MYCOTOXINS BIANNUAL REPORT 2012/13 (1 April 2012 to 30 September 2012)

DETAILS

PROJECT NUMBER		M141/17
PROJECT TITLE		Modelling the incidence of *Fusarium verticillioides* and *Aspergillus* spp. and their mycotoxins in maize
PROJECT MANAGER		B Janse van Rensburg
CO-WORKER(S)	Internal	BC Flett, A Schoeman, D Biya
	External	University of the Free State, University of Stellenbosch
PROJECT STATUS		Continue
DURATION		01/04/2008 to 31/03/2013

ACTIONS TAKEN TO DATE

All samples were subjected to HPLC and quantitative RT-PCR.

PROGRESS MADE

This project is almost completed. Final data were sent to Prof. N. McLaren at the University of the Free State for further model development. A PhD was submitted to the University of the Free State. Outputs from this project include: 10 popular articles, two radio talks, 15 congress presentations, in-house training of colleagues and training of one agricultural student.

RESULTS ACHIEVED TO DATE

1. The objectives of this research project were to quantify the incidence of fumonisin and aflatoxin producing *Fusarium*- and *Aspergillus* spp. in commercial maize samples from different production localities in South Africa by means of quantitative (q)RT-PCR and HPLC. Incidence maps were used to highlight fungal and toxin occurrence in localities from 2007 - 2009 (action completed).
2. The genotype X environment interactions associated with fumonisin contamination of maize were also studied.
3. Weather data, fumonisin and fungal data will be statistically analysed to identify important factors to be included into an epidemiological model to predict disease and mycotoxin incidence in maize in South Africa (final season).
4. The effect of a fungicide spray regime on the incidence of fumonisin producing *Fusarium* spp. and fumonisins on selected cultivars at various localities will also be studied (to be completed in 2012/13).

OBJECTIVE 1 & 2

Fumonisins associated with the colonisation of commercial South African maize grain by *Fusarium* spp.

The natural occurrence of fumonisin-producing *Fusarium* spp. and fumonisins as well as aflatoxin-producing *Aspergillus* spp. and aflatoxin contamination of maize grain was quantified in various maize production areas of South Africa. Maize grain samples (1 kg each) were collected from cultivar trials from 16, 20 and 14 localities, during the 2007 - 2009 planting seasons, respectively. Each sample was analysed using quantitative (q) real-time PCR to determine the respective biomasses fumonisin-producing *Fusarium* spp. as well as aflatoxin-producing *Aspergillus* spp. Fumonisin and aflatoxin levels were quantified by means of High Performance Liquid Chromatography (HPLC). G x E interactions were quantified using regression analysis ($Y=ax^b$) whereby the relationship between disease/fumonisin potential and observed disease/fumonisin level of each cultivar was determined. Kernels from the 2007 samples were also plated onto Fusarium selective medium and subsequently, split plates containing PDA & CLA and *Fusarium* spp. were quantified and identified after 14 days. Simple linear regression analysis was used to determine the relationship between qRT-PCR, HPLC and the plating out method. Results indicated high natural infection by fumonisin-producing *Fusarium* spp. and fumonisin levels in warmer production areas such as Northern Cape, North West and Free State Provinces. High fumonisin producing fungal biomass and concomitant fumonisin levels (above 2 ppm in certain localities) quantified in this study, could negatively impact grain quality food safety and security due to the potentially harmful effects of this mycotoxin on humans and animals. Trace amounts of aflatoxin and *Aspergillus* spp. biomass were detected from maize and therefore it is not considered a threat to animal and human health. Caution should however be taken with stored maize because low concentrations of aflatoxin can sharply increase during storage under sub-optimal conditions if contaminated with *Aspergillus* spp.. Spearman ranking correlations of cultivars for fumonisin producing *Fusarium* spp. and concomitant fumonisin contamination were poorly correlated over localities/seasons. Regression analyses between disease/fumonisin potential and observed disease/fumonisin level in cultivars

suggested differential responses of cultivars at the various disease potentials. Regression analysis yielded a significant relationship between qRT-PCR and HPLC data, but not with the plating out of grain data.

OBJECTIVE 3

Use of weather variables to quantify the potential risk of grain colonisation by fumonisin-producing *Fusarium* spp. and fumonisin synthesis in commercial maize in South Africa.

