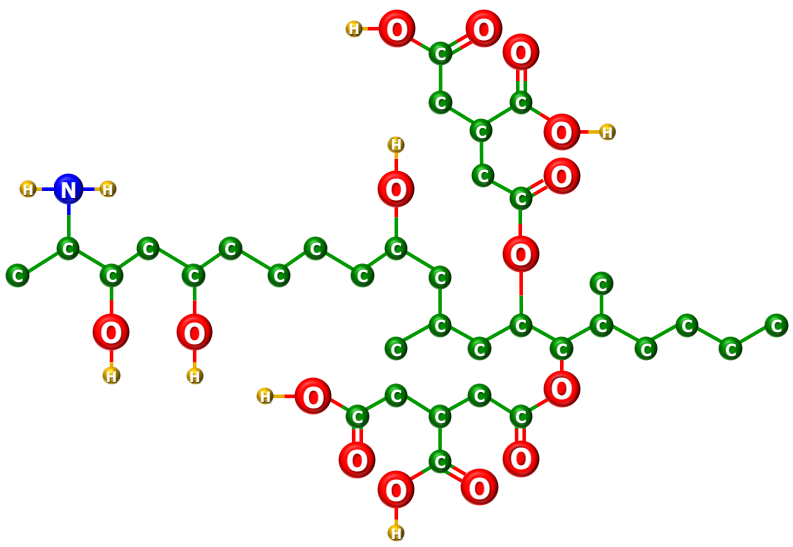
# Maize Trust Mycotoxin Research Symposium

## PROGRAMME AND ABSTRACTS



**THE GRAIN BUILDING PRETORIA**

**1 March 2012**

## The Maize Trust Mycotoxin Research Symposium

It is a great pleasure to welcome all the participants at the first Maize Trust Mycotoxin Research Symposium. The aim of the Symposium is to enable researchers to present their current results on a wide range of issues concerning mycotoxins and to freely interact with sponsors, peers and maize industry stakeholders.

In 2009, a Strategy for Mycotoxin Research was developed, directed at fostering world-class research at South African universities and research institutions in order to ensure that safe maize is supplied to the food and animal feed industries, consumers and export markets. The following main objectives were set in the Strategy:

- To support and determine the magnitude of mycotoxin contamination of maize during the stages of its production, storage, and processing.
- To support the regular monitoring of the occurrence of the fumonisins, aflatoxins, zearalenone, and trichothecenes in locally produced and imported maize.
- To support the determination of the factors which contribute to mycotoxin contamination during the production, storage and processing of maize**.**
- 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.
- To support the development of sound mycotoxin risk management practices in the maize supply chain, including both commercial and emerging/subsistence farmers to ensure the delivery of safe products to the consumer.

We believe that the Symposium will contribute to food security, ensuring that all people have access to sufficient, safe and nutritious food and to sustainable development. These two key areas affect the wellbeing of people in Southern Africa and in the developing world.

We trust that all participants are inspired by the credo of our Symposium: *Local relevance --- international excellence.*

Here's to a fruitful and enjoyable time together.

Piet Steyn

Coordinator: Mycotoxin Research at the Maize Trust

## Symposium programme

### Thursday, 1 March 2012

| | | |
|---|---|---|
| 08:00 – 08:30 | **Registration: Tea and coffee** | |
| | Chairperson | **Leon du Plessis** |
| 08:30 – 08:45 | Welcome address and goals of the meeting | **Jannie de Villiers** |
| 08:45 – 09:00 | The Maize Trust Mycotoxin Research Programme | **Piet Steyn** |
| 09:00 – 09:30 | Perspectives on mycotoxins in South African maize | **W.C.A. Gelderblom**, H-M. Burger, M.J. Lombard, G. S. Shephard, J.P. Rheeder, and L.. van der Westhuizen |
| 09:30 – 10:00 | Approaches to the management of mycotoxins in maize | **B.C. Flett**, A.. Viljoen, and N.W. McLaren |
| 10:00 – 10:20 | **Tea and coffee** | |
| | Chairperson | **Mark Laing** |
| 10:20 – 10:45 | Development of an epidemiological model of fumonisin producing *Fusarium* species on commercial maize in South Africa | **B. Janse van Rensburg**, N.W. McLaren, A. Viljoen, and B.C. Flett |
| 10:45 – 11:10 | Resistance in South African maize to mycotoxigenic *Fusarium* species and their secondary metabolites | **A. Viljoen** and L.J. Rose |
| 11:10 – 11:35 | Effect of *Busseola fusca* and *Fusarium verticillioides* interaction on Fusarium ear rot and fumonisin production in maize | **E. Ncube**, B.C. Flett, J.B.J. van Rensburg, J. van den Berg, and A. Viljoen |
| 11:35 – 12:00 | Conventional and unconventional improvement of South African maize for resistance to *Fusarium verticillioides* and fumonisins | **L.J. Rose** and A.. Viljoen |

