle by reducing primary inoculum.

6. Objectives *(refer to application)*
6.1 Strategic objectives *(alignment with Maize Trust objectives)*

Strategic objectives	Yes/No
To support the establishment of the magnitude of mycotoxin contamination of maize during the stages of its production, storage, and processing in South Africa.	X
To support the regular monitoring of the occurrence of the fumonisins, aflatoxins, zearalenone, and trichothecenes (DON and NIV) in locally produced and imported maize.	
To support the determination of the factors which contribute to mycotoxin contamination during the production (pre-harvest), storage (post-harvest) and processing of maize.	X

To support the development of practical, affordable and environmentally sound methods to manage toxigenic fungi in maize, with particular emphasis on the introduction of resistance in local maize cultivars.	X
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.	

6.2 Project objectives *(list main objectives)*

- To determine if prophylactic fungicide sprays for control of leaf diseases in maize could reduce *F. verticillioides* and *F. boothii* maize ear rots by adjusting time of spraying.
- To determine if strobilurins and or triazoles are responsible for elevated mycotoxin production in grain

7. Work plan *(refer to application)*

7.1 Work plan as stated in the application *(list main tasks)*

- Completely randomised experiments of two maize cultivars (BT and non-BT isohybrid) will be conducted with three replicates at three different locations (Cedara, Potchefstroom and Vaalharts) for two planting seasons (Mr. F.K. Mashinini, Dr. A. Abrahams, Dr. B. Janse van Rensburg). Planting will commence during November-December of 2017 and 2018.
- Experimental plots will be monitored for the 5-12 leaf stage, pre-flowering, flowering and soft dough stage of plant growth (Dr. Abrahams, Mr. F.K. Mashinini).
- Four rows of each cultivar in an experimental block will be planted and the middle two rows will be sprayed to prevent fungicide drift to other experimental blocks.
- Fungicide spray combinations and time of application can be seen in the methods section. Dr. Abrahams will be responsible to carry out spray combinations at corresponding time frames.
- During silking maize ears of one row will be inoculated with *F. verticillioides* and the other row with *F. boothii* (Dr. Janse van Rensburg, Dr. Abrahams).

- Plants will be scouted throughout for leaf diseases and stalk borers and leaf disease ratings will be made if it occurs. Stalk borer damage will be observed in the field and on maize ear ratings after harvest (Mr. F. Mashinini, Dr. Abrahams).
- Date of flowering, soft dough, hard dent and physical maturity will be noted to establish “stay green” effects (Dr. Abrahams).
- Ears will be hand harvested during June/July of 2018 and subjected to visual ratings, 1000 kernel weight and moisture measurements (Mr. F. Mashinini, Dr. Abrahams).
- Samples will then be milled to conduct qPCR (target DNA fungal biomass) and HPLC/LC-MS analyses (August to October 2018, Dr. Janse van Rensburg, Dr. Abrahams, Dr. M. Stander).
- The second season planting during November – December 2018, scouting, inoculation, harvest and sample preparation and quantifications will be the same as described above.
- Data analyses and writing of reports and a scientific article will take place from November 2019 to March 2020.

7.2. Achieved tasks according to the stated work plan *(list measurable units as milestones and provide an indication of progress made, e.g. tasks achieved or not [add additional rows if necessary])*

Milestones	**Achievements**
Plant field trials	Achieved. Two seasons field trials planted at Cedara, Potchefstroom and Vaalharts.
Fungicide spray treatments	Achieved. Administered according to spraying schedule.
Inoculation with *F. verticillioides* and *F. boothii*	Achieved. Inoculum of *F. verticillioides* and *F. boothii* were prepared and plants were inoculated during silking stage at the three localities over two seasons.
Harvest and milling of samples	Achieved. A total of 540 individual samples were milled during July – August 2018 and again in 2019.
DNA extraction and mycotoxin analyses	qPCR and mycotoxins analysed for the 2017/18 and 2018/19 field trials.

Popular article	Published on the basis of the 2017/18 results in SA Graan.
Scientific article	Need to be completed.

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

INTRODUCTION

Maize plants are important crops which are grown worldwide, serving different functions of economic value particularly as a staple diet for millions of people in South Africa especially in the rural population (Walker & Schulze, 2006). South Africa is the second largest maize production region in Africa, producing an estimated 10-12 million tons of maize annually (www.jse.co.za/commodities). It is produced throughout the South African community under various agro ecological conditions and is grown under commercial, small-scale and subsistence farming levels. The production of the crop depends on the correct application of management practices, ensuring both environmental and agricultural sustainability (Nape, 2011). Its productivity could be limited due to a combination of factors such as low soil fertility, unfavourable environmental conditions, poor agricultural management as well as pests and diseases. Maize also serves as a convenient substrate for naturally occurring fungal pathogens which may result in mycotoxin contamination when environmental conditions are suitable. Economically important maize fungal ear rots are the *Fusarium spp.*: *F. graminearum* (*sensu lato*), *F. verticillioides*, *F. proliferatum* and *Stenocarpella maydis. Fusarium verticillioides* and *F. proliferatum* are the causative agents that produce fumonisins, B_1, B_2, and B_3, which are toxic secondary, possible carcinogenic metabolites that occur naturally as contaminants of agricultural products such as maize. The distribution and predominance of these *Fusarium* spp. and their concomitant fumonisin production varies depending on season, geographic locality, climatic factors such as temperature and moisture, host genotype and agricultural practices (Nyaka *et al*., 2010). The limit of allowable Fumonisins (B^1+B^2) in South African maize grain intended for further processing, may not be more than 4000 µg/kg (Government Gazette, 2016).

To date there are 16 phylogenetic species that have been identified within *F. graminearum* (*sensu lato*) which contribute to the *F. graminearum* species complex (FgSC). Five species of the complex have been shown to be associated with maize. These are *F. asiaticum, F.*

austroamericanum, F. boothii, F. meridionale, and *F. graminearum sensu stricto*. These species differ in the degree to which they cause disease (virulence) and tissue specificity. In South Africa, these five species were isolated and identified from maize roots and crowns. However, in maize ears, only *F. boothii* has been recorded (O'Donnell *et al*., 2004). Of these species, *F. boothii* are the most virulent and *F. meridionale* the least. The estrogenic metabolite zearalenone (ZEA) together with the trichothecenes Nivalenol (NIV) and Deoxynivalenol (DON) are closely associated with FgSC. DON is the most widely produced FgSC mycotoxin, but is largely associated with *F. boothii* (lineage 3) on maize. *F. boothii* is known to produce 3-A-DON and is most commonly found in contaminated wheat and maize. DON is commonly found in hyphae of fungal colonised grains and is most frequently distributed in wheat bran fractions. Low amounts of DON have been recorded in eggs, indicating that DON can be transferred embryonically if lay hens consume contaminated feed (Bhat *et al*., 2010). Limited information is available regarding the effects of DON on human health and more research is necessary. The limit of allowable DON in South African maize grain intended for further processing may not be more than 2000 µg/kg (Government Gazette, 2016). There is no legislation for ZEA in South Africa, however worldwide limits range from 300 – 2000 µg/kg (FAO, 2003).

