

Key areas of mycotoxin research in South Africa

Scientists involved in mycotoxin research and the management of the Maize Trust discussed mycotoxin research at a meeting held in January 2010 at Stellenbosch. The following eight key areas of mycotoxin research and their rationale were discussed. Substantial consultation occurred between researchers representing the various institutions and disciplines involved in mycotoxin research. It was agreed that these eight key areas, as accepted by the Maize Trust, should provide additional direction to the funding of research and be conducive to collaborative research between South African researchers as well as involving international experts.

1 The importance of mycotoxins as contaminants of maize and maize-derived products, their role in the aetiology of animal and human diseases, and intervention methods to reduce the exposure of humans to mycotoxins

Mycotoxins are naturally occurring fungal contaminants produced in maize and are associated with several animal disease syndromes. They pose health risks to humans as they have been implicated in the development of cancer and neural tube defects. Based on these findings, risk assessments have been performed by FAO and WHO, while IARC has classified some mycotoxins such as aflatoxins, ochratoxins and fumonisins as either human carcinogens or possible human carcinogens. Based on toxicological studies and the use of safety factors, the maximum tolerable daily intakes for certain mycotoxins have been defined while for others, such as aflatoxins, levels must be as low as reasonably achievable, due to the absence of a no observed effect level. Mycotoxins in maize are a health risk as maize is a staple food commodity in South and southern Africa, and known to be frequently contaminated with high levels of mycotoxins, especially fumonisins. High risk population groups include rural communities and/or subsistence farmers having a low socio-economic status and poor food security who consume large quantities of maize as part of their daily diet. The ultimate aim will be to reduce mycotoxin exposure thereby minimizing the risk in adults, children and babies, who are often weaned on maize-based foods. Recent developments regarding mycotoxin reduction by simple intervention methods and the utilization of urinary and/or serum biomarkers of exposure provide ample opportunities to effectively integrate mycotoxin contamination of maize and the level of exposure which will form the basis of applicable intervention programmes. Additional approaches included health promotional and educational campaigns involving the community to ensure the sustainability of the mycotoxin reduction intervention programmes.

2 The importance of reliable/accurate mycotoxin analysis, including method development and an accredited mycotoxin reference laboratory.

2.1 Introduction to the need for reliable and accurate mycotoxin analysis

The provision of reliable and accurate chemical analytical results is of fundamental importance for the provision of the basic data needed so that meaningful and correct decisions can be made in the applications and policy flowing from these results.

Adequate resources should be available for maintenance of an infrastructure that provides this information and for the upgrading of methods and instrumentation to meet the ongoing developments in mycotoxin analytical research. Chemical analysis of mycotoxins in South Africa is performed by a number of laboratories, using a range of screening, semi-quantitative or fully quantitative HPLC-based methods, which meet the requirements of various local industries or exporters. A very few can undertake the type of multi-mycotoxin analytical methods by HPLC with tandem mass spectrometry, which are currently being adopted in many laboratories of the developed world and South Africa's trading partners. The current costs for multi-mycotoxin analyses are high and impact negatively on conducting large scale research projects.

Regular monitoring of mycotoxin levels in food and feed is necessary to determine the extent of the mycotoxin residue problem. For the maize industry the monitoring programme should cover a representative number of samples tested for a range of mycotoxins including at least the following 5 mycotoxins: aflatoxin, deoxynivalenol, zearalenone, fumonisins and ochratoxin A.

2.2 The importance of the analysis of masked and bound mycotoxins

At present, South African scientists do not have the capacity to analyze the masked and bound mycotoxins formed in maize. Local researchers in collaboration with the Southern African Grain Laboratory (SAGL) intend to develop the expertise to elucidate the structures of these complex molecules, and to develop appropriate analytical methodology. The South African researchers benefit from collaboration in large international collaborative projects such as the MoniQa project (Monitoring and Quality Assurance in the Food Supply Chain) through the International Association for Cereal Science and technology (ICC) and by collaboration with several international experts.

The masking and binding of mycotoxins, including fumonisins, can shed some light on their stability and toxicity in maize-based foods. Apart from the free mycotoxins which are commonly found, mycotoxins also occur as conjugated compounds. Two types occur namely a soluble conjugated form or the so-called "masked" type, and an insoluble conjugated form also known as the "bound" type. Several mechanisms exist which can convert free mycotoxins into the masked or bound form. These involve conjugation during plant growth (the typical case with fumonisins), fungal conjugates (fungi excreting mycotoxins as part of a larger molecule), food processes conjugates (treatments such as heat processing induce binding of mycotoxins to other organic molecules especially proteins and amino acids) and mammalian conjugates, where the mycotoxin is bound by the serum albumin. It has emerged that mycotoxins are actually not destroyed, but rather bound during food processing. A typical example is the reaction of fumonisin B₁ in the presence of reducing sugars to yield N-carboxymethyl fumonisin. This typically occurs in the wide range of high temperature extruded maize products including snacks, breakfast cereals and instant porridges for weaning foods.