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|---|---|---|
| 12:00 – 12:25 | The diversity and population structure of *Fusarium verticillioides* in South African maize | **A. Schoeman**, B.C. Flett, and A Viljoen |
| 12:25 – 12:50 | FUM1 Gene expression and fumonisin production of *Fusarium verticillioides* MRC 826 subcultures | **L.M. Moses**, **H. F. Vismer,** and W.F.O. Marasas |
| 12:50 – 13:45 | **Lunch** | |
| | Chairperson | **Robert Vleggaar** |
| 13:45 – 14:10 | Monitoring of mycotoxin levels in maize: different seasons, different techniques – what does the result tell us? | **W. Louw**, J Nortjé, C. Erasmus, and P. van Niekerk |
| 14:10 – 14:35 | Post harvest occurrence of free, bound and masked Fusarium mycotoxins in the maize processing chain, with specific emphasis on fumonisins | **C. Erasmus,** J. Kruger, L.S. da Silva, P. J. Jooste, and W. Louw |
| 14:35 – 15:00 | *Fusarium graminearum* mycotoxins associated with grain mould of maize in South Africa | **B.C. Flett, N.W. McLaren**, M. Mavhunga, A-L. Boutigny, and A.. Viljoen |
| 15:00 – 15:25 | Biological control of mycotoxins in food and feed grain commodities | **J.F. Alberts,** W.H. van Zyl, and W.C.A. Gelderblom |
| 15:25 – 17:00 | Chairperson | **Piet Steyn** |
| | Discussion on mycotoxin research requirements of the maize industry | |
| 17:00 – 17:05 | Concluding remarks | **Wynand van der Walt** |
| 17:05 – 19:00 | **Social function sponsored by the Maize Trust** | |

## Perspectives on mycotoxins in South African maize

**W.C.A. Gelderblom**[1*],H-M. Burger[1], M.J. Lombard[2], G. S. Shephard[1], J.P. Rheeder[1] and L. van der Westhuizen[1]

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*Aspergillus* and *Fusarium* spp are the major fungal genera which infect maize and lead to contamination with mycotoxins namely the aflatoxins, fumonisins, ochratoxins, trichothecenes and zearalenone. These structurally different mycotoxins cause a diverse range of toxicological effects in experimental animals and are associated with the development of different diseases, the so-called human mycotoxicoses, in man. Mycotoxicological investigations in experimental animals led to the identification of acute and chronic adverse effects resulting in the characterisation of the underlying mechanisms responsible for their toxic and carcinogenic properties. The development of sensitive analytical techniques enables determination of the level of contamination in food and feed and the assessment of the exposure in humans. These aspects form part of the hazard and exposure components of assessing the risk they pose to human health. This is of relevance as mycotoxin regulation differs between countries resulting in a shift in the risk paradigm in accordance with economic factors and international trade agreements to the country to which the commodity is imported, specifically to developing countries where regulations do not apply. Comprehensive population characterisation and the use of validated analytical tools need to be developed to increase the accuracy of assessing exposure at an individual level. This will contribute to the development of a quantitative relationship between intake (diet) and exposure to the relevant mycotoxins. These parameters are valuable to both risk characterisation and management, where specific lifestyles and/or traditions will impact on intervention strategies and its long-term efficacy.