Fungicide spray programmes have been employed by maize producers to prevent leaf diseases and companies claim that fungicide applications result in better yields (due to leaf disease control) and that plants remain green and healthy for an extended period which improves standability and grain quality. Maize foliar diseases can be managed using agricultural practices such as the selection of resistant cultivars, crop rotation, tillage practices as well as through the use of curative fungicide sprays. Since 2007, significant increase in the use of fungicides in maize production has become prominent to prevent and/or control foliar diseases, as well as an increase in the market price of maize which makes fungicide sprays economically viable. Such spray programmes, for example, usually include a combination of strobilurin and triazole at the 5-8 leaf stage of plant growth, followed by a second spray of triazole 28-30 days after the first spray. New fungicides have recently been registered and are extensively being marketed (Wise and Mueller, 2011). No fungicides are currently registered to control Fusarium ear rot diseases and their subsequent mycotoxins. In a previous study by Janse van Rensburg *et al*. (2016), it is shown that a prophylactic spray regime as used by producers, had no effect on the colonisation of grain by fumonisin producing *Fusarium* spp. This was possibly due to the timing of fungicide application relative to the plant growth stages critical to the colonisation of kernels by ear rot fungi. The study did show an elevation in fumonisins with fungicide spray applications compared to control plants (not sprayed). This could be an indication that strobilurin and/or

triazole could have attributed to fumonisin synthesis in planta. A combination of azoxystrobin (strobilurin) and difenoconazole (triazole) was applied 40 - 45 days after planting which corresponds with the pre-tassling period while flusilazole (silicone triazole) in combination with carbendazim (benzimidazole) was applied 28 - 30 days later which corresponds to the flowering stage. De Curtis *et al*. (2011) emphasised the importance of fungicide application at flowering when maize ears are the most vulnerable to colonisation by *Fusarium* species through the silks (Munkvold *et al*., 1997; Pascale *et al*., 2002; Duncan and Howard, 2010). Therefore, this study will focus on the possible use of prophylactic fungicide sprays (used for control of leaf diseases in maize) to reduce maize ear rot diseases by adjusting time of sprays to align with flowering. These can add value by controlling both leaf diseases, maize ear rots and their associated mycotoxins. Strobilurin, or Quinone-outside inhibitor fungicides (Qol) are widely marketed in maize production for the management of biotic stresses, with the suggestion that these fungicides can increase yield (Hershman *et al*., 2011) even in the absence of disease (Wise and Mueller, 2011). In plants, Qol fungicides may increase water and nitrogen use efficiency, improve chlorophyll retention and delay senescence, thus lengthening the growing period. Literature has indicated that delayed grain maturation can give *F. verticillioides* more time to synthesize fumonisins and could lead to a major risk of mycotoxin contamination (Maiorano *et al*., 2009). With this study we want to elucidate the role of stay green on mycotoxin synthesis in maize grain. Several other studies have also drawn reference to the use of fungicides that may stimulate the production of various mycotoxins. In order to establish the justifiable use of possible additional fungicide applications for controlling *F. verticillioides and F. boothii* ear rot infection and their associated mycotoxins, one must determine the fungicide to be applied, the phenological growth stage for spray application as well as dose rate. This study will align with three strategic objectives as it will elucidate the effect of fungicide formulations (strobilurins and or triazoles) on maize ear rots and mycotoxins pre-harvest. By determining the effect of timing of applications on possible control of maize ear rots and the effect of fungicide formulations and/or the stay green effect on mycotoxin production, valuable information can be conveyed to the maize industry. If strobilurins and/or triazoles is responsible for elevated mycotoxin production in grain, corrective measures can be taken by changing spray programmes.

METHODS

Field trials

Randomised split-split plot experiments were conducted with three replicates at Potchefstroom (warm, dry production areas in the North-West Province), Cedara (subtropical production area in the KwaZulu-Natal Province) and Vaalharts (semi-desert area in the Northern Cape Province) for the 2018/19 planting season under dry land conditions. The whole plot factor was days of spray applications and the second main effect spray combinations. The sub-plot effect was cultivar. Two maize cultivars (BT and non-BT isohybrid) were used and trials were maintained according to "Best Practise" appropriate to the respective production areas. Experimental plots were monitored for the 5-12 leaf stage, pre-flowering, flowering and soft dough stages of plant growth. The fungicide spray combination was administered (Table 1). The controls included naturally infected plants with no fungicide treatment.

Four rows of each cultivar in an experimental block were planted and the middle two rows were sprayed to prevent fungicide drift to other experimental blocks. The outer two rows were the controls. During silking, maize ears of one middle row were inoculated with *F. verticillioides* and the other middle row with *F. boothii*. Plants was scouted throughout for leaf diseases and stalk borers. Individual plots were harvested. Individual samples were rated, threshed and milled for qPCR and HPLC analyses.

Table 1 Fungicide spray combinations and time of application

	First spray	Second spray
1st combination	Amistar Top (Azoxystrobin 200g/l + Difenoconazole 125g/l) 5 leaf stage* 8 leaf stage* 8 leaf stage* 10 leaf stage* 12 leaf stage*	Artea (Cyproconazole 80g/l + Propiconazole 250g/l) 28 days later (pre- flowering stage) 28 days later 42 days later (flowering stage) 28 days later (flowering stage) 56 days later (soft dough stage)
2nd combination	Abacus (Pyraclostrobin 62.5g/l + Epoxiconazole (62.5 g/l)	PunchXtra (Flusilazole 125g/l + Carbendazim 250g/l)
3rd combination	Amistar Top (Azoxystrobin 200g/l + Difenoconazole 125g/l)	PunchXtra (Flusilazole 125g/l + Carbendazim 250g/l)
4th combination	Abacus (Pyraclostrobin 62.5g/l + Epoxiconazole (62.5 g/l)	Artea (Cyproconazole 80g/l + Propiconazole 250g/l)

*Application times will be the same for all fungicide combinations

Product information as supplied online on product labels by respective companies

	Active ingredients	Fungicide group codes	Mode of action
Abacus® - BASF	Pyraclostrobin (methoxy-carbamates) 62,5 g / l Epoxiconazole (triazole) 62,5 g / l	11 and 3	Contact and translaminar fungicide for preventative control of maize leaf diseases. Apart from fungicidal activity, F500®, one of the active ingredients of Abacus®, exhibits the potential to increase plant physiological effects, which are beneficial to the crop. In growing number of regions, including the USA, UK, Europe, Brazil and Argentina, F500® is also registered and recommended as a plant health remedy, to increase yields. Research conducted locally, has proven that the use of Abacus®, according to label recommendations, can also increase yield, even in situations when low disease pressure occur.
Amistar Top - Syngenta	azoxystrobin (strobilurin) 200 g/l difenoconazole (triazole) 125 g/l	11 and 3	A broad spectrum suspension concentrate fungicide with systemic, translaminar and contact properties for the preventive control of different leaf diseases of maize.
Artea - Syngenta	propiconazole (triazole) 250 g/l cyproconazole (triazole) 80 g/l	3	An emulsifiable concentrate systemic fungicide for the preventive control of different diseases in crops. Both active ingredients, cyproconazole and propiconazole, are absorbed by the assimilating parts of the plant, the majority within one hour. They are transported acropetally (upwards) in the xylem. This systemic translocation contributes to good distribution of the active ingredients within the plant tissue and prevents them from being washed off.
PUNCH®XTRA - DuPont	Flusilazole (silicone triazole) 125g/l Carbendazim (benzimidazole) 250g/l	3 and 1	Organic systemic suspension concentrate fungicide combination for the control of diseases in maize. PUNCH® XTRA consists of two non-related systemic fungicides with protective and post infection curative action. The efficacy of PUNCH® XTRA is not influenced by rain occurring after 3 hours after application due to rapid uptake of the active ingredients by plant tissue.

Silk channel inoculation of *F. verticillioides* (MRC826)

A conidial suspension with a concentration of 1 x 10^6 conidia/ml of the *F. verticillioides* isolate was prepared according to Small *et al*. (2012). Primary ears were inoculated using a cattle inoculator fitted with an 18 G x 1.5-in (1.20-by38-mm) Terumo needle (sterile, nontoxic and non-pyrogenic). The conidial suspension (2 ml) was injected down the silk channel six days after silk emergence.