Fumonisins are mycotoxins produced mainly by the maize pathogens *Fusarium verticillioides* and *F. proliferatum.* Mycotoxins are secondary carcinogenic metabolites and have been reported in maize in every part of the world. Since no commercial cultivar in South Africa has been developed with resistance to *F. verticillioides* and *F. proliferatum* and their fumonisins, alternative disease management strategies have to be used to reduce contamination of the local maize crop with these *Fusarium* spp. and their toxins. An epidemiological model that acts as an instrument to constantly monitor and assess the risk of fumonisin contamination in maize grain could aid agronomic decisions during the crop production cycle and, thereby, reduce the risk of infection by *Fusarium* spp. Maize samples (1 kg each) were collected from cultivar trials (2007 - 2009) and planted at 16, 20 and 14 localities respectively in the maize production areas of South Africa. Site-specific weather data, including temperature, radiation, humidity and evapo-transpiration were provided by ARC-ISCW. Grain colonisation by *Fusarium* spp. was determined by quantitative real-time PCR and contamination with fumonisins by HPLC. Fungal incidence ranged from 0 - 15.7 pg in 2007, 0 - 20.9 pg in 2008, 0 - 31.5 pg in 2009. From 2007 - 2009, fumonisin levels from 0 - 2.09 ppm, 0 - 6.13 ppm and 0 - 12.26 ppm were recorded, respectively. *Fusarium* colonisation of grain and fumonisin levels were related to prevailing weather conditions during early post-flowering and grain development stages, respectively. Both colonisation and fumonisin production were significantly inversely correlated with mean maximum temperature ($r = -0.77$ and $r = -60$, respectively) and minimum relative humidity ($r = -0.83$ and $r = -0.79$, respectively) during the critical growth periods. A preliminary model has been developed and evaluated. Additional data from the 2011 - 2012 season will be analysed by Prof N McLaren in further model development. A scientific article will be submitted to *Plant Disease.*

OBJECTIVE 4

Effect of a fungicide spray regime for foliar diseases on the incidence of fumonisin producing *Fusarium* spp. and fumonisin on selected maize cultivars.

Field trials were carried out in five maize production areas of South Africa to study the effect of a prophylactic fungicide regime for the control of foliar diseases on the infection of grains by fumonisin producing *Fusarium* spp. and fumonisin production. Azocystrobin + difenoconazole (strobilurin, 200 g/l + triazole, 125 g/l), was applied 40 - 45 days after planting followed by flusilazole + carbendazim (silicone triazole, 125 g/l + benzimidazole, 250 g/l) with petroleum as adjuvant 28 - 30 days later. Fumonisins were analysed using High Performance Liquid Chromatography (HPLC) and fumonisin producing *Fusarium* spp. were quantified by means of quantitative RealTime-PCR (qRT-PCR). Results from field trials showed that the natural colonisation of maize kernels by fumonisin producing *Fusarium* spp. and fumonisin contamination were high at Vaalharts and Greytown (4.23 - 10.54 ng and 11.57 - 32.19 ppm), moderate at Cedara (675.33 pg - 3.51 ng and 2.31 - 7.43ppm) and low at Potchefstroom and Buffelsvlei (61.63 pg - 3.02 ng and 0.12 - 6.11 ppm). Anova showed no significant differences between sprayed and control treatments on colonisation of grain by fumonisin producing *Fusarium* spp. colonisation or fumonisin contamination. A cultivar x locality interaction was recorded. No significant differences were observed between Bt and non-Bt cultivars at Buffelsvlei, Potchefstroom and Cedara for total fumonisin, fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3). No differences between Bt and non-Bt cultivars at Greytown were observed for total fumonisin, FB_1 and FB_2, however cultivars DKC80-12B and DKC78-15B had significantly lower levels of FB_3 than their isohybrids DKC80-10 and CRN3505. In contrast to this, total fumonisin, FB_1 and FB_3 from Vaalharts were significantly higher in DKC80-12B than in the isohybrid DKC80-10, whereas these cultivars reacted similarly for FB_2. Further investigation is needed to evaluate the effect of different spray regimes on *Fusarium* spp. infection and fumonisin contamination in South Africa. Application of these fungicides, phenological stage of the maize plant as well as stalk borer infestation need to be taken into account. Data from the 2011 - 2012 field trials need to be analysed and incorporated with the above data. A scientific article will be written for submission to *Crop Protection*.

PROBLEMS ENCOUNTERED

None.