## Approaches to the management of mycotoxins in maize

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Maize is an important staple food for millions of people in South Africa with consumption levels as high as 400 and 500 g per person per day in certain areas. *Fusarium verticillioides* and *F. proliferatum*, and members of the *F. graminearum* species complex (FGSC), cause two common maize diseases, Fusarium ear rot and Gibberella ear rot, respectively. These ear rot pathogens produce mycotoxins, which are secondary metabolites and include fumonisins (*F. verticillioides* and *F. proliferatum)*, and deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA). Various biotic and abiotic factors affect infection and growth of these pathogens and thus, mycotoxin production. Fumonisins have been classified as Group 2B carcinogens by the World Health Organisation's International Agency for Research on Cancer meaning they are probably carcinogenic to humans . Fumonisins are also associated with various mycotoxicoses in animals. The trichothecenes, DON and NIV, have been strongly correlated with chronic and fatal toxicoses of humans and animals. ZEA is not acutely toxic and have not been associated with fatal mycotoxicoses in humans and animals. Zearalenones are non-steroidal estrogenic mycotoxins associated with estrogenic syndromes in various animals. Recently research has focused primarily on taxonomy and population genetics, genomics, mycotoxin biosynthesis, incidence and occurrence of various mycotoxins, new biochemical and molecular detection technologies, toxicology and risk assessments. Resistance and disease epidemiology have been studied to a lesser degree. Although sources of resistance to the ear rots have been found, limited inclusion into commercial breeding programs has occurred. Therefore, resistance of commercial maize cultivars is poor. There is paucity in research on pathogen, host and environment interactions and their effect on mycotoxin production. Research must focus on reducing mycotoxin levels at the initial stage of the maize chain, and that is during production. To achieve this we need a thorough understanding of the various interactions between the host, pathogen and environment, and the effect this has on mycotoxin production in grain. This will enable us to identify and develop intervention strategies to reduce mycotoxin levels thereby reducing their impact in food and feeds. With no legislation on the allowable limits of mycotoxins in maize grain, and a lack of a reliable, cheap, fast and easy to use quantification technique, role players in the local maize industry are not obliged to take steps to reduce mycotoxin levels. It is, therefore, important that the required legislation be drafted and suitable analytical technologies be developed to ensure that all role players implement management strategies to reduce mycotoxin levels.

## Development of an epidemiological model of fumonisin producing *Fusarium* species on commercial maize in South Africa

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Fumonisins are mycotoxins produced mainly by *Fusarium verticillioides* and *F. proliferatum.* Mycotoxins are secondary fungal metabolites and have a world-wide distribution. Disease management strategies to reduce contamination of the local maize crop with these *Fusarium* spp. and their toxins include the identification of high disease/fumonisin potential areas, understanding the epidemiology and the use of chemical control. High natural infection of grain by fumonisin-producing *Fusarium* spp. and fumonisin levels >5 ppm were recorded in warmer production areas. Poor correlations in the responses of cultivars to *Fusarium* spp. and concomitant fumonisin contamination over seasons/localities suggest differential responses of cultivars at different disease potentials. *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 3-dimentional model using mean maximum temperature and mean minimum humidity during the early post silking period could account for 86 % to 89% of the variation in grain colonization. A second sub-routine including *Fusarium* biomass, together with mean maximum temperature during the dough stage of grain development suitably predicted fumonisin production subsequent to colonisation ($R^2$=0.89 and $R^2$=0.94 respectively). Further development and validation of this model is required. A fungicide spray regime for foliar diseases on selected maize cultivars at various localities, yielded no significant differences in fungal colonisation or fumonisin contamination. Phenological stage of the maize plant, as indicated by the model, as well as stalk borer infestation need to be taken into account in further chemical control studies.