Other food processing systems such as fermentation and acidic processes have shown to release large quantities of these bound mycotoxins, and it is now generally believed that the human digestive tract also plays a major role in releasing mycotoxins from their conjugated forms, with resulting increases in toxicity much higher than initially believed. In South Africa the measurement of mycotoxin

conjugates has not adequately been done and thus it places a significant limitation on the understanding of the interaction between fungal contaminants in the maize value chain and the expression of the mycotoxin, linked to the health of consumers.

Bound fumonisins are present in unprocessed maize, but the exact nature of the substances is not understood. The bound fumonisins are commonly associated with the Osborne prolamin and glutelin fractions from maize. The elucidation of the formation of the bound fumonisins and their molecular structures remain a great challenge.

2.3 The importance of a national accredited mycotoxin analytical laboratory

In addition to the monitoring programme, there is also the need for an accredited analytical facility to support mycotoxin research. Such a facility needs to be able to supply accurate reliable analytical data at an affordable price to researchers and industry in South Africa and should include the following services:

Research into new analytical techniques (methods), provision of routine methods to routine analytical laboratories, support laboratories in quality assurance based on ISO 17025, organize inter-laboratory comparisons, offer technical advice to laboratories, identify and determine sources of disagreements between laboratory's results, offer training opportunities and assist government with technical and scientific expertise.

The National Metrology Institute of South Africa (NMISA) maintains and develops primary methods for chemical analysis to certify reference materials for SA and the region. Their capabilities are benchmarked through participation in international proficiency tests. As the NMISA is a public entity funded by the Department of Trade and Industry – they offer traceability to the measurement laboratories in support of trade and industry. In order to provide a mechanism for scientific and technical cooperation in the analysis of organic contaminants in grain and related products, the SAGL and NMISA, agreed to pursue scientific and technical cooperation in the accurate analysis of organic contaminants, such as mycotoxins in grain and related products.

3 Importance of regulations/legislation for controlling mycotoxins in foods and feeds

Mycotoxins are a diverse range of fungal secondary metabolites with widely differing chemical structures. Many are genotoxic, carcinogenic, teratogenic, oestrogenic, haemorrhagic, immunotoxic, nephrotoxic, hepatotoxic or neurotoxic. As a consequence, mycotoxin contamination of the food supply represents a health risk to the population, the nature and extent of which depends on the mycotoxins present, their respective levels of contamination and the degree to which the contaminated foodstuffs are consumed. For these reasons, many national governments, particularly in the developed world, have instituted legislated regulations for both their national food supply and for the importation of foods into their markets. In certain instances, these regulations are complex and have been designed to take account of the lowering of contamination levels in certain unprocessed cereals that occurs during the processing chain, the lowest regulated levels being reserved for foods destined for direct human consumption. The concept of the graded regulation is that should the raw material or cereal meet the standards required, the final products

should similarly meet the lower levels. On the other hand, maize sold by small scale or emerging farmers in small local informal markets generally falls outside regulatory controls, which can represent a serious challenge to food safety.

4 The importance of expertise on the identification, characterization and detection of mycotoxin-producing *Fusarium* spp.

Several *Fusarium* spp. have been associated with diseases and mycotoxin production in maize. *F. verticillioides*, *F. graminearum*, *F. proliferatum* and *F. subglutinans* cause ear rot, seedling diseases, and root and stalk rots. Fumonisin are produced mainly by *F. verticillioides* and *F. proliferatum*, while *F. graminearum* produces the toxins deoxynivalenol and zearalenone. The threat of mycotoxins becomes more important if food supply is limited, and animals and humans are forced to consume mycotoxin-infected food. The taxonomic classification in *Fusarium* has been refined in the past two decades, especially with the availability of advanced methods in molecular biology. Reliable and rapid identification and detection procedures, thus, need to be developed and established to determine maize kernel infections with mycotoxin-producing *Fusarium* spp. The identification of large numbers of infected kernels to quantify pathogen levels after harvest is a laborious, expensive and time-consuming exercise. Molecular technologies, such as multiplex PCR and quantitative real-time PCR, can help to significantly reduce the time and effort required to accurately measure toxin-producing *Fusarium* spp. in maize. In the modern era of molecular biology, these techniques have also become essential for studies on the distribution, epidemiology, ecology and control of *Fusarium* spp. and their mycotoxins in maize. Despite their significant value, molecular techniques should serve as additional tools to conventional mycological methods in studying *Fusarium* species only, and not entirely replace them.

5 Relevance of fungal genetics and genomics, especially that of *Fusarium verticillioides*

Fusarium verticillioides is a sexually reproducing fungus that causes Fusarium ear rot and may result in mycotoxin contamination of maize grain. The fungus has an endophytic nature that can infect maize plants without causing any visual symptoms, while high toxin levels may be present in maize ears. Due to this unpredictable behaviour and because individual isolates of *F. verticillioides* have the ability to produce varying levels of mycotoxins in maize, populations of *F. verticillioides* strains, rather than individuals, should be investigated as pathogens and toxin producers in maize. Investigating the molecular regulatory system and toxigenic potential of *F. verticillioides* in order to ascertain why certain strains produce different amounts of fumonisins is, therefore, necessary. Furthermore, the genetic structure of the fungus in South Africa, and its ability to cause different levels of Fusarium ear rot, is unknown. Isolates that are non-pathogenic and/or non-toxigenic can form important components of a future disease management strategy. Whether fungal virulence and toxin production in *F. verticillioides* are functions of pathogenic fitness and/or the genomic composition of the fungus, the physiological state of the plant, or prevailing environmental conditions, is still unknown. A better understanding of the genetic composition and genomics of *F. verticillioides* and the maize plant, and the interaction between plant and pathogen using molecular and genomic tools, might lead to the development of novel disease management practices that prevent Fusarium ear rot and fumonisin contamination of maize.

