MYCOTOXINS
MANAGING MYCOTOXINS IN MAIZE: CASE STUDIES

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Abstract

Mycotoxin contamination of Australian maize is neither common nor extensive, but has the capacity to seriously disrupt ordering marketing. Low to moderate levels of aflatoxins and fumonisins can be widespread in some seasons, with zearalenone and trichothecenes confined to small growing localities. A strategy for managing such situations is tested by an analysis of several case studies. It is concluded that communication across the industry, prediction and prevention of contamination, rapid detection and assessment of contamination, effective use of contaminated maize, and breeding for resistance are reasonable strategies for the purpose, and a current project is addressing these.

Introduction

It is not always possible to produce maize free of mycotoxins, because the fungi responsible are always present, requiring only suitable conditions for growth and mycotoxin production. However, it is practical to ensure that the extent of contamination meets accepted standards for different purposes, whether that is milling, manufacturing, pet-food or stock-foods. This paper attempts to dissect the mycotoxin problem and clarify the underlying causes of failure to meet market specification through an analysis of several case studies, and suggests approaches to aid industry to find solutions.

Mycotoxins of concern

Previously available information about mycotoxin contamination of Australian maize was reviewed at the fifth maize conference in Toowoomba (Blaney 2003), but some of the key points about mycotoxin control are discussed here. In the last 3 years, The Grains Research and Development Corporation has supported a project on Managing Mycotoxins in Maize being conducted by officers of the Qld and NSW Departments of Primary Industries and the Universities of Queensland and Sydney. Progress on the project will be discussed here and by other speakers at this conference.

Aflatoxins

Aflatoxins are usually present at low frequency and concentration in maize grown in temperate regions of Qld and NSW, but occasional samples can contain high concentrations. Invasion of maize by the fungi Aspergillus flavus and A. parasiticus is favored by high temperatures, insect attack, and premature drying of the ear during filling. Once the fungus has invaded certain kernels, aflatoxin production is then favoured by persistent high humidity during grain maturation, and very high concentrations can quickly develop if the grain is stored moist (16-20% moisture). Pre-harvest contamination can involve a very small number of kernels yet significantly contaminate an entire crop. In moist storage, the fungus can quickly spread to adjacent sound maize kernels. Hence, critical control steps for aflatoxin include: avoiding planting situations (region and time) and rainfall/irrigation systems that subject the developing kernel to high temperatures (30-40 ºC); control of insects; and harvest and storage at recommended moisture contents (<14%).

Ochratoxins

Ochratoxin A is rare unless maize heats in storage. The causative fungus in maize is Aspergillus alutaceus (previously A. ochraceus) which is less prevalent than aflatoxin-producing fungi, and seems to prefer slightly higher moisture contents, which are most commonly provided once moisture migration is well underway in heating maize. Control steps are similar to those for aflatoxin.
Fumonisins

Fumonisins produced by *F. verticillioides* are very common in maize. *F. verticillioides* was previously called *F. moniliforme*, but the latter is now considered to include several related fungi, and should no longer be used (Seifert et al. 2003). *F. verticillioides* causes kernel rot, but is present even in apparently sound grain. Low concentrations of fumonisin are consequently very common. Increased stress due to water restrictions and insect attack has been associated with increased ear rot in NSW (Watson et al. 2004). Occasionally, extremely high concentrations of fumonisin can be produced and the cause is not clear, although hybrid susceptibility and climate are involved. Until these factors are explored, control measures cannot be fine tuned. However, selecting suitable hybrids for each region and not restricting water during grain maturation will certainly help.

Zearalenone

Zearalenone can be produced by several *Fusarium* species, but the main producer in maize is the ear- and stalk-rot pathogen *Fusarium graminearum*. This fungus can be associated with a deep purple colouration of infected kernels. The fungus is present on crop debris in the soil and release of spores, and infection of developing maize ears during silking, are both favoured by moderate temperatures and persistent high humidity at that time. Thus, infection is higher in situations when persistently moist and overcast conditions occur during maize silking. Such conditions tend to be limited to the higher-rainfall regions of the far-northern Qld tablelands and the northern rivers of NSW, but can occasionally occur in certain seasons in the Liverpool Plains of NSW. Overall, the extent of zearalenone contamination of maize in Australia is very low, except on parts of the tablelands of far-north Qld. Control of zearalenone contamination is best achieved through use of resistant hybrids.