DETAILS

PROJECT NUMBER		M141/18
PROJECT TITLE		Modelling the incidence of *Fusarium graminearum* and associated toxins in maize
PROJECT MANAGER		BC Flett
CO-WORKER (S)	Internal	B Janse van Rensburg, A Belgrove, FK Mashinini
	External	ARC-PPRI, University of the Free State, University of Stellenbosch
PROJECT STATUS		Continue
DURATION		01/04/2008 to 31/03/2013

ACTIONS TAKEN TO DATE

The distribution and co-occurrence of four *Fusarium* species and their mycotoxins were investigated in maize samples from two cultivars collected at 14 localities. Real-time PCR was used to quantify grain colonization by the respective *Fusarium* species and multi-toxin analysis HPLC-MS was used to quantify mycotoxins.

A total of 308 maize samples representing six cultivars were collected from maize producing areas of South Africa over three growing season's i.e. 2006/07 to 2008/09. DON (Deoxynivalenol) and ZEA (Zearalenone) were initially quantified using ELISA technique and LCMS/MS.

PROGRESS MADE

As this project is completed and results are being written up for submission as part of a M.Sc. thesis and each chapter is being prepared for publication of various scientific manuscripts, a brief overview of the abstracts of each chapter are included. Progress on this project has been highly satisfactory with the last LCMS/MS results being finalised prior to final report preparation in early 2013. Climate data has been collected and is being analysed by Prof Neal McLaren to develop an epidemiological model.

RESULTS ACHIEVED TO DATE

Colonisation of cereal grain by fungi of the *Fusarium graminearum* species complex (FGSC) often results in mycotoxin contamination. Early detection and management of this disease complex is crucial to preventing toxins from entering the food and feed chain. The distribution and occurrence of *Fusarium graminearum* in six maize cultivars collected from various localities over three growing seasons between 2006 and 2009 was determined using quantitative real-time PCR. DNA was extracted from ground grain samples using a commercial DNA extraction kit and analysed in a LightCycler system using specific primers and fluorogenic TaqMan probes. The TaqMan method was able to detect the species of the FGSC found in infected maize grain samples. Species of the FGSC occur in variable concentrations as a natural contaminant of local maize grain, ranging from 1 - 3920 pg. Analysis of the translation elongation factor gene revealed that *F. boothii* was the predominant species infecting maize while *F. graminearum* occurred to a lesser extent.

The aim of the present study was to investigate the occurrence of *F. graminearum* mycotoxins Deoxynivalenol (DON), Nivalinol (NIV) and Zearalenone (ZEA) in South African maize cultivars, to estimate the risk of contamination. A total of 308 maize samples were collected over three seasons from 2006/07; 2007/08 and 2008/09. DON and ZEA were determined using Enzyme-Linked Immuno-Sorbent Assay (ELISA) test kits. Results show that maize grain produced in South Africa is frequently contaminated with mycotoxins at high concentrations. Over the respective seasons, ZEA was frequently found in maize (67 % positives; median 1105 µg/kg, maximum 11 804 µg/kg). Confirmatory analyses for DON, ZEA, and NIV were conducted on 56 maize samples using liquid chromatography-mass spectrometry (LC-MS/MS). The LC-MS/MS results showed that DON, NIV and ZEA occurred in maize grain and that NIV was a contaminant of all the samples tested, with high concentrations found in maize.

Species within the *Fusarium graminearum* species complex (FGSC) are capable of producing mycotoxins, which include the trichothecene derivatives, deoxynivalenol and nivalenol. The occurrence of trichothecene chemotypes and species of the FGSC is reported here for maize grain samples produced in the 2007/08 and 2008/09 seasons in South Africa. Sixty-four maize isolates were screened for trichothecene chemotypes using polymerase chain reaction (PCR) based on the *Tri*12 and *Tri*6 portion of the trichothecene gene. All the maize FGSC isolates screened were of the 15-ADON chemotype.

PROBLEMS ENCOUNTERED

The student responsible for completing the write up has experienced personal problems and missed her hand in date. This has since been discussed with her supervisor and she is in the process of finalising her manuscripts and thesis.

DETAILS

PROJECT NUMBER	M141/19
PROJECT TITLE	Interactions between *Busseola fusca* and *Fusarium verticillioides* on ear rot and fumonisin production in maize
PROJECT MANAGER	E Ncube
CO-WORKER(S) Internal	BC Flett, A Erasmus, B Janse van Rensburg, A Schoeman, N de Klerk, F Mashinini, DB Biya
External	University of Stellenbosch, North West University
PROJECT STATUS	Continue
DURATION	01/04/2010 to 31/03/2015

ACTIONS TAKEN TO DATE

Three trials were planted at Potchefstroom during October/November 2011 and harvested during April/May 2012:

Trial 1: To determine whether stalk borer infestation enhances Fusarium ear rot (FER) and fumonisin contamination.