Silk channel inoculation of *F. boothii* (M0100)

The method of Beukes (2015) was used to grow mycelia for maize silk inoculations due to *F. boothii* possibly not producing sufficient macroconidia. The *F. boothii* isolate M0100 (Stellenbosch University) was inoculated in 100 ml potato dextrose broth and incubated at 25°C (rotated at 100 rpm) for one week. Mycelia was harvested through filtration and rinsed with sterile water and allowed to dry for 30 minutes. Mycelia (3 g) was weighed into 100 ml sterile water and pulverised to form a homogenous suspension. The viability of the inoculum was confirmed by plating 1 ml suspension onto potato dextrose agar after the completion of inoculations. Primary ears were inoculated using a cattle inoculator fitted with an 18 G x 1.5-in (1.20-by38-mm) Terumo needle (sterile, nontoxic and nonpyrogenic). The conidial suspension (2 ml) was injected down the silk channel six days after silk emergence.

Quantification of stalk borer damage

No *Busseola fusca* damage observed in the 2017 and 2018 seasons. In the 2018 season, the presence of *Spodoptera frugiperda* (Fall armyworm) in the field trial in Cedara was reported at the 6 leaf stage (identified by Dr. Annemie Erasmus, ARC-GC). Cultivar BG3492B showed more protection against FAW infections, with cultivar BG3292 showing damage on the leaves. The field trial was sprayed with Chlorypriphos 480 which controlled the FAW.

Quantification of leaf diseases

Northern corn leaf blight (*Exserohilum turcicum*) was quantified at 20% in Cedara in the 2017 growing season and at 50% in 2018, even though prophylactic fungicide regimes were administered. It could have an effect on Fusarium ear rot infection and mycotoxin production which will be elucidated after statistical analyses.

Quantification of *F. verticillioides* and *F. boothii*

DNA extraction: Maize ears of the 2017 season 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 the 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.

A high fumonisin-producing *F. verticillioides* isolate (MRC826) and *F. boothii* isolate was used to generate standard curves. The respective fungi were plated out on potato dextrose agar (PDA) and DNA will be extracted from mycelia after 1 week using the CTAB method adapted from Winnepenninckx *et al*. (1993).

Quantification of *F. verticillioides* and *F. boothii* target DNA: A 10-fold dilution of *F. verticillioides* DNA was used to generate a standard curve for quantification (Waalwijk *et al*. 2008; Janse van Rensburg *et al*. 2015). *Fusarium verticillioides* target DNA was determined as described by Janse van Rensburg *et al*. (2015). For *F. boothii*, a 4-fold standard dilution was used to generate a standard curve for quantification (Nicolaisen *et al*. 2009). Primers in combination with SYBRGreen, as tested by Nicolaisen *et al*. (2009), was used. A 96- well reaction plate will be prepared consisting of a total volume of 25 µL of 12.5 µL of SYBRG green and 0.625 µL (250mM) of each primer, 9.25 µL of nuclease free water and 2 µL of DNA. Negative controls that contain no template DNA will be treated similar to the reaction samples. A CFX96TM Real-Time PCR detection system (Bio-Rad, Hercules, USA) with a 96 well reaction plate was used for all qPCR assays. Cycling conditions for *F. boothii* 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 were generated from the amplification curves.

Mycotoxin quantification

Fumonisins: Fumonisins were analysed using the HPLC-VICAM method (Janse van Rensburg *et al*., 2015). Fumonisin standards were obtained from CPUT. To generate a standard curve, standards were evaporated and reconstituted with a calibration standard solution ranging from 0.31 to 5 µg/kg. 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 LOD of the method used was 16 µg/kg and R^2 values were ≥99%. Total fumonisins were determined as the sum of FB^1+FB^2+FB^3.

Zearalenone: Zearalenone were 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 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 a 24-cm fluted filter paper, the filtrate (4 mL) was mixed with 96 mL HPLC grade water (18 MΩ.cm) and filtered again through a microfiber filter paper. The diluted extract (100 mL) was passed through the ZearaTest affinity column (VICAM) at a rate of approximately 3 drops per second and the column was washed with 25 mL HPLC grade water. Zearalenone were eluted with 0.75 mL methanol followed by 0.5 mL water. The eluate (50 µL) were injected into the HPLC system of which the mobile phase consisted of acetonitrile: methanol: water (30:30:40 v/v/v) set to a flow rate of 1 mL/min. Zearalenone standards were obtained from Sigma-Aldrich Missouri, USA). To generate a standard curve, standards were evaporated and reconstituted to 100-, 250-, 500-, 1250- and 2500 µg/kg. Fluorescence were 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 LOD of the method used was 1.9 µg/kg and R^2 values were ≥99%.

Deoxynivalenol: Deoxynivalenol were 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 and the filtrate collected. The filtrate (10 mL) was mixed with 40 mL phosphate-buffered saline (PBS) (8.0 g NaCl, 1.2 g Na2HPO4, 0.2 g KH2PO4, 0.2 g KCl, made to 1L; pH 7.0) and filtered inside a funnel (11 cm). The filtered extract (5 mL) was passed through a deoxynivalenol WB affinity column (VICAM) at a rate of approximately 1 drop per 2 seconds. The deoxynivalenol WB affinity column was washed with 10 mL PBS followed by 10 mL purified water at a rate of about 1 drop/second. Deoxynivalenol was eluted by 0.5 mL HPLC grade methanol and 1.5 mL HPLC acetonitrile. Standards for deoxynivalenol were obtained from Sigma-Aldrich Missouri, USA). To generate a standard curve, standards were evaporated and reconstituted to 100-, 500-, 2500- and 5000 µg/kg. 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 LOD of the method used was 30 µg/kg and R^2 values were ≥99%.

Statistical analysis

A split-split plot analysis was done on the data with the first main effect being days of spray applications and the second main effect spray combinations. The sub-plot effect was cultivar. The data was analysed in SAS 9.4 by Mrs. N. Cochrane from the ARC-Biometry.

Mean *F. verticillioides, F. boothii* target DNA as well as fumonisins, deoxynivalenol and zearalenone was mainly higher in the 2018/19 production season, compared to the 2017/18 production season (Table 2), therefore each experiment was analysed separately by location and year. Weather data (Table 3) shows an increase of 2 - 3 °C between Cedara, Potchefstroom and Vaalharts.

Table 2 Mean *F. verticillioides, F. boothii* target DNA, fumonisins, deoxynivalenol and zearalenone levels for the 2017/18 and 2018/19 production seasons.

Mean target DNA and mycotoxin levels at three localities over 2 seasons.	**Cedara**		**Potchefstroom**		**Vaalharts**	
	2017/18	**2018/19**	**2017/18**	**2018/19**	**2017/18**	**2018/19**
***F. verticillioides* target DNA (P<.0001) expressed as pg μg^{-1}**	1120.63	1742.05	7.02	1682.61	322.76	953.43
Total fumonisins (FB1+FB2+FB3) (P<.0001) expressed as µg/kg	3284.00	3326.09	107.54	2716.46	2038.45	2126.74
***F. boothii* target DNA (P<.0001) expressed as pg µg-1**	28.37	507.13	20.20	3.94	117.24	202.21
Deoxynivalenol expressed as µg/kg (P<.0001) (DON log transformed)	300.00 (19.00)	1800.10 (810.00)	9.00 (7.00)	100.00 (20.00)	160.00 (90.00)	1570.00 (790.00)
Zearalenone expressed as µg/kg(P<.0001)	136.77	234.07	19.55	109.34	25.49	252.71

Table 3 **Weather data for the 2017/18 and 2018/19 production seasons at Cedara, Potchefstroom and Vaalharts, supplied by the ARC - ISCW.**