Nivalenol and deoxynivalenol

The trichothecene mycotoxins, nivalenol and deoxynivalenol, are produced in maize by *Fusarium graminearum* in addition to zearalenone. As explained above, this fungus is only common in Australia on the cool, wet tablelands of far north Qld, where for reasons not completely clear, the fungus produces mainly nivalenol. In southern Qld and in NSW, the fungus produces mainly deoxynivalenol (also called vomitoxin or DON). Control of nivalenol and deoxynivalenol is best achieved with resistant hybrids in higher risk areas, but suitable crop rotations and removal of crop residues can also assist in lower risk areas.

How serious is the risk of contamination overall?

Mycotoxin testing over the last few years is consistent with the previous conclusions that the majority of Australian maize meets the most stringent milling standards, and that all but a very small proportion of remaining crops are suitable as stockfood. There are some localities where the risk of contamination with certain mycotoxins is always higher (such as zearalenone and nivalenol on the Atherton Tableland), and seasons where the risk increases (such as the impact of drought on rainfed crops in hotter localities like central Queensland). Over the last 30 years, there are no indications that contamination has ever been sufficient that it could not be managed, at least potentially, in a way that achieved satisfactory outcomes for both producer and end-user of maize.

Problems that have arisen in the past three years appear to be due to several factors:

(i) General lack of information about mycotoxins in a form that is accessible and easily understood by industry participants. Related to this is the ‘outrage factor’ arising from the shock of finding unexpected contamination, through not knowing how to respond to that situation, and who to discuss it with in order to find a resolution.

(ii) The sporadic seasonal nature of contamination, and our inability to predict situations where the risk of contamination increases. Sometimes, failure to use good storage and transport practices to avoid increases in mycotoxin contamination.
(iii) Our inability to test maize for contamination within the current truck turnaround times for grain deliveries to end-user, and the appropriateness of general grain quality standards for assessing mycotoxin contamination. Related to this is the availability of cost-effective mycotoxin testing methods.

(iv) Failure to set contractual standards for mycotoxin concentrations that are practicable and appropriate for the intended end-use, based on solid scientific data on tolerances of humans and livestock to mycotoxins. Related to this is lack of awareness of, and failure to meet, the expectation of international trading partners in respect to mycotoxin levels.

(v) Use of maize hybrids with innate susceptibility to certain fungi in high risk localities.

What should our management strategies be?

Our current project has set the basic hypothesis that mycotoxins in maize can be managed by addressing five broad strategies that relate to the factors discussed above. Under the guidance of a steering group comprised of a cross-section of industry participants, the project team has been engaged in various activities aimed at providing the tools to help industry address these strategies.

**Communication and coordination across the industry**

- Devising a communication plan to ensure distribution of relevant information to key industry and regulatory authorities, based on a detailed stakeholder analysis.
- Undertaking a formal risk analysis of the food safety hazards from mycotoxins, based on known and projected hypothetical levels of contamination, as part of a PhD study program.
- Adapting the CODEX guidelines for Good Agricultural Practice for managing mycotoxins in grain to the specifics of mycotoxins in Australian maize.
- Developing information packages on managing mycotoxins in maize.

This strategy will be effective if the project team and steering group work effectively, if a national strategy is endorsed by stakeholders, and if information on managing mycotoxins in maize is distributed and adopted across industry.

**Prediction and prevention of contamination outbreaks**

- Investigating outbreaks of contamination to determine key factors contributing.
- Identifying the fungi involved in diseases giving rise to mycotoxin contamination.
- Developing a model to predict mycotoxin contamination of maize from climatic variables, starting with a similar approach used for aflatoxin in peanuts.
- Developing similar predictive models for fumonisin production by *F. verticillioides* and trichothecene production by *F. graminearum*.

This strategy will be effective if the epidemiology and aetiology of the plant pathogens producing mycotoxins are well understood, and control measures are available. In addition, if maize growers and other industry participants are able to predict seasons with a high risk of contamination, and take measures to minimise the impact of this on their operation.

**Rapid detection and assessment of contamination**

- Developing sampling protocols appropriate to Australian maize.
- Compiling and promulgating information on physical indicators of contamination.
- Investigating NIR technology for rapid assessment of contamination.
- Validating chromatographic assay and ELISA methods for mycotoxins of interest.
• Maintaining a list of Australian laboratories capable of mycotoxin assay.
• Assaying maize from all major production regions during the project (three to four seasons).