Trial 2: To determine the effect of mechanical damage of maize ears on *F. verticillioides* and fumonisin contamination in maize in the field as compared to stem borer damage.

Trial 3: To compare the effect of maize infestation with *B. fusca* and contamination with *F. verticillioides* on fumonisin accumulation between a Bt hybrid and its susceptible isohybrid.

To determine the effect of *F. verticillioides* x *B. fusca* interaction on Fusarium ear rot and fumonisin contamination in a non-Bt maize hybrid (PAN 6723), the following treatments were done: *B. fusca* only, *F. verticillioides* only (refers to MRC 826, unless otherwise stated), both *F. verticillioides* and *B. fusca*, and a control treatment with neither *B. fusca* nor *F. verticillioides*. To determine the same interaction in a Bt hybrid (PAN 6236B) and its isohybrid (PAN 6126), the field trial included the same treatments as above. Finally, to determine the effect of mechanical damage to maize ears on Fusarium ear rot and fumonisin contamination, field experiments were conducted as follows: cork borers with diameters of 1.55, 1.75, 2.23 and 2.39 cm were used to stab through the husk to create single or multiple wounds on kernels 7 - 10 days after silk emergence. The ears were inoculated with *F. verticillioides,* and a control where maize ears were not damaged. For *F. verticillioides* inoculation in all trials, a 2-ml spore suspension containing 2×10^6 spores ml^{-1} of *F. verticillioides* isolate was injected into the silk channel of each primary ear at the blister stage. For *B. fusca* infestation, aliquots of 10-15 neonate larvae obtained from a mass rearing facility at the ARC-GCI were deposited into the whorl of each plant 6 weeks after emergence. All ears per treatment were hand harvested at physiological maturity. FER was visually rated by expressing visible FER as a percentage of the total ear surface. *Busseola fusca* damage was quantified by measuring feeding tunnel length on each ear. Fumonisins were quantified on milled maize samples using HPLC. A multifactor ANOVA was performed on all data using StatGraphics5^+.

PROGRESS MADE

All trials were harvested and laboratory analyses were performed according to plan. A manuscript on this work has been prepared for publication.

RESULTS ACHIEVED TO DATE

Taken together, the results of trials 1, 2 and 3 (listed above) indicate that the *B. fusca* x *F. verticillioides* interaction significantly increased Fusarium ear rot and fumonisin contamination in non-Bt hybrids but not in a Bt maize hybrid (MON810 event). *Busseola fusca* possibly act as a vector in this interaction by spreading *F. verticillioides* spores from the plant surface and providing them access to maize kernels through its feeding wounds in non-Bt hybrids. This study indicated that *B. fusca* is mainly a vector of *F. verticillioides* rather than a plant stressor when compared to other stem borers such as *Ostrinia nubilalis* (European corn borer). Since an increase in mechanical damage significantly increased both Fusarium ear rot and fumonisin production, this also indicated that *B. fusca* did not sufficiently damage maize ears in the non-Bt hybrid (PAN6723) to the extent of inducing plant stress which is associated with fumonisin production. However, *B. fusca* damage did cause a significant increase in fumonisin contamination in the non-Bt isohybrid (PAN6126). *Ostrinia nubilalis* is highly mobile and due to multiple infestations occurring in the same planting, poses a threat to maize production throughout the season in the USA. However, in the case of *B. fusca*, only one generation of larvae occur in one planting. This could be a reason why *O. nubilalis* results in an increase in both Fusarium ear rot and fumonisin contamination as several studies have shown in the USA while *B. fusca* only increases Fusarium ear rot as this study has shown. This also implies that the timing of insect pressure is very important in this interaction. For instance, if *B. fusca* infestation pressure is high at about the

same time the maize plants reach the blister growth stage, then significant damage to maize ears will likely occur, leading to an in increase in fumonisin production.