6 Importance of epidemiology and pre-harvest management of *Fusarium* ear rot and mycotoxin production in maize.

Various maize ear rot pathogens that produce mycotoxins under a variety of practices and conditions occur in South Africa. The three most important ear rot-causing organisms include *F. verticillioides*, *F. graminearum* and *Stenocarpella maydis*. Ear rot fungi primarily produce mycotoxins during crop development and harvest, and only some will produce toxins under favourable conditions during storage. In the field, mycotoxin contamination in maize depends on the coincidence of host susceptibility, presence of inoculums, environmental conditions favourable for infection and, in some cases, vector activity. As the interaction between these factors affects the incidence and severity of ear rots and mycotoxin production, disease development and mycotoxin production in the field may yield contradictory results, dependent on the conditions under which trials were conducted. Many agricultural practices, including hybrid selection, crop rotation, tillage, planting date, insect management, chemical control and management of irrigation and fertilization, can affect fungal infection and mycotoxin accumulation in maize grain. Under endemic conditions the use of integrated management systems will go a long way in ensuring low mycotoxin production and food safety. When environmental conditions or genotypes planted are highly favourable for a specific ear rot epidemic and increased mycotoxin contamination, cultural practices alone are often not sufficient to prevent unacceptable levels of contamination and alternate control strategies need to be included in the integrated management system. Mycotoxin management methods used in commercial agricultural systems may not always be suitable for use in subsistence production systems because of the differences in food systems and technological infrastructure. Understanding holistic epidemiological models, which take all factors that contribute to disease development into consideration, will enable us to develop realistic integrated control programs to reduce mycotoxin levels in various farming systems.

7 Evaluation and improvement of maize for resistance to *F. verticillioides* and fumonisin contamination

The planting of resistant cultivars, as part of an integrated disease management strategy, can be considered the most efficient approach to reduce maize ear rot diseases and minimise the risk of fumonisin accumulation in maize. Maize hybrids resistant to *Fusarium* ear rot and fumonisin contamination have been found internationally, but no highly resistant genotypes suited to the South African production regions are known to exist and, thus, have to be developed. Once available, these cultivars will provide producers with affordable, practical and environmentally sound means of disease control. Plant resistance to pathogens can be obtained through conventional (plant breeding) and unconventional (genetic engineering) strategies. For conventional breeding, inbred lines with resistance to *Fusarium* ear rot and fumonisin contamination first need to be identified for breeders to develop locally adapted hybrids, as hybrids grown outside of their adapted range are known to accumulate high concentrations of fumonisin. Such inbred lines should also be evaluated for resistance against other important ear rot diseases, such as Diplodia (caused by *S. maydis*) and Gibberella ear rot (caused by *F. graminearum*). While conventional breeding is time-consuming and labour-intensive, unconventional breeding offers a faster alternative for developing maize plants with resistance to ear

rot pathogens and their mycotoxins. Genetic engineering is the most attractive unconventional strategy available to reduce the production of fumonisins in maize, and mutation breeding has been used before to find valuable plant selections with superior properties, such as herbicide-resistance. Resistance in maize cultivars best adapted to South African growing conditions can also be enhanced by inducing their natural defence responses by treating them with biotic and abiotic resistance inducers.

8 The importance of postharvest contamination of maize and maize products by fungi

The maize value chain consists of all the steps from developing of the seed by plant breeding technologies to the end products destined for human and animal consumption. This includes all the steps of transportation, storage, processing and production. Recent research has shown that inside maize mills, there are definite trends and significant development of storage fungi at specific processing unit operations causing a shift in the amount and type (species) of fungal spore populations in the end food products. These storage fungi are currently a major cause of food spoilage in both the commercial and emerging markets, especially during storage and transport. Although mycotoxins are not necessarily produced from these storage fungi, the vegetative forms of the fungi cause severe spoilage resulting into post harvest losses and a serious risk to food security. The interaction between these fungi, the triggers necessary for their germination and/or mycotoxin production, and the links with the formation of mycotoxin conjugates and the eventual risk to the consumers are poorly understood. Specific proposed topics of research include:

- The study of the mechanisms which trigger fungal contamination and the routes of contamination in mills and processing plants. These investigations should result in the development of appropriate management and cleaning/preventative practices
- The investigation of the growth patterns of storage fungi during transport and warehousing systems (including silos).
- The development of novel rapid and accurate methods for the detection of fungal contamination
- The study of the toxicity of mycotoxin conjugates in human and animal models