Cedara			Average				
	Year	Month	Tx	Tn	RHx	RHn	Rain
	2017/18	11	24,13	10,54	89,26	36,27	4,52
		12	23,54	12,46	92,6	52,39	3,17
	2018/19	1	27,07	14,09	92,87	44,35	2,11
		2	26,47	15,18	93,5	49,71	8,13
		3	25,67	13,56	93,92	48,23	5,02
		4	24,25	12,39	94,5	46,71	2,16
		5	21,54	7,06	92,88	38,77	1,23
Mean average			24,66	12,18	92.79	45,20	3,76
	2018/19	11	24,21	10,94	93,39	46,83	1,36
		12	26,81	14,85	94,18	50,03	4,75
	2018/19	1	25,71	14,56	94,83	53,29	1,85
		2	26,03	14,52	94,46	50,36	4,4
		3	25,9	14,63	94,69	51,54	1,98
		4	22,95	11,86	95,08	53,26	3,7
		5	23,73	8,4	94,08	35,36	0,78
Mean average			25,05	12,82	94,39	48,67	2,69
Potchefstroom			Average				
	Year	Month	Tx	Tn	RHx	RHn	Rain
	2017/18	11	29,12	12,67	78,14	20,46	2,31
		12	29,29	15,69	84,56	29,15	2,02
	2018/19	1	31,04	16,09	76,81	23,64	1,52
		2	27,68	15,64	90,59	39,67	2,44
		3	27,54	14,55	90	36,35	1,9
		4	25,33	11,13	91,77	36,63	1,19
		5	22,78	4,86	87,51	26,38	0,36
Mean average			27,54	12,95	85,63	30,33	1,68
	2018/19	11	30,7	14,03	69,07	16,1	0,57
		12	32,85	16,22	75,12	19,46	1,37
	2018/19	1	31,47	16,74	83,13	27,14	2,29
		2	28,74	15,6	89,09	34,98	1,75
		3	30,22	15,07	85,76	27,5	1,11
		4	23,84	11,74	92,87	44,58	4,86
		5	24,26	5,82	87,7	24,04	0
Mean average			28,87	13,60	83,25	27,69	1,71
Vaalharts			Average				
	Year	Month	Tx	Tn	RHx	RHn	Rain
	2017/18	11	31,81	12,12	67,33	13,74	0,09
		12	33,31	15,49	82,98	18,39	1,25
	2018/19	1	34,12	17,16	76,93	19,33	2,02
		2	31,32	16,98	95	33,1	2,98
		3	29,51	14,41	93,85	33,69	4,75

		4	26,11	11,67	98,07	40,15	1,49
		5	24,15	3,71	95,4	26,34	0,27
Mean average			30,05	13,08	87,08	26,39	1,84
	2018/19	**11**	32,93	13,29	59,45	11,72	0,03
		12	35,61	16,11	64,13	12,91	0,62
	2018/19	**1**	35,96	17,32	68,41	15,17	1,22
		2	33,36	16,39	80,32	17,36	1,14
		3	32,87	15,97	85,95	24,49	1,08
		4	26,35	11,81	96,54	35,95	5,82
		5	26,12	6,98	89,7	24,91	0,19
Mean average			31,89	13,98	77,79	20,36	1,44

RESULTS

2017/18 season Cedara

F. verticillioides

Mean *F. verticillioides* infection of maize grain in Cedara was 1120.63 pg μg^{-1}. Anova results showed that only cultivar as main factor had a highly significant effect (<.0001) on infection of grain with *F. verticillioides*. The mean fungal infection for cultivar BG3292 was significantly higher (2044.90 pg μg^{-1}) when compared to its isoline BG3492B (196.30 pg μg^{-1}).

Fumonisins

Anova results showed that only cultivar as main factor, had a highly significant effect (<.0003) on the mean total fumonisin synthesis in grain. The mean total fumonisin in cultivar BG3292 was significantly higher (3992.50 µg/kg) when compared to its isoline BG3492B (2577.3 µg/kg).

F. boothii

Mean *F. boothii* infection of maize grain in Cedara was 282.37 pg μg^{-1}. Anova results showed that spray combination and cultivar (as main factors) had a significant influence on *F. boothii* fungal infection in Cedara (P=0.01 and P=0.03, respectively). The Abacus/PunchXTra (140.7 pg μg^{-1}) and Abacus/Artea (185.3 pg μg^{-1}) spray combinations had significantly less fungal infection when compared to the Amistar Top/Artea (451.00 pg μg^{-1}) and Amistar Top/PunchXTra (352.4 pg μg^{-1}) spray combinations. The mean fungal infection for cultivar BG3292 was significantly higher (365.94 pg μg^{-1}) when compared to its isoline BG3492B (198.80 pg μg^{-1}).

Deoxynivalenol (DON) and Zearalenone (ZEA)

DON data was skew and was log transformed. The mean DON and ZEA levels were 30 and µg/kg and 136.77 µg/kg respectively. Application date of fungicide sprays as main effect had a significant effect (P=0.05) on DON synthesis in maize grain (Table 4). Cultivar as main effect had an influence on ZEA synthesis in maize (P=0.07) with a mean concentration of 180.96 µg/kg and 92.58 µg/kg in cultivar BG3292 and BG3492B respectively.

Table 4 The effect of fungicide spray application dates on DON synthesis in maize grain at Cedara for the 2017/18 season.

Days of application First spray (expressed as leaf stage) / second spray (days following the first spray)	DON expressed as µg/kg	DON Log transformed
5/28	270.00bc	190.00abc
8/28	590.00a	360.00a
8/42	80.00c	70.00c
10/28	150.00bc	120.00bc
10/56	430.00ab	240.00ab

2018/19 season Cedara

F. verticillioides

Mean *F. verticillioides* infection of maize grain in Cedara was 1742.05 pg μg^{-1}. Anova results showed that none of the parameters had an effect on infection of grain with *F. verticillioides*.

Fumonisins

Mean fumonisins quantified in maize grain was 3326.08 µg/kg. Anova results showed that none of the parameters had an effect on infection of grain with fumonisins.

F. boothii

Mean *F. boothii* infection of maize grain in Cedara was 507.12 pg μg^{-1}. Anova results showed that application date of fungicide sprays as main effect had a significant effect (P=0.02) on *F. boothii* infection of maize grain (Table 5). *F. boothii* infections of maize grain was higher in cultivar BG3492B (627.9 pg μg^{-1}) than in cultivar BG3292 (388.30 pg μg^{-1}) with a P-value of 0.07.

Deoxynivalenol (DON) and Zearalenone (ZEA)

Anova results showed that none of the parameters had an effect on infection of grain with DON. Cultivar as main effect had a significant influence on ZEA synthesis in maize (P=0.01)

with a mean concentration of 284.00 µg/kg and 184.15 µg/kg in cultivar BG3292 and BG3492B, respectively. Date of fungicide sprays as main effect had an influence on ZEA synthesis in maize with a P-value of 0.09 (Table 6).

Table 5 The effect of fungicide spray application dates on *F. boothii* infection of maize grain at Cedara for the 2018/19 season.

Days of application **First spray (expressed as leaf stage) / second spray (days following the first spray)**	***F. boothii* expressed as pg µg^{-1}**
5/28	653.20ab
8/28	860.50a
8/42	455.60bc
10/28	347.80bc
10/56	211.80c

Table 6 The effect of fungicide spray application dates on ZEA production in maize grain at Cedara for the 2018/19 season.

Days of application **First spray (expressed as leaf stage) / second spray (days following the first spray)**	***ZEA* expressed as µg/kg**
5/28	285.68a
8/28	292.74a
8/42	246.51ab
10/28	186.18b
10/56	159.25b

2017/18 season Potchefstroom

F. verticillioides

Mean *F. verticillioides* infection of maize grain in Potchefstroom was 7.02 pg µg^{-1}.