In conclusion, this study showed that injuries to maize ears is an important factor in Fusarium ear rot development and fumonisin production and the use of Bt hybrids to control *B. fusca* also indirectly reduces Fusarium ear rot and fumonisin production. This study also showed that Bt hybrids suffer less stem borer damage and are therefore less inclined to develop Fusarium ear rot. However, in situations where there is a high concentration of *F. verticillioides* inoculum, they tend to simultaneously have high levels of fumonisin production and less Fusarium ear rot symptoms. This implies that grading procedures such as sorting visibly mouldy grain from apparently non-mouldy grain to reduce fumonisin contamination, as suggested in other studies will not be entirely effective in reducing fumonisin exposure to consumers in such situations. This has implications in subsistence farming systems where grain sorting is usually the only practice used for the reduction of fumonisin or mycotoxin exposure in grain. Therefore, other measures to reduce mycotoxin exposure such as routine screening and testing of grain for mycotoxins should be applied.

This study and others have shown that herbivorous insects could serve as vectors of *Fusarium* spp., therefore, the importance of other ear-feeding insects in the Fusarium disease epidemiology should not be ignored. In South Africa, the Nitidulidae beetles have been reported as one of the most abundant groups of insects feeding on maize ears. These insects are, other than stem borer larvae, capable of moving between ears and could contribute to spread of *Fusarium* spp. in fields.

PROBLEMS ENCOUNTERED

None.

DETAILS

PROJECT NUMBER		M141/21
PROJECT TITLE		The effect of maize plant stressors on *Fusarium verticillioides* and fumonisin production
PROJECT MANAGER		B Janse van Rensburg
CO-WORKER(S)	Internal	BC Flett, A Schoeman, E Hugo, W Deale, K Mashingaidze
	External	University of the Free State, University of Stellenbosch, North West University,
PROJECT STATUS		Continue
DURATION		01/04/2011 to 31/03/2016

ACTIONS TAKEN TO DATE

This project started on the 1st of April 2011. An increasing number of studies have demonstrated the role of stress factors in mycotoxin production. Fumonisins produced by *F. verticillioides* are amongst the most dangerous fungal toxins, as they have been associated with various animal diseases and human oesophageal cancer. This study will aim to investigate the effect of maize plant stressors such as N levels, drought, plant density and heat on *F. verticillioides* infection and fumonisin production.

Greenhouse trials

Greenhouse drought trials were scheduled to be planted in April - June 2011, however due to glasshouse maintenance procedures the planting date moved to 30 January 2012. We were able to replicate the trial three times thereby saving time in the long run. Maize ears have been inoculated and leaf samples have been taken for the determination of PR proteins. The extractions of proteins from leaf samples have been completed. Furthermore chlorophyll fluorescence measurements were also taken. Maize ears were harvested by hand at the end of August 2012.

Field trials

We planted the cultivars PAN6P-110 and CRN3505 at plant densities of 10 000, 20 000, 30 000, 40 000 and 50 000 per hectare in Potchefstroom. Leaf samples have been collected at the 8 leaf stage, silking, milk stage and soft dough stage. Samples were sent to Eco-Analytica (North West University) for the determination of N, C and S in dried leaves. Literature indicates higher fumonisin contamination with N deficiencies and it is therefore important to determine the amount of nutrient uptake by the maize and how this could affect *Fusarium* infection and fumonisin production. From each growth stage leaf samples have been collected to determine PR proteins and chlorophyll fluorescence measurements were also taken. The extraction of PR proteins from leaf samples is completed. Soil samples have been taken from each plot to determine the nutrient status according to the different plant densities at each respective plot. These soil samples were sent to the ARC-IIC for analysis. This trial was hand-harvested on 28 June 2012.

PROGRESS MADE

Greenhouse trials

HPLC analyses to quantify fumonisins are completed. Quantitative RT-PCR to determine the amount of fumonisin producing *Fusarium* spp. biomass need to be conducted. Presently PR-Proteins extracted are total proteins, chitinase and peroxidase. We are currently busy to extract B-1, 3-glucanase from samples. A total of 1 350 greenhouse samples will be analysed in triplicate for each individual protein. Dr B Berner of the North West University is busy analysing the chlorophyll fluorescence data.

Field trial

The field trial has been processed (weighed, moisture taken, threshed by hand). All HPLC and qRT-PCR analyses are completed. Presently PR-Proteins extracted are total proteins, chitinase and peroxidase. We are currently busy to extract B-1, 3-glucanase from samples. A total of 360 field samples will be analysed in triplicate for each individual protein. Dr B Berner of the North West University is busy analysing the chlorophyll fluorescence data.