This strategy will be effective if: a suite of sensitive, specific and rapid assay methods, and sampling protocols are available to industry for testing maize; detailed information is obtained on mycotoxin contamination of the Australian maize crop over four seasons; and if indices are assessed for predicting value of grain based on physical parameters.

**Effective use of contaminated maize**

• Collating available data on tolerances of livestock to different mycotoxins, and providing these data to industry.
• Performing risk assessments on the potential for reduced livestock production by different levels of contamination.
• Helping to establish industry and regulatory standards for mycotoxins in maize, based on good science, which balance the ability of growers to produce quality grain with the requirements of end-users.

This strategy will be effective if rational and transparent standards for acceptable levels of mycotoxins in maize are incorporated in livestock feeding standards, and if markets accept these standards and respond in an economically rational manner.

**Breeding maize for mycotoxin resistance**

• Collecting data that might indicate variable susceptibility of maize cultivars to mycotoxin contamination.
• Developing germplasm combining resistance to certain mycotoxigenic fungi with other desirable characteristics, and making this available for production of commercial cultivars.

This strategy will be effective if cultivars with appropriate resistance to mycotoxins are planted in higher risk situations.

**Testing the hypothesis: case studies**

The appropriateness of these management strategies can be tested via case studies of contamination incidents that arose over the last three seasons. Collectively, these cases provide ample examples of the problems that can arise and lessons for their effective resolution.

**Case study A: aflatoxins in central NSW**

In 2001, ‘extremely high’ levels of aflatoxin (0.2 - 0.3 mg/kg) were detected in some maize grown in NSW, possibly affected by crop flooding, by member companies of the Australian Food and Grocery Council. The confidential report raised the concern that the matter could develop into a serious food scare if not handled with sensitivity. Members were all advised to be extra vigilant in regard to aflatoxin, to ensure appropriate screening procedures (not specified) were in place, and to advise members and regulatory authorities if high levels of aflatoxin were detected. The problem appeared to be confined to a small area of NSW.

In examining the case response, it is clear that the problem was identified and appropriately communicated across those industry participants in the AFGC.

What does not appear to have been dealt with was predicting the problem in the first place, quickly defining the extent of the contamination once detected, specifying what screening procedures should be adopted, what standards should be met for what end-use, what should happen in case of dispute, and advising the growers about their rights and responsibilities in the matter. The response was constrained by natural concern over potential adverse publicity, which is a continuing dilemma for all industries.
Our opinion is that concealing information about contamination might have short-term benefits, but in the long run, simply impairs credibility and leaves the whole industry vulnerable. In a report to the grains industry about 20 years ago, the senior author argued that Australia was in a fortunate position in regard to mycotoxins compared to other countries – mainly because of climate patterns, dry harvests and fewer storage problems. We stand to benefit from a full and open scrutiny of our grain quality. Industry is naturally very concerned that instances of contamination are not blown out of proportion, but this should not occur if we have the evidence of responsible testing, and managing incidents as they arise.

**Case study B: fumonisins in the MIA**

This case got our project off to a flying start in April 2003, when a milling company in the Murrumbidgee Irrigation Area (MIA) rejected a large number of deliveries of contracted maize because of high fumonisin contents – some also had excessive aflatoxin concentrations. It was proposed to offer the maize to local feedlots, but there was concern on both sides about acceptable concentrations for this purpose (and of course, the price that should be set for contaminated maize). Grain prices were high at about $360/tonne, and at least one feedlot rejected grain as poor quality.

The response was led by officers of NSW DPI. Handling the outbreak was helped by the closeness of growers in the MIA. About 60 samples were collected for fumonisin testing at a commercial laboratory (paid for by GRDC via our project) in order to assess the problem and check on tolerances. Only about 40 samples had detectable fumonisin, and only 20 exceeded 5 mg/kg. A few samples contained 10-50 mg/kg. Gravity grading removed a large proportion of fumonisin into the lightweight fraction. A field day was held in the midst of the outbreak and 110 growers attended. Information on fumonisin was got to growers quickly and this was aided by timely release of an IREC Farmers newsletter that provided management information. There was not too much focus on aflatoxin although it was known that some growers had problems. Detailed information about the *Fusarium* outbreak was provided in a report to the maize growers, and a summary was also published in ‘The Cob’ (O’Keeffe 2003) (4000 copies of this magazine are regularly circulated to maize industry participants across Australia www.maizeaustralia.com.au). Also involved were radio interviews, addresses to farmer groups, and presentations to district agronomists who extended the message. The project also provided detailed information on tolerances of livestock to mycotoxins and the impact of nutritional changes in infected grain on livestock.