## Resistance in South African maize to mycotoxigenic *Fusarium* species and their secondary metabolites

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A number of *Fusarium* species cause Fusarium ear rot (FER) of maize, a disease responsible for loss of yield and quality in the crop. *Fusarium verticillioides* is considered the most important species associated with FER, as it produces fumonisins that are hazardous to the health of humans and animals. Other pathogens associated with maize ear rots include *F. graminearum*, *F. proliferatum* and *F. subglutinans*; all producers of mycotoxins. Reliable and rapid testing procedures were developed to determine the severity of maize kernel infections with mycotoxin-producing *Fusarium* spp and their mycotoxins in South Africa. Infected maize ears were collected from 14 localities over two seasons and analyzed for contamination using quantitative (q) real-time PCR and multi-toxin analysis. Since no maize cultivars and breeding lines with resistance to *F. verticillioides* are known in South Africa, 24 genetically diverse and locally adapted inbred lines were evaluated for resistance to Fusarium ear rot and fumonisin accumulation in the greenhouse and in the field. Susceptible cultivars were also treated with plant growth regulators to induce systemic resistance. In 2008, *F. verticillioides* was found to be the predominant species in the Northwest, the western Free State and the Northern Cape provinces, while *F. graminearum* was predominant in the eastern Free State, Mpumalanga and KwaZulu-Natal provinces. In 2009, *F. graminearum* became the predominant species found in the Northwest Province. *Fusarium subglutinans* was associated with maize ear rot in both years at most of the localities, while *F. proliferatum* was not detected from any locality. Trichothecenes B toxins were well correlated with the amount of *F. graminearum*, and fumonisins with *F. verticillioides*. This information on the distribution and epidemiology of *Fusarium* species in South African maize can help to predict mycotoxin contamination risks and implement preventative disease management strategies. Of the maize inbred lines tested, five consistently showed a low FER (< 5%) incidence, and two of these accumulated fumonisin levels < 5 mg $kg^{-1}$. These lines, therefore, could potentially be used as sources of resistance within a maize breeding programme. They are now being tested over multiple locations, and will be tested in 2013 for resistance to other ear rot diseases and for agronomic properties such as superior yield and drought resistance. None of the five chemical elicitors or the fungicide consistently reduced FER and/or fumonisin contamination significantly. A reduction in diseased and toxic maize would not only increase the yield and quality of the crop, but will also ensure that markets are accessible to local producers.

## Effect of *Busseola fusca* and *Fusarium verticillioides* interaction on Fusarium ear rot and fumonisin production in maize

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*Fusarium verticillioides* and *Busseola fusca* are among the most significant maize production constraints in South Africa. This project involves the study of the interaction between *F. verticillioides, B. fusca* and mechanical damage. The effect that this interaction may have on Fusarium ear rot (FER) and fumonisin production in both Bt and non-Bt maize hybrids was studied. Results indicated that the *B. fusca* x *F. verticillioides* interaction significantly increased FER severity but it did not significantly increase fumonisin contamination. Mechanical damage significantly increased the severity of FER in both naturally infected and artificially *F. verticillioides*-inoculated maize plants while both FER and fumonisin contamination significantly increased only in artificially *F. verticillioides*-inoculated plants. Results also indicated a lower incidence of FER in Bt than in non-Bt maize but there was no significant difference in fumonisin contamination. This study showed that the *B. fusca* x *F. verticillioides* interaction increases the incidence of FER in non-Bt-hybrids compared to Bt-hybrids, thus Bt-technology is an effective method for the management of Fusarium ear rot but it does not reduce fumonisin contamination. Fumonisin contamination of maize grain therefore remains problematic in both Bt- and non-Bt-hybrids.

## Conventional and unconventional improvement of South African maize for resistance to *Fusarium verticillioides* and fumonisins

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*Fusarium verticillioides* is a fungal species commonly associated with maize world-wide. Infection of maize by *F. verticillioides* may result in Fusarium ear rot (FER), a disease that reduces grain quality, and potentially contaminates the grain with mycotoxins (fumonisins). Due to the threat of fumonisins to human and animal health, and the economic losses associated with reductions in grain quality, strategies aimed at the prevention of FER and fumonisin contamination are required. The planting of maize genotypes with enhanced host resistance potentially offers the most efficient, cost effective and environmentally sound method to reduce FER and fumonisin contamination. No commercial cultivars in South Africa are resistant to *F. verticillioides*, which prompted the development and use of conventional and unconventional technologies for the improvement of local germplasm for FER and fumonisin resistance. Locally adapted maize inbred lines with resistance to FER and fumonisin contamination had been identified in the MTM 11/01 project entitled: Exploiting plant resistance to manage *Fusarium verticillioides* and fumonisins in maize in South Africa. In the current project (MTM 11/02), we plan to link this resistance to quantitative trait loci (QTL) that can support breeders with the introduction of resistance into new cultivars by means of marker assisted selection (MAS). Preliminary QTL mapping will be done on the $F_2$ generation during the development of the recurrent inbred line (RIL) population. The outcome will be the first step in identifying genetic factors contributing to resistance and to the identification of flanking markers to be used in MAS in future. An RIL mapping population will then be used to dissect the genetic components responsible for FER and fumonisin resistance in maize. In addition, maize plants have also been subjected to unconventional plant improvement approaches, such as mutation breeding and genetic modification to enhance their resistance to *F. verticillioides* and fumonisin accumulation. A mutant population had been planted in Potchefstroom, self-pollinated and tested for resistance to *F. verticillioides*. The $M_2$ population is currently being tested in the field, and from these mutant plants with superior properties, such as disease resistance, plant height, ear height and yield will be selected. Maize callus has also been genetically modified with genes preventing FER and fumonisin contamination, and introduced genes will be back-crossed into high yielding local germplasm. Considering the difficulties associated with screening maize plants for FER and mycotoxin resistance, the availability of DNA markers may serve as an excellent tool to help breeders speed up their breeding programs. Unconventionally improved plants could also provide us with an opportunity to have locally adapted maize cultivars with resistance to *F. verticillioides*.