Anova results showed that cultivar (P<.0001) as main factor had a significant effect on *F. verticillioides* fungal infection of maize grain in Potchefstroom. Cultivar BG3292 yielded significantly higher mean *F. verticillioides* infections (11.39 pg µg^{-1}) compared to its isoline, cultivar BG3492B (2.72 pg µg^{-1}).

Fumonisins

Mean fumonisin levels at Potchefstroom was 107.54 µg/kg. Only cultivar as main factor influenced total fumonisins (FB^1+FB^2+FB^3), with BG3292 having a mean fumonisin level of 138.31 µg/kg and BG3492B having a mean fumonisin level of 75.71 µg/kg (P=0.005).

F. boothii

Mean *F. boothii* infection of maize grain in Potchefstroom is 42.15 pg μg^{-1}. Cultivar (P=0.002) as main variable had a significant effect on *F. boothii* fungal infection with mean quantifications of 53.31 pg μg^{-1} in cultivar BG3292 and 31.28 pg μg^{-1} in cultivar BG3492B, respectively.

Deoxynivalenol (DON) and Zearalenone (ZEA)

Mean DON and ZEA levels at Potchefstroom were 80.00 µg/kg and 19.55 µg/kg respectively. Anova results showed that none of the parameters had an effect on infection of grain with DON or ZEA.

2018/19 season Potchefstroom

F. verticillioides

Mean *F. verticillioides* infection of maize grain in Potchefstroom was 1682.60 pg μg^1. Anova results showed that application date of fungicide sprays as main effect had a significant effect (P=0.04) on *F. verticillioides* infection of maize grain (Table 7).

Anova results showed that cultivar (P=0.09) as main factor had an effect on *F. verticillioides* fungal infection of maize grain in Potchefstroom. Cultivar BG3292 yielded lower mean *F. verticillioides* infections (1484.10 pg μg^{-1}) compared to its isoline, cultivar BG3492B (1877.80 pg μg^{-1}).

Fumonisins

Mean fumonisin levels at Potchefstroom was 2716.46 µg/kg. Only cultivar as main factor influenced total fumonisins (FB^1+FB^2+FB^3), with BG3292 having a lower mean (P<.0001) fumonisin level of 1838.90 µg/kg compared to BG3492B (3594.00 µg/kg).

F. boothii

Mean *F. boothii* infection of maize grain in Potchefstroom is 3.94 pg μg^{-1}. Cultivar (P=0.08) as main variable had an effect on *F. boothii* fungal infection with mean quantifications of 3.50 pg μg^{-1} in cultivar BG3292 and 4.38 pg μg^{-1} in cultivar BG3492B, respectively.

Deoxynivalenol (DON) and Zearalenone (ZEA)

Mean DON and ZEA levels at Potchefstroom were 50.00 µg/kg and 109.34 µg/kg respectively. Anova results showed no significant effects regarding the tested parameters on DON production in maize grain at Potchefstroom. Cultivar (P=0.01) as main variable had an effect on ZEA production in maize grain infection with mean quantifications of 170.06 µg/kg in cultivar BG3292 and 48.62 µg/kg in cultivar BG3492B, respectively.

Table 7 The effect of fungicide spray application dates on *F. verticillioides* infection of maize grain at Potchefstroom for the 2018/19 season.

Days of application **First spray (expressed as leaf stage) / second spray (days following the first spray)**	***F. verticillioides* expressed as pg μg^{-1}**
5/28	1811.5ab
8/28	2345.6a
8/42	1452.2b
10/28	1329.2b
10/56	1464.9b

2017/18 season Vaalharts

F. verticillioides

Mean *F. verticillioides* infection of maize grain in Vaalharts was 175.49 pg μg^{-1}. Anova results showed that cultivar (P<.0001) as main factor had a significant effect on *F. verticillioides* fungal infection of maize grain in Vaalharts. Cultivar BG3292 yielded

significantly higher mean *F. verticillioides* infections (273.75 pg µg^{-1}) compared to its isoline, cultivar BG3492B (78.06 pg µg-^{1}).

Fumonisins

Mean fumonisin levels were 2038.45 µg/kg and cultivar as main variable had a highly significant effect on fumonisin levels in maize grain (P<.00001). Fumonisins were quantified at 3074.9 µg/kg in cultivar BG3292 and 1002.0 µg/kg in cultivar BG3492B respectively.

F. boothii

Mean target DNA levels of *F. boothii* were 117.24 pg µg^{-1} in Vaalharts. Cultivar (P=0.001) as main variable had a significant effect on *F. boothii* fungal infections. Mean quantifications of *F. boothii* target DNA was 61.98 pg µg^{-1} in cultivar BG3292 and 172.50 pg µg^{-1} in BG3492B, respectively. A trend could be observed where fungicide spray combination had an effect on *F. boothii* fungal infections (P=0.08) of maize grain. The Abacus / Artea combination had higher mean *F. boothii* infections (189.58 pg µg-1) compared to the Amistar Top / Artea (94.55 pg µg-1), Amistar Top / PunchXtra (97.20 pg µg-1) and Abacus / PunchXtra (87.64 µg-1) spray combinations.

Deoxynivalenol (DON) and Zearalenone (ZEA)

The mean DON and ZEA levels were 160.00 µg/kg and 25.49 µg/kg respectively. Anova results showed that application date of fungicide sprays as main effect had a significant effect (P=0.05) on DON production in maize grain (Table 8). Application date of fungicide sprays x cultivar had a significant (P=0.01) effect on ZEA production in maize grain (Table 9).

2018/19 season Vaalharts

F. verticillioides

Mean *F. verticillioides* infection of maize grain in Vaalharts was 953.43 pg µg-1. Anova results showed that cultivar (P=.0003) as main factor had a significant effect on *F. verticillioides* fungal infection of maize grain in Vaalharts. Cultivar BG3292 yielded significantly higher mean *F. verticillioides* infections (1220.60 pg µg^{-1}) compared to its isoline, cultivar BG3492B (686.30 pg µg^{-1}).

Fumonisins

Mean fumonisin levels were 2126.73 µg/kg and cultivar had no effect on fumonisin levels in maize grain. Application date of fungicide sprays x fungicide spray combination had an effect on mean total fumonisin infection of maize grain (Table 10).

F. boothii

Mean target DNA levels of *F. boothii* were 202.21 pg μg^{-1} in Vaalharts. Cultivar (P=0.008) as main variable had a significant effect on *F. boothii* fungal infections. Mean quantifications of *F. boothii* target DNA was significantly higher in cultivar BG3292 (292.64 pg μg^{-1}) compared to cultivar BG3492B111.78 pg μg^{-1}.

Deoxynivalenol (DON) and Zearalenone (ZEA)

The mean DON and ZEA levels were 1570.00 µg/kg and 252.71µg/kg respectively. Application date of fungicide sprays x fungicide spray combination had a significant effect on mean DON levels in maize grain (Table 10). Cultivar (P=0.01) as main variable had a significant effect on DON production in maize grain with mean quantifications of 910.00 µg/kg in cultivar BG3292 and 660 µg/kg in cultivar BG3492B, respectively. Cultivar (P=0.02) as main variable had a significant effect on ZEA production in maize grain with mean quantifications of 212.25 µg/kg in cultivar BG3292 and 293.17 µg/kg in cultivar BG3492B, respectively.

Table 8 The effect of fungicide spray application dates on DON production in maize grain at Vaalharts for the 2017/18 season.