RESULTS ACHIEVED TO DATE

Data generation are almost completed and will then be statistically analysed, therefore we have no statistical results or tendencies to report at this stage.

PROBLEMS ENCOUNTERED

None.

DETAILS

PROJECT NUMBER		M141/80
PROJECT TITLE		Variation between *Fusarium verticillioides* isolates in their ability to produce fumonisins and infect maize
PROJECT MANAGER		A Schoeman
CO-WORKER(S)	Internal	BC Flett, B Janse van Rensburg
	External	University of Stellenbosch, Forestry and Agricultural Biotechnology Institute, University of Pretoria
PROJECT STATUS		Continue
DURATION		01/04/2007 to 31/03/2013

ACTIONS TAKEN TO DATE

Five field trials were planted for the 2011/12 season. Two trials were planted in Potchefstroom and the other three trials were planted in Ermelo, Cedara and Makhathini. In April to July these field trials were harvested when the moisture content was 20 % and the Fusarium ear rot symptoms caused by the different strains of *Fusarium verticillioides* were scored visually. The harvested maize was milled and HPLC analysis was performed. The four field trials that were planted in Potchefstroom, Cedara, Ermelo and Makhathini were inoculated with five high fumonisin producers that were isolated from different provinces in South Africa (GCI1608 isolated from NW, GCI432 isolated from MP, GCI340 isolated from KZN, GCI51 isolated from EC and MRC826, isolated from the Transkei). One *F. verticillioides* isolate is a low-fumonisin producer (GCI282 isolated from LP) and was chosen based on results from experiments conducted in the lab and in previous field trials in Potchefstroom. The aim of these trials was to determine if high/low fumonisin producers remain high/low fumonisin producers in their province of origin or if their fumonisin production abilities remained unaffected by the different environmental conditions. The fifth trial that was conducted in Potchefstroom was performed in order to investigate if 51 *F. verticillioides* isolates do differ in their virulence or only in fumonisin production, as was found in greenhouse trials and lab experiments.

Primers for the simple sequence repeats (SSR's) method were tested and optimized. Thirty six primers were tested. The primers that showed the most promising results, thus showing the most diversity were used to test 296 *F. verticillioides* isolates from different geographic locations in South Africa.

RESULTS ACHIEVED TO DATE

Four field trials planted on dry land in Potchefstroom, Cedara, Ermelo and Makhathini

$\underline{FB_1}$

The overall FB_1 levels measured at the four localities showed that Makhathini (17.052ppm) produced significantly higher levels than the other three localities. Potchefstroom (10.435ppm) produced significantly higher levels than Cedara (8.059ppm) and Ermelo (4.799ppm), with Cedara significantly higher than Ermelo. Isolates GCI51 (13.736ppm) and GCI340 (13.954ppm) produced significantly higher levels than MRC826 (9.471ppm), GCI1608 (9.177ppm), the control (8.376ppm) and GCI282 (6.067ppm). GCI282 produced significantly lower FB_1 levels than all the isolates tested. When comparing each treatment over the four localities the control plants and MRC826 (20.216ppm) produced significantly higher FB_1 at Makhathini than the other localities. For GCI282 (0.989ppm) at Potchefstroom significantly lower levels were produced than at Cedara (8.83ppm) and Ermelo (10.748ppm). At Ermelo significantly higher FB_1 levels were produced than at Makhathini (3.418ppm) and Potchefstroom. GCI51 and GCI340 produced significantly higher FB_1 levels at Potchefstroom (GCI51 produced 29.349ppm, GCI340 produced 24.522ppm), followed by Makhathini (GCI51 produced 16.870ppm, GCI340 produced15.936ppm). The isolate GCI432 produced significantly higher levels in Makhathini (25.117ppm) than at the other three localities and at Cedara (11.635ppm) produced significantly higher FB_1 than at Ermelo (1.325ppm) and Potchefstroom (5.355ppm). GCI1608 produced significantly higher FB_1 levels in Makhathini (15.635ppm) than at the other localities. When comparing all the treatments per locality, Cedara GCI432 (11.635ppm) produced significantly higher levels than the other isolates. In Ermelo GCI282 (10.748ppm) produced significantly higher FB_1 than the other isolates except for MRC826 (6.814ppm). Isolate GCI432 (25.117ppm) produced significantly higher FB_1 levels than GCI282 (3.418ppm), GCI51 (16.87ppm), GCI340 (15.936ppm) and GCI1608 (15.635ppm) in Makhathini. GCI282 significantly produced the lowest FB_1 levels in Makhathini. In Potchefstroom GCI51 (29.439ppm) and GCI340 (24.522ppm) produced significantly higher FB_1 levels than the other isolates. GCI1608 (7.445ppm) also produced significantly higher levels than GCI282 (0.989ppm) and the control plants (1.163ppm).