## The diversity and population structure of *Fusarium verticillioides* in South African maize

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*Fusarium verticillioides* is the predominant fungus associated with maize ear rot in South Africa, and is responsible for contamination of maize grain with fumonisins. The fungus reproduces both sexually and asexually, which means that it has the ability to diversify by recombination to adapt to changing environments, and to then rapidly reproduce clonally to establish itself in the new environment. The more diverse the population structure of *F. verticillioides*, the easier it is to adapt to adverse environments and to overcome disease management strategies. In this study, 300 isolates of *F. verticilioides* were collected from maize kernels and stems in Limpopo, Mpumalanga, Northwest Province, KwaZulu-Natal, the Free State and the Eastern Cape. All isolates were screened for the presence of *Fum*1 and *Fum*19 genes which underlie fumonisin production, after which their toxigenic potential to produce fumonisin was determined *in vitro*. From these, a subpopulation, representative of high, medium and low fumonisin producers were selected and tested for causing Fusarium ear rot. All *F. verticillioides* isolates were also tested for the presence or absence of *Mat*1 and *Mat*2 genes, which determine their ability to mate, and subjected to phylogenetic analysis to determine their genetic relatedness. All the *F. verticillioides* isolates tested contained *Fum*1 and *Fum*19 genes, but they varied significantly in the amount of fumonisins produced. Similarly, the isolates also differed in their ability to cause ear rot. Fumonisin production did not correlate with disease severity, suggesting that the fungus does not require the toxin to cause disease. The ratio between *Mat*1 and *Mat*2 genes was almost 1:1, with 44% of the population containing the *Mat*2 gene. This suggests that recombination in nature takes place regularly. Phylogenetic analysis grouped all *F. verticillioides* isolates together, but separated them from other *Fusarium* species within the Gibberella species complex, which confirms its species identity. In future, microsattelite markers will be used to investigate the reproductive mode, gene flow and migration among F*. verticillioides* populations from different areas in South Africa in order to develop appropriate disease management strategies. This project will substantially contribute to elucidating the role of the fungus in the epidemiology of Fusarium ear rot and fumonisin contamination of maize in the different production areas in South Africa.

## *FUM1* Gene expression and Fumonisin production of *Fusarium verticillioides* MRC 826 subcultures

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*F. verticillioides* strain MRC 826 isolated from maize collected from the Transkei region in 1975 originally produced unsurpassed high levels of fumonisin B (FB) toxins, particularly $FB_1$. Several subcultures of this fungal isolate were established over the past 35 years and a deviation in the ability to produce fumonisins has occurred. The FB biosynthetic gene cluster consists of 17 genes, namely *FUM* genes. In order to elucidate the mechanism by which *F. verticillioides* produces FBs, a study into the regulation of *FUM* genes in a set of MRC 826 subcultures with varying FB levels is being conducted. Real-time PCR, was first performed to ascertain whether all 17 *FUM* genes are differentially expressed or absent in the maize patty cultures of strains at 3 weeks incubation. This analysis revealed that strains of low, medium and high FB producing groups displayed similar expression patterns for all *FUM* genes. However, since the results were not in conformity with the levels of FBs produced, a subsequent expression analysis of four selected *FUM* genes and a regulatory gene *FCK*1 is currently being performed at nine time points from a second batch of MRC 826 subcultures along with HPLC analyses to determine concurrent production of FB. Determining what genes are expressed to a different extent in high producing strains could potentially identify possible target areas for reduction in the synthesis of the carcinogenic mycotoxins.