Days of application **First spray (expressed as leaf stage) / second spray** **(days following the first spray)**	**DON expressed as µg/kg**	**DON log transformed**
5/28	130.00b	90.00b
8/28	460.00a	220.00a
8/42	100.00b	70.00b
10/28	50.00b	40.00b
10/56	40.00b	40.00b

Table 9 The effect of application date of fungicide sprays x cultivar on ZEA production in maize grain at Vaalharts in the 2017/18 season.

Cultivar	Days of application First spray (expressed as leaf stage) / second spray (days following the first spray)	ZEA expressed as µg/kg
BG3292	**5/28**	30.97ab
BG3292	**8/28**	32.85a
BG3292	**8/42**	22.67abcd
BG3292	**10/28**	17.38cd
BG3292	**10/56**	13.17d
BG3492B	**5/28**	27.91abc
BG3492B	**8/28**	21.30bcd
BG3492B	**8/42**	27.52abc
BG3492B	**10/28**	30.77ab
BG3492B	**10/56**	30.38ab

Table 10 The effect of application date of fungicide sprays x fungicide spray combination on mean total fumonisin and DON infection of maize grain in Vaalharts in the 2018/19 season.

Days of application First spray (expressed as leaf stage) / second spray	Fungicide spray combinations	Fumonisins expressed as µg/kg	DON expressed as DON expressed as µg/kg	DON log transformed
5/28	**Amistar Top / Artea**	2719.20b-d	2380.00a	1110.00a
5/28	**Amistar Top/PunchXtra**	1885.80b-g	710.00de	480.00c
5/28	**Abacus/PunchXtra**	1862.70b-g	1090.00a-e	840.00abc
5/28	**Abacus/Artea**	2677.9b-d	1120.00b-e	570.00bc
8/28	**Amistar Top / Artea**	3104.60b	1690.00a-e	840.00abc
8/28	**Amistar Top/PunchXtra**	2145.00b-g	2520.00a	1180.00a
8/28	**Abacus/PunchXtra**	2351.00b-f	1070.00b-e	2570.00bc
8/28	**Abacus/Artea**	1585.60d-g	1950.00abc	990.00ab
8/42	**Amistar Top / Artea**	1762.70b-g	2540.00a	1210.00a
8/42	**Amistar Top/PunchXtra**	2997.40bc	170.004a-e	890.00abc
8/42	**Abacus/PunchXtra**	900.40g	1090.00b-e	510.00bc
8/42	**Abacus/Artea**	2573.10b-e	2010.00ab	970.00ab
10/28	**Amistar Top / Artea**	4553.10a	740.00cde	510.00bc
10/28	**Amistar Top/PunchXtra**	2347.80b-f	2410.00a	1070.00a
10/28	**Abacus/Punch**	1722.90b-g	1010.00b-e	470.00c
10/28	**Abacus/Artea**	1719.70b-g	590.00e	420.00c
10/56	**Amistar Top / Artea**	1092.00fg	910.00b-e	480.00c
10/56	**Amistar Top/PunchXtra**	1705.40c-g	1530.00a-e	830.00abc
10/56	**Abacus/PunchXtra**	1572.8d-g	2050.00ab	970.00ab
10/56	**Abacus/Artea**	1264.30e-g	1920.00a-d	850.00abc

DISCUSSION

In a previous study funded by the Maize Trust, the use of a prophylactic fungicide spray regime for control of leaf diseases in maize did not reduce Fusarium ear rot in maize, however, significantly elevated fumonisin levels were recorded (Janse van Rensburg, *et al*. 2016) . In this study, azoxystrobin + difenoconazole (strobilurin, 200 g/L + triazole, 125 g/L) at a rate of 500 ml/ha, were applied 40 - 45 days after planting. This was followed 28 - 30 days later by a second fungicide application of flusilazole + carbendazim (silicone triazole, 125 g/L + benzimidazole, 250 g/L) at a rate of 750 m[/ha with petroleum as adjuvant (500 ml/ha). It was hypothesized that the lack of efficacy may be attributed to the timing of fungicide application relative to the plant growth stages critical to the colonisation of kernels by ear rot fungi. Therefore, the current study focussed on the possible use and inclusion of more prophylactic fungicide sprays (used for control of leaf diseases in maize) to reduce maize ear rots such as *F. verticillioides* and *F. boothii* by adjusting time of sprays to align with flowering.

The research show that cultivar and season as main variables had an influence on the infection of *F. verticillioides* and *F. boothii*, as well as mycotoxin production in maize grain. In the 2017/18 season in Cedara, levels of *F. verticillioides* and *F. boothii* as well as fumonisins and ZEA were significantly higher in cultivar BG3292 compared to its isoline BG3492B. In the 2018/19 season, cultivar had no effect on *F. verticillioides* infection or DON production in maize grain. *F. boothii* target DNA levels and ZEA levels were lower in maize grain of cultivar BG3492B.

In the 2017/18 season at Cedara, spray combination (as main factor) had a significant influence on *F. boothii* fungal infection in Cedara (P=0.01). The Abacus/PunchXTra (140.7 pg μg^{-1}) and Abacus/Artea (185.3 pg μg^{-1}) spray combinations had significantly less fungal infection when compared to the Amistar Top/Artea (451.00 pg μg^{-1}) and Amistar Top/PunchXTra (352.4 pg μg^{-1}) spray combinations. In the same season, application date of fungicide sprays as main effect had a significant effect (P=0.05) on DON synthesis in maize grain. It showed that an application at 8 leaf stage and then 42 days later (which correspond with the flowering stage), had the lowest concentration of DON (80.00 µg/kg) when compared to the application at 8 leaf stage and then 28 days later (which correspond to the pre-flowering stage) with the highest concentration of DON (590 µg/kg).

F. verticillioides and *F.boothii* infection as well as fumonisin and DON production in maize grain were the highest at Cedara in the 2018/19 season. This correspond with the outbreak

of Fall armyworm in the 2018/19 season and an infection of 50% Northern corn leaf blight (*Exserohilum turcicum*). These could have predisposed plants to ear rot infections, thereby possibly masking the effects of fungicide spray combinations and applications at this locality. In the 2018/19 season at Cedara, the application date of fungicide sprays as main effect had a significant effect (P=0.02) on *F. boothii* infection of maize grain. It showed that an application at 10 leaf stage and then 56 days later (which correspond to the soft dough stage), had the lowest concentration of *F. boothii* (211.80 pg μg^{-1}) compared to an application at 8 leaf and then 28 days later (pre-flowering stage) with the highest *F. boothii* concentration (860.50 µg/kg).

Overall fungal infection and mycotoxin production was low in Potchefstroom in both seasons, except for *F. verticillioides* target DNA levels and total fumonisins with levels of 1682.61 pg ug^{-1} and 2716.46 µg/kg in the 2018/19 season respectively. In the 2017/18 season, *F. verticillioides* and *F. boothii* infections as well as fumonisin levels were higher in cultivar BG3292 than its isoline BG3492B. Contradictory to this finding, these levels were all lower in cultivar BG3292 compared to its isoline BG3492B in the 2018/19 season. ZEA levels were higher in this season in cultivar BG3292 compared to its isoline BG3492B. In the 2018/19 season, fungicide applications at 8 leaf and then 28 days later (pre-flowering stage) had significant higher *F. verticillioides* target DNA (2345.6 pg ug^{-1}) in maize grain compared to the other date of application regimes.

In the 2017/18 season at Vaalharts, mean *F. verticillioides* infection of maize grain and fumonisin levels were higher in cultivar BG3292 compared to its isoline BG3492B. *F. boothii* target DNA were lower in BG3292 compared to its isoline BG3492B. An application at 8 leaf stage and then 28 days later (which correspond to the pre-flowering stage), had the highest concentration of DON (460.00 µg/kg) compared to all the other date of application combinations. A cultivar x date of application interaction showed that BG3292 x the application at the 8 leaf stage and then again 28 days later, had the highest level of ZEA (32.85 µg/kg) compared to the other interactions. BG3292 x the application at the 10 leaf stage and then again 56 days later (soft dough stage), had the lowest level of ZEA (13.17 µg/kg) compared to the other interactions.