$\underline{FB_2}$

FB_2 levels were significantly higher at Makhathini (10.386ppm), while there were no significant differences in FB_2 levels measured at the other three localities. Isolate GCI340 (2.293ppm) produced significantly the highest FB_2 levels compared to the other isolates. There were no significant differences between the other

isolates except that GCI51 (1.457ppm) produced significantly higher FB_2 levels than the control plants (0.637ppm). When comparing each isolate per the four localities the control plants and MRC826 produced significantly higher FB_2 levels in Makhathini (control produced 16.581ppm, MRC826 produced 12.162ppm) than at the other localities. There were no significant differences between localities for GCI282. GCI51 produced significantly higher FB_2 levels in Makhathini (8.528ppm) than the other three localities, followed by Potchefstroom (4.863ppm) that were significantly higher than Cedara (0.8ppm) and Ermelo (0.711ppm). GCI340 also produced significantly higher fumonisin levels in Makhathini (16.771ppm) and in Potchefstroom (3.675ppm) higher FB_2 levels were measured than in Ermelo (0.865ppm). Isolates GCI432 (9.489ppm) and GCI1608 (8.022ppm) produced significantly higher FB_2 levels in Makhathini than at the other three localities. When comparing all the isolates per locality significant differences between isolates were observed for Makhathini and Potchefstroom. In Makhathini the control plants (16.581ppm) and GCI340 (16.771ppm) produced significantly the highest FB_2 levels. MRC826 (12.162ppm) produced significantly higher fumonisin levels than GCI51 (8.582ppm), GCI282 (1.095ppm) and GCI1608 (8.022ppm). Significantly the lowest FB_2 levels were produced by GCI282. In Potchefstroom GCI51 (4.863ppm) produced significantly the highest FB_2 levels than the other isolates, except GCI340 (3.675ppm). GCI340 produced significantly higher FB_2 levels than the control (0.166ppm), MRC826 (0.34ppm) and GCI282 (0.682ppm).

FB_3

Cedara (4.799ppm) produced significantly higher FB_3 levels than Ermelo (2.724ppm) and Makhathini (2.428ppm). Potchefstroom (4.045ppm) produced significantly higher levels than Makhathini. GCI51 (4.002ppm) produced significantly higher FB_3 levels than all the isolates except GCI340 (3.319ppm). GCI340 produced significantly higher FB_3 levels than GCI282 (1.514ppm), GCI432 (1.817ppm) and the control (2.382ppm). GCI282 produced significantly lower FB_3 levels than MRC826 (2.836ppm), GCI1608 (2.624ppm), GCI340 and GCI51. When comparing each isolate per locality in Cedara the control (4.577ppm) produced significantly higher FB_3 levels than the other localities. MRC826 and GCI1608 did not show significant differences in FB_3 levels at the different localities. GCI282 produced the lowest FB_3 levels in Potchefstroom (0.196ppm) and was significantly lower than Ermelo (4.655ppm). In Potchefstroom GCI51 (12.638ppm) produced significantly the highest FB_3 levels than at the other localities. GCI340 produced significantly higher FB_3 levels in Cedara (9.694ppm) and Potchefstroom (9.872ppm) compared to Ermelo (3.345ppm) and Makahatini (2.547ppm). GCI432 produced significantly higher FB_3 levels in Cedara (5.459ppm) compared to Ermelo (1.004ppm). In Cedara (9.694ppm) GCI340 produced significantly the highest FB_3 levels compared to the other isolates. In Ermelo GCI282 (4.655ppm) produced significantly higher FB_3 levels than the control plants (0.38ppm). In Makhathini there were no significant differences between the isolates in their ability to produce FB_3 levels. In Potchefstroom GCI51 (3.685ppm) and GCI340 (2.547ppm) produced significantly higher FB_3 levels than the other isolates.