## Monitoring of mycotoxin levels in maize: different seasons, different techniques – what does the result tell us?

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With mycotoxin contamination becoming increasingly more important nationally and internationally, there is a need to accumulate reliable analytical data on the current situation in South Africa. The maize industry is being called upon to comment on proposed legislative levels and therefore needs a clear knowledge of the mycotoxin contamination usually seen over different seasons and regions of the country. During 2010 SAGL implemented a multi-mycotoxin screening method using UPLC-MS/MS capable of determining residues of Aflatoxin $G_1$; $B_1$; $G_2$; $B_2$, Fumonisin $B_1$ and $B_2$, Deoxynivalenol, T2-toxin, Zearalenone and Ochratoxin A in a single run. With the financial support of the Maize Trust, SAGL started accumulating analytical data on 125 maize crop samples of the 2009/2010 season to establish a clear baseline on mycotoxin contamination. This data aims at obtaining a clear indication of mycotoxin levels and their distribution in the maize growing areas. Once at least three season's data are available, a more targeted approach can be adopted for survey purposes. The 2009/2010 crop quality survey samples which SAGL received from the commercial grain stores were composite samples, proportionally representing white and yellow maize per class and grade, produced in the various production regions throughout South Africa. Based on the environmental conditions for this production season, industry indicated that maize produced in Mpumalanga, Gauteng and KwaZulu-Natal during the 2009/2010 season, might be of higher risk with regards to mycotoxin contamination and therefore the 125 samples for analysis were selected from regions 30 to 32 (Mpumalanga), 34 (Gauteng) and 36 (KwaZulu-Natal).

Results obtained during this first phase of the survey will be presented. The presentation will also include a comparison with historical data generated using different screening techniques.

## Post harvest occurrence of free, bound and masked *Fusarium* mycotoxins in the maize processing chain, with specific emphasis on fumonisins

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Exposure to fumonisin contamination in maize is diverse due to bound and masked forms of fumonisins occurring along with the free forms. The effects of processing and the toxicity of the bound forms in biological systems are currently being debated. Evidence in the literature suggests that the toxicity of masked and bound fumonisins upon release in the body can be as high as the free form. Existing databases on fumonisin occurrence in foods are based only on the detection of the free forms and thus useful information on the bound forms, especially in South Africa, does not exist. Maize with a previously acceptable fumonisin limit has the risk of being unfit for human consumption due to the undetected quantities of bound fumonisins. An analytical support system for accurate detection of masked and bound mycotoxins that will be available to the broader maize industry (for human and animal product testing) is being developed and implemented at the Southern African Grain Laboratory (SAGL). Validation is done according to a combination of the accredited methods of the SAGL (in-house for free fumonisins using a UPLC-MS/MS) and the method developed by Dall' Asta et al, 2009 (Italy) for masked and bound forms as detected by analysing the hydrolysed forms (HFB1, HFB2 and HFB3). Reference standards were purchased from Biomin and the Medical Research Council (PROMEC). A clean maize reference sample was imported from Canada (Dr. David Miller, Carleton). Twelve whole grain maize samples were selected from regular local cultivar trials and analysed for free and bound fumonisin contents. Of these samples, six did not contain any total free fumonisins. Three samples contained low levels of total free fumonisins (<100µg/kg), but no bound fumonisins. Bound fumonisins were found in three maize samples with higher levels of free fumonisins. There were no samples measured yet that contained bound fumonisins, when the samples did not contain free fumonisins as well. The levels of bound fumonisins varied between 121-1216 µg/kg, 59-536 µg/kg and 0-159 µg/kg for bound FB1, FB2 and FB3 respectively. This represented an increased amount of as much as 45% of measured fumonisins when added to the detected free fumonisins. These preliminary results indicate that bound fumonisins might only start forming at a certain level of free fumonisin occurrence. The next step of this research will be to analyse a large amount of samples to determine if such a cut-off point of free fumonisins can be established, below which it can be assumed there will be no bound fumonisins. It is also necessary to establish a pattern in terms of occurring levels of bound fumonisins in terms of locality and cultivar susceptibility. In order to determine whether bound fumonisins might be associated with specific proteins in the maize as suggested by literature, analyses is currently being done on the bound fumonisin contents of the Osborne fractions of the maize samples that tested positive for bound fumonisins. Specific challenges experienced during method transfer, development and validation will be discussed.