In the 2018/19 season at Vaalharts, mean *F. verticillioides* and *F. boothii* infections of maize grain and DON levels were higher in cultivar BG3292 compared to its isoline BG3492B. Mean ZEA levels were lower in cultivar BG3292 compared to its isoline BG3492B. Application date of fungicide date of spray x fungicide spray combination had an effect on mean total fumonisin levels in maize grain with the Abacus / PunchXtra interaction with the

spray at 8 leaf stage followed by a second spray after 24 days having the lowest fumonisin level (900.40 µg/kg). The Amistar Top / Artea interaction with the spray at 10 leaf stage followed by a second spray after 28 days having the highest fumonisin level (4553.10 µg/kg).

Application date of fungicide sprays x fungicide spray combination in the 2018/19 season at Vaalharts also had an effect on mean DON levels in maize grain with the Abacus / Artea interaction with the spray at 10 leaf stage followed by a second spray after 28 days having the lowest DON level (590.00 µg/kg). The Amistar Top / Artea x 5/28 days of application, Amistar Top / PunchXtra x 8/28 days of application, Amistar Top / Artea x 8/42 days of application and Amistar Top / PunchXtra x 10/28 days of application all had significantly highest DON levels (2380.00 µg/kg, 2520.00 µg/kg, 2540 µg/kg and 2410 µg/kg) respectively.

CONCLUSION

Although the effect of spray combinations, application date of fungicide sprays and interactions between the two were recorded on the reduction of maize ear rot and associated mycotoxins, results were not consistent. It seems that spray applications at flowering or during soft dough stage can decrease either DON levels or *F. boothii* infections of maize grain at Cedara and Vaalharts, while earlier applications (pre-flowering) show significantly higher levels of *F. boothii* or DON. The same trend was observed in Potchefstroom where earlier fungicide applications (pre-flowering) showed higher levels of *F. verticillioides* target DNA in maize grain. Similar results were obtained in Vaalharts in the 2017/18 season where cultivar BG3292 x the application at the 8 leaf stage and then again 28 (pre-flowering) days later, had the highest level of ZEA compared to the other interactions. BG3292 x the application at the 10 leaf stage and then again 56 days later (soft dough stage), had the lowest level of ZEA compared to the other interactions.

In the 2017/18 season (Cedara), fungicide combinations with Amistar Top resulted in higher levels of *F. boothii* when compared to the other spray combinations without Amistar Top. Similar results were observed in Vaalharts in the 2018/19 season, where Amistar Top / Artea x 5/28 days of application, Amistar Top / PunchXtra x 8/28 days of application, Amistar Top / Artea x 8/42 days of application and Amistar Top / PunchXtra x 10/28 days of application all had the highest DON levels. Similar to this, the Amistar Top / Artea interaction with the spray at 10 leaf stage followed by a second spray after 28 days also had the highest fumonisin level at Vaalharts (2018/10 season). In a previous study by Janse van Rensburg

et al. (2016) a prophylactic spray regime using Amistar Top showed no effect on the infection and colonization of *F. verticillioides* in maize grain and described the lack efficacy due to timing of the fungicide application relative to the plant growth stages. In this study it is shown that Amistar Top should not be considered for the possible additional control of maize ear rots and mycotoxin contamination. Amistar Top is the only fungicide used in this study with the active ingredient strobilurin, which could explain the higher infection levels of *F. boothii* as well as DON and FUM synthesis in maize grain.

The inconsistent reactions of fungicide combinations and/or date of application against *F. verticillioides*, *F. boothii* as well as FUM, DON and ZEA synthesis can be due to the fungal infection process and plant morphology (Luna and Wise, 2015). Foliar fungicides are commonly applied over the top of the maize plant with ground or areal equipment and it is possible that these barriers limit the amount of fungicide to penetrate the plant to come into contact with the fungus.

Prophylactic foliar fungicides used in this study can be effective as management tools for leaf diseases such as *Cercospora zea maydis* and *Escerohilum turcicum,* but because they do not consistently reduce ear rots and mycotoxins tested in this study, it is not economically beneficial to change spray dates to co-incide with the flowering or soft dough plant stages. It is recommended that producers continue to manage maize ear rots using cultural methods such as cultivar selectin, crop rotation and residue management (where applicable). In conservation agriculture systems, crop rotation will be greatly beneficial to break the disease cycle by reducing primary inoculum.

REFERENCES

Anonymous, 2012. Vicam fumonitest instruction manual. Vicam, L.P. 313 Pleasant Street, Watertown, MA 02472, United States of America.

Beukes, I. 2015. Pathogenicity and mycotoxin production of *Fusarium graminearum* species complex in South African grains. MSc in AgriSciences at Stellenbosch University, pp 137.

Bhat, R., Rai, R.V. & Karim, A.A. 2010. Mycotoxins in food and feed: Present status and future concerns. Comprehensive Reviews in Food Science & Food Safety 9:57-81.

De Curtis, F.D, De Cicco, V, Haidukowski, M, Pascale, M, Somma, S and Moretti, A. 2011. Effects of agrochemical treatments on the occurrence of Fusarium ear rot and fumonisin concentration of maize in southern Italy. Field Crops Research. 123: 161-169.

Duncan, K.E and Howard, R.J. 2010. Biology of maize kernel infection by *Fusarium verticillioides*. Molecular Plant Microbe International. 23: 6 -16.

FAO. Food and Agriculture Organization of the United Nations, 2003. Worldwide regulations for mycotoxins in food and feed. Available at: http://www.fao.org/3/y5499e/y5499e00.htm. Accessed 24 June 2020.

Government Gazette, 2016. Regulations Governing Tolerances For Fungus-Produced Toxins in Foodstuffs: Amendment. Section 15 (1) of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972).

Hershman, D.E. Vincelli, P and Kaiser, C.A. 2011. Foliar fungicide use in corn and soybeans. Plant pathology fact sheet PPFS-MISC-05, UK Cooperative extension service, University of Kentucky, College of Agriculture.

Janse van Rensburg, B, McLaren N.W, Flett, B.C. and Schoeman, A. 2015. Fumonisin producing *Fusarium* spp. and fumonisin contamination in commercial South African maize. European Journal of Plant Pathology. 141: 491-504.

Janse van Rensburg, B, McLaren N.W, Schoeman, A and Flett, B.C. 2016. The effect of cultivar and prophylactic fungicide spray for leaf diseases on colonisation of maize ears by fumonisin producing *Fusarium* spp. and fumonisin synthesis in South Africa. Crop Protection. 79: 56 - 63.

Kruger, S.C., Kohn, B., Ramsey, C.S. and Prioli, R. 1999. Rapid Immunoaffinity-Based Method for Determination of Zearalenone in Corn by Fluorometry and Liquid Chromatography, Journal of AOAC INTERNATIONAL. 82: 1364–1368, https://doi.org/10.1093/jaoac/82.6.1364.

Luna, M.P.R. and Wise, K.A. 2015. Timing and efficacy of fungicide applications for Diplodia ear rot management in corn. Plant Health Progress. 16: 123 - 131.

Maiorano, A, Reyneri, A, Magni, A and Ramponi, C. 2009. A decision tool for evaluating the agronomic risk of exposure to fumonisins of different maize crop management systems in Italy. Agricultural Systems. 102: 17 - 23.