Total

In the locality Makhathini (29.866ppm) significantly the highest total fumonisin was produced compared to the other localities. In Cedara (14.181ppm) and Potchefstroom (16.147ppm) the isolates produced significantly higher fumonisins than in Ermelo (8.194ppm). Isolates GCI340 (27.166ppm) and GCI51 (24.778ppm) produced significantly the highest fumonisins. GCI1608 (14.617ppm), MRC826 (14.949ppm) and GCI432 (16.098ppm) produced significantly higher fumonisins than the control (13.791ppm) and GCI282 (9.101ppm). GCI282 produced the lowest total fumonisin levels. When comparing each isolate per locality the control (41.466ppm), MRC826 (35.172ppm) and GCI1608 (26.158ppm) produced significantly the highest total fumonisins in Makhathini. GCI282 produced significantly higher fumonisins in Ermelo (16.71ppm) than in Makhathini (5.497ppm) and Potchefstroom (1.866ppm). In Makhathini (29.137ppm) and Potchefstroom (46.94ppm) isolate GCI51 produced significantly higher fumonisins than in Ermelo (7.563ppm) and Cedara (10.203ppm). The fumonisin levels in Potchefstroom were significantly higher than in Makhathini for GCI51. GCI340 produced significantly higher fumonisin levels in Makhathini (35.255ppm) than at Potchefstroom (38.069ppm) Cedara (23.906ppm) and Ermelo (9.032ppm). The fumonisin levels measured in Cedara were significantly higher than in Ermelo. In Makhathini (36.376ppm) GCI432 produced significantly higher fumonisin levels than in the other localities. In Cedara (18.656ppm) significantly higher fumonisin levels were produced by GCI432 than in Ermelo (2.783ppm) and Potchefstroom (7.56ppm). When comparing the different isolates per locality GCI340 (23.906ppm) produced significantly the highest fumonisin in Cedara compared to the other isolates except for GCI432 (18.656ppm) and GCI282 (14.155ppm). In Ermelo (16.710ppm) GCI282 produced significantly higher total fumonisin than the control (2.433ppm). In Makhathini GCI282 produced significantly the lowest total fumonisin levels (5.497ppm). The control plants (41.466ppm) produced significantly higher fumonisn levels than GCI51 (29.137ppm) and GCI282 in Mkahatini. In Potchefstroom GCI51 (46.940ppm) produced significantly the highest fumonisin levels compared to the other isolates except for GCI340 (38.069ppm).

Trial conducted under irrigation in Potchefstroom testing 51 *F. verticillioides* isolates and their ability to cause Fusarium ear rot and produce fumonisin

The isolates tested did not differ significantly in their ability to produce Fusarium ear rot symptoms. These isolates also did not differ significantly in their ability to produce different levels in fumonisin. In this trial very low levels of fumonisin were produced and in most cases none were detected. This could be because the trial was conducted under irrigation. The plants did not suffer any drought stress as opposed to the field trial planted without irrigation and this could have contributed to very low fumonisin levels to be produced.

SSR's

Thirty six primers were tested that were selected out of seven published articles. From these 18 primers did not work and no PCR product was formed after various optimization protocols. Five of these primers that did work, were not highly diverse and were not suitable for analyzing the diversity of the *F. verticillioides* population. Thirteen primers have been optimized and have proven to be highly diverse when nine *F. verticillioides* isolates were tested. Currently, all 296 *F. verticillioides* isolates are being screened with the thirteen primers.

PROGRESS MADE

- High fumonisin producers seemed to remain high fumonisin producers regardless of different environmental conditions.
- The low-fumonisin producer seemed to be the most sensitive to differing weather conditions and in Ermelo and Cedara high fumonisin levels were produced.
- The environment significantly impacted the production of fumonisin under irrigation and dryland conditions. In the dry land trial the maize plants suffered drought stress and high fumonisin levels were observed, but under irrigation very low to zero fumonisin levels were produced.
- Thriteen SSR primers have been optimized and are currently being used to screen the genetic diversity of 296 isolates. This method will help to understand the reproductive mode, genetic drift and gene flow (how fast new genotypes will be introduced into distant fields) of *F. verticillioides* in South Africa.

TECHNOLOGY TRANSFER

Schoeman, A., 2012. Mikotoksiene in mielies. RSG Landbou 18 July 2012 4.30am.

Schoeman, A., 2012. Mycotoxins. Grain SA, September 2012. Pp. 106-107.

Schoeman, A., 2012. Diplodia ear and stalk rot of maize in the spotlight. Grain SA, June 2012, p.66.

PROBLEMS ENCOUNTERED

Difficulty was experienced in optimisation of the published SSR markers.