## *Fusarium graminearum* mycotoxins associated with grain mould of maize in South Africa

**B.C. Flett**[1*], **N.W. McLaren**[2*], M. Mavhunga[3]; A-L. Boutigny[4] and A. Viljoen[4]

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The *Fusarium graminearum* species complex (FGSC) is a major mycotoxin producer in cereal grain samples. The most prevalent mycotoxins include deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA). Trichothecenes such as DON and NIV are known protein synthesis inhibitors and consumption of grain contaminated with these mycotoxins can cause anaemia and immunosuppression, haemorrhage, diarrhoea and emesis. 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. *F. graminearum* was the predominant species in the eastern Free State, Mpumalanga and KwaZulu-Natal while *F. verticillioides* was predominant in the Northwest, western Free State and Northern Cape provinces during 2008. Although higher levels of ear rot infection were found in 2009 similar tendencies were noted but *F. graminearum* became predominant in the Northwest province. DON and ZEA were well correlated with *F. graminearum* biomass in grain. Studies on the composition of the FGSC using 100 Gibberella ear rot isolates indicated that only *F. boothii* was associated with maize with one exception which appears to be an interspecific hybrid between *F. boothii* and *F. graminearum*. It appears that different species in the FGSC may vary in host preference. A total of 308 maize samples representing six cultivars were collected from maize producing areas of South Africa over three growing seasons i.e. 2006/07 to 2008/09. DON and ZEA were initially quantified using ELISA technique. No DON was detected during the 2006/07 season. In the 2007/2008 and 2008/2009 seasons, DON was detected in 17% of the samples, with concentrations ranging from 0.3 to 5.5 ppm. ZEA was detected in 69% of the samples with an average concentration of 325 ppb. Quantitative real-time PCR (qPCR) was used for quantification of *F. graminearum* biomass in plant tissue, and fungal DNA was detected in 53% of the samples in concentrations ranging from 1 to 3920 pg. PCR was used to determine species identity and LCMS to quantify the trichothecenes in 57 isolates of the FGSC (including some isolates from sorghum). Analysis of the sequence data generated from the TEF-1α gene confirmed that *F. boothii* was the predominant species in maize whilst at least three species were found in sorghum, namely *F. meridionale*, *F. acaciae-mearnsii* and *F. cortaderiae*. 15-ADON was the only chemotype detected in maize FGSC isolates. The NIV chemotype was detected in sorghum isolates. Reanalysis of toxin concentrations using HPLC-MS indicated that contrary to chemotype data, NIV production in maize was high with a mean level of

5.87 ppm.  The mean levels of ZEA and DON were 28.05 and 0.22 ppm, respectively. The ZEA and NIV values exceeded the acceptable levels for these toxins as set by the FDA of the USA.

## Biological control of mycotoxins in food and feed grain commodities

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The focus of this research initiative is to develop practical, affordable and environmentally sound methods to biologically detoxify fumonisin $B_1$ ($FB_1$). The aim is to eliminate $FB_1$ contamination from food and feed grain commodities by treatment with food-grade recombinant enzyme preparations. Fungi capable of degrading $FB_1$ are selected by screening fungal isolates from culture collections and by enrichment from compost-rich soil. Selection is based on the ability to utilize molecules containing groups related to the terminal end (2-amino-3-hydroxy butyl moiety) of $FB_1$ as nitrogen source. Fungal genes encoding degradation enzymes (carboxylesterases and aminotransferases) are subsequently targeted and expressed in *Saccharomyces cerevisiae*, a food-grade yeast with Generally Regarded As Safe (GRAS) status. *Aspergillus niger* SU 10864 was able to grow in minimal growth media with $FB_1$ as nitrogen source. Species identification by microscopic examination, sequencing of the ITS regions and BLAST sequence comparison resulted in 100% homology with *A. niger* CBS 513.88. The enrichment procedure from compost-rich soil produced one fungal isolate capable of maintaining growth in a medium with 1,4 diaminobutane as nitrogen source as well as in $FB_1$ minimal medium and was identified as *Fusarium solani*. *A. niger* is widely employed in biotechnology for the production of food ingredients, pharmaceuticals and industrial enzymes and many of these products have obtained GRAS status. Expression of microbial enzymes originating from *A. niger* strains in *S. cerevisiae* could support the development of safe recombinant cultures and enzymatic preparations for degrading $FB_1$ in food and feed.

## List of delegates

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