Munkvold, G.P, McGee, D.C and Carlton, W.M.1997. Importance of different pathways for maize kernel infection by *Fusarium moniliforme*. Phytopathology. 87: 209 -217.

Nape, K.M. 2011. Using Seasonal Rainfall with APSIM to Improve Maize Production in the Modder River Catchment. Bloemfontein: University of the Free State, South Africa. Thesis-MSc.

Nicolaisen, M, Suproniene, S, Nielsen, L.K, Lazzaro, I, Spliid, N.H, Justesen, A.F. 2009. Real-time PCR for quantification of eleven individual Fusarium species in cereals. Journal of Microbiological Methods. 76: 234 - 40.

Nyaka, S.C, Shankar, A.C.U, Niranjana, S.R, Wulff, E.G, Mortensen, C.N and Prakash, H.S. 2010. Detection and quantification of fumonisin from *Fusarium verticillioides* in maize grown in southern India. World Journal of Microbial Biotechnology. 26: 71 - 78.

O'Donnell, K, Ward, T.J, Geiser, D.M, Kistler, H.C, Aoki, T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genetics Biology. 41: 600 – 623.

Pascale, M, Visconti, A and Chelkowsky, J. 2002. Ear rot susceptibility and mycotoxin contamination of maize hybrids inoculated with *Fusarium* spp. under field conditions. European Journal of Plant Pathology. 108: 645 - 651.

Small, I.M, Flett, B.C, Marasas, W.F.O, McLeod, A, Viljoen, A. 2012. Resistance in maize inbred lines to *Fusarium verticillioides* and fumonisin accumulation in South Africa. Plant Disease. 96: 881 – 888.

Waalwijk, C, Koch, S, Ncube, E, Allwood, J, Flett, B.C, de Vries, I. and Khema, G.H.J. 2008. Quantitative detection of *Fusarium* spp. and its correlation with fumonisin content in maize from South African subsistence farmers. World Mycotoxin Journal 1: 37 - 45.

Walker, N.J and Schulze, R.E. 2006. An assessment of sustainable maize production under different management and climate scenarios for smallholder agro-ecosystems in KwaZulu-Natal, South Africa. Physical Chemistry Earth. 31: 995 - 1002.

Wise, K and Mueller, D. 2011. Are fungicides no longer just for fungi? An analysis of foliar fungicide use in corn. [Available on Internet:] http://www.apsnet.org/publications/apsnetfeatures/Pages/fungicide.aspx [Date of access: 10 October 2011].

Winnepenninckx, B., Backelgau, T. and De Wachter, R. 1993. Extractions of high molecular weight DNA from molluscs. Trends in Genetics. 9: 407

9. Scientific outputs

9.1 Outputs of project as stated in the application

Expected outputs	**Numbers**	**Achieved (yes/no or n.a.)**	**Nature of output e.g. title of papers, description, etc.**
Scientific papers:	1	Not yet	Scientific paper to be written after submission of final report.

Technical reports:		Yes. Annually as required.	
Databases:			
Procedures/methods:		HPLC, qPCR, sampling, fungicide sprays, calibrations.	
Human capacity development:	2	Trained two new personnel of the ARC.	
Technology transfer:	1	yes	Popular article in SA Grain. 2020
Other outputs:			

9.2 Reasons for outputs not achieved

Initially there was a problem with purchasing of SybrGreen for qPCR. With the Covid situation, there was a delay in the import of this product. However, it was managed to obtain it and to finalize the qPCR quantifications at the end of February 2020. Therefore the scientific paper is behind schedule.

10. Successful institutional and inter-institutional collaboration

Researcher	Institution	Role
Dr. Henry Njom – replaced Dr. A. Abrahams	ARC-GC	Molecular techniques
Mr. D. Nkoko – field technician that replaced F.K. Mashinini	ARC-GC	Field trials and spray regimes
Mr. J. van Schalkwyk – field technician at Vaalharts	ARC-GC	Field trials and spray regimes
Mr. P. MacU – field technician at Cedara	ARC-GC	Field trials and spray regimes
Ms. M. van der Walt – research technician at weed sciences	ARC-GC	Calculations and calibrations of spray equipment.

11. Benefit of the outputs to the maize industry

Although the effect of spray combinations, application date of fungicide sprays and interactions between the two were recorded on the reduction of maize ear rot and associated mycotoxins, results were not consistent. It seems that spray applications at flowering or during soft dough stage can decrease either DON levels or *F. boothii* infections of maize grain at Cedara and Vaalharts while earlier applications (pre-flowering) show significantly higher levels of *F. boothii* or DON. The same trend was observed in Potchefstroom where earlier fungicide applications (pre-flowering) showed higher levels of *F. verticillioides* target DNA in maize grain. Similar results were obtained in Vaalharts in the 2017/18 season where cultivar BG3292 x the application at the 8 leaf stage and

then again 28 (pre-flowering) days later, had the highest level of ZEA compared to the other interactions. BG3292 x the application at the 10 leaf stage and then again 56 days later (soft dough stage), had the lowest level of ZEA compared to the other interactions.

In the 2017/18 season (Cedara), fungicide combinations with Amistar Top resulted in higher levels of *F. boothii* when compared to the other spray combinations without Amistar Top. Similar results were observed in Vaalharts in the 2018/19 season, where Amistar Top / Artea x 5/28 days of application, Amistar Top / PunchXtra x 8/28 days of application, Amistar Top / Artea x 8/42 days of application and Amistar Top / PunchXtra x 10/28 days of application all had the highest DON levels. Similar to this, the Amistar Top / Artea interaction with the spray at 10 leaf stage followed by a second spray after 28 days also had the highest fumonisin level at Vaalharts (2018/10 season). In a previous study by Janse van Rensburg *et al*. (2016) a prophylactic spray regime using Amistar Top showed no effect on the infection and colonization of *F. verticillioides* in maize grain and described the lack efficacy due to timing of the fungicide application relative to the plant growth stages. In this study it is shown that Amistar Top should not be considered for the possible additional control of maize ear rots and mycotoxin contamination. Amistar Top is the only fungicide used in this study with the active ingredient strobilurin, which could explain the higher infection levels of *F. boothii* as well as DON and FUM synthesis in maize grain.

The inconsistent reactions of fungicide combinations and/or date of application against *F. verticillioides*, *F. boothii* as well as FUM, DON and ZEA synthesis can be due to the fungal infection process and plant morphology (Luna and Wise, 2015). Foliar fungicides are commonly applied over the top of the maize plant with ground or areal equipment and it is possible that these barriers limit the amount of fungicide to penetrate the plant to come into contact with the fungus.

Prophylactic foliar fungicides used in this study can be effective as management tools for leaf diseases such as *Cercospora zea maydis* and *Escerohilum turcicum,* but because they do not consistently reduce ear rots and mycotoxins tested in this study, it is not economically beneficial to change spray dates to co-incide with the flowering or soft dough plant stages. It is recommended that producers continue to manage maize ear rots using cultural methods such as cultivar selectin, crop rotation and residue management (where applicable). In conservation agriculture systems, crop rotation will be greatly beneficial to break the disease cycle by reducing primary inoculum.

12. Progress with regards to human resource development *(e.g. training of post-graduate students in mycotoxin research)*

Training of Dr. Henry Njom (molecular techniques and quantification of mycotoxins with HPLC) and Mr. D. Nkoko (field trial layout, maintenance, sampling procedures, inoculation techniques and application of fungicides).

13. Statement whether funds were adequate to complete the project

yes

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

15. Signature of the Project Leader

Potchefstroom	25 June 2020
Project Leader	**Place**	**Date**

16. Signature of Responsible Authority

ARC-Grain Crops	Potchefstroom	29 June 2020
Name / Institution	**Place**	**Date**