The cause of the outbreak is not perfectly clear. After severe heat in December, 32 mm of storm rain at the start of January, crops received about 40 mm rain on 21st February with high humidity for the following few days. Two weeks after this, some growers had ‘pushed the system’ a bit by stretching out irrigation water and noticed quality problems on harvest in March/April. While ‘stress’ clearly contributed, the timing of that stress in relation to *F. verticillioides* growth is speculative – probably heat stress (>40°C at times) and premature drying (and insect damage to allow an entry point) in early-mid February reduced plant resistance to the fungus, and high rainfall and humidity after 21st February provided perfect conditions for fungal growth and fumonisin production (18% is the minimum moisture content for growth of *F. verticillioides*). In any case, recommendations now are to plant on time (to sow late September), to adjust irrigation intervals (not extend), to manage nitrogen (avoid excess), and avoid softer varieties, which might be more stress susceptible.

At this local level, the contamination episode was managed quite well after the initial shock - the problem was recognised; the risks were clarified; accurate information was provided to those who needed to know; and appropriate decisions were made by most stakeholders. A positive outcome was the subsequent establishment of levels for aflatoxins and fumonisins in trading standards of the National Agricultural Commodities Marketing Association (NACMA) (Cogswell 2003). Ongoing needs identified were better prediction of mycotoxin problems, and faster (and cheaper) assay methods.

**Case study C: aflatoxins in central Qld**

In mid 2004, the project team detected aflatoxin in a large number of small (0.5 kg) ‘grower samples’, supplied by a bulk handler, grown on one farm in central Qld. Concentrations ranged up to 0.24 mg/kg, but averaged 0.045 mg B1/kg. This level exceeded the Qld Stockfood regulation limit of 0.02 mg/kg for ‘grain, crushed grain and seeds’.
The average level would meet the limit of 0.05 mg/kg for ‘stock food for beef cattle, horses or sheep’, but the regulation does not specify a process whereby grain becomes stock food for beef cattle, horses or sheep!

It was recognised that the samples tested were far too small to accurately represent the aflatoxin content of bulk maize. According to the Aflatoxin Handbook of the Grains Inspection, Packers and Stockyards Administration (GIPSA) of the USDA (http://www.usda.gov/gipsa), a minimum of 2 pounds (908 g) should be taken per truckload. Even then, the aflatoxin content of that sample might vary between 0.003 and 0.039 mg/kg, if the ‘true’ concentration in the truck was 0.02 mg/kg. Obviously, the 1 kg sample is satisfactory for detecting potential contamination, but for regulatory purposes, larger samples (5-10 kg per truckload) need to be taken. The entire 5 kg sample must be milled before sub-sampling, and certain mills like the Romer mill are available for this purpose - the logistics of testing such large samples have only been addressed so far by certain milling companies.

The supplier, once aware of the potential problem, elected to place the grain under quarantine, and also submitted larger samples representing bulk maize from that region. These samples all met the Qld Stockfood standard of 0.02 mg B1/kg, suggesting substantial dilution by other negative deliveries of maize. Although the regulations were apparently met, it was recognised that some portions of the bulk maize could have higher concentrations, so to minimise risk the maize was sold to a cattle feed-lot, and this appeared to have been an appropriate course of action.

What did we learn? The industry already has sufficient evidence to indicate that mycotoxin testing, at least for aflatoxin and fumonisin, should be regularly performed, although the frequency of this might be low except in certain high risk circumstances. Now that NACMA has set standards for maize, pressure will increase for suppliers to provide evidence that their product meets those standards! Appropriate sampling procedures for aflatoxin must be used. Do we still need regulations, and if regulations are to be retained, should they be brought into line with the NACMA standards? Some wordings of the current Qld Stockfood Regulations are not at all clear, e.g. in what manner does ‘grain’ become ‘cattle feed’. However, it is clear that nothing will happen unless industry pushes for change.

**Case study D: aflatoxins in an export consignment**

In January 2005, a single container of bulk maize from the MIA was rejected on arrival in Japan for aflatoxin residues. Japan has a limit of 0.005 mg aflatoxins/kg, and the container tested at 0.027 mg/kg. The Department of Agriculture, Fisheries and Forestry (DAFF) was notified of this by the Japanese Ministry of Health, Labour and Welfare, and requested to investigate the cause of the incident, to introduce measures to reduce contamination and to ensure that it did not happen again. Under an ‘enhanced inspection order,’ the next 300 maize shipments or all shipments over the next 3 years would be tested for aflatoxin, and a second breach would prompt exclusion from the Japanese market. The investigation was a good example of cooperation and communication at the National level. It was coordinated by members of the Grains Council, Maize Association of Australia, NSW DPI, Qld DPI and GRDC. The investigation revealed the following story. The maize was grown in 2003/2004 over a particularly hot and dry summer in the MIA – conditions known to favour *Aspergillus flavus* invasion. Harvesting took place during cool and wet conditions and the harvest moisture content ranged from 13.5-16% (14% is regarded as the maximum safe level for storage). Noticing some quality problems, the maize was gravity graded and about 90% of physically damaged grain removed. Follow-up testing by our project as part of the trace-back investigation found 0.002 mg aflatoxins/kg in graded grain, and 0.005 mg/kg in ungraded grain – clear indication of the presence of the fungus, although aflatoxin levels were probably acceptable before shipment. However, the grain was then placed in bulk in non-aerated transport containers, which spent several weeks on docks and ships at temperatures ranging up to 50°C before testing in Japan. Under these extreme conditions, any slight excess of moisture becomes concentrated into pockets through the alternate heating and cooling of container sides, an ideal situation for aflatoxin production by the fungus.

As a consequence of this case, Australian exporters have been made aware of Japan’s increased testing regimen, and The Maize Association of Australia has recommended all exporters test for mycotoxins prior to export (in addition to existing testing being carried out by milling companies). The key lesson is the need to manage moisture levels in stored maize at all times.
In shipping containers, maize in bags is of lower risk than bulk maize since migrating moisture will condense outside the bags, and inert adsorbents like diatomaceous earth in the container might help to mop up some condensation. Some exporters pay a little more to ensure their containers are in the hold of ships, rather than on deck, because it is cooler.

Even with these precautions in place, some serious risks remain: firstly, that some occasional or first-try exporter might send untested maize overseas, either through ignorance or overconfidence, and put all our exports grain markets at risk; and secondly, that the aflatoxin testing process used by certain laboratories itself might be insufficiently rigorous to ensure that certain batches will meet a stringent limit of 0.005 mg aflatoxin/kg (see the requirements for testing discussed in Case study C). At least the latter risk can be reduced if clients specify an appropriate sampling system like the GIPSA system, and only use laboratories that can supply evidence of method validation and an accreditation system like that of the National Association of Testing Authorities (NATA).

**Case study E: Effective use of contaminated maize screenings**

In mid 2004, a sample of maize screenings was submitted to our project by a grower in mid-west NSW. Alert to visible damage and the possibility of mycotoxin contamination, his agent had gravity graded several hundred tonne of lightweight material out of a 30,000 tonne crop. We detected 0.06 mg aflatoxins/kg and over 600 mg fumonisins/kg in the screenings! The most lenient NACMA standard for maize used in stock food is 0.08 mg/kg aflatoxins, and 40 mg/kg fumonisins.

Our advice to this grower was that there was a high risk of toxicity if the undiluted material was fed to livestock. If he intended to feed the grain to his own mature beef cattle or sheep, it should be diluted substantially or used only as a feed supplement. The aflatoxin level should be tolerable by adult ruminants, but the fumonisin content was too high. In published experimental trials, cattle fed 100 mg/kg fumonisin for 30 days had slightly reduced gain and slight liver damage. Cattle fed 200 mg/kg fumonisin for 14 days had more substantial liver damage and lower weight gain (compared to cattle fed a fumonisin-free ration). Consequently, it would seem best to feed no more than 1kg/head/day to cattle. It was noted that the material must not be fed to horses, which are very susceptible to fumonisin, nor to pet species of unknown susceptibility. Given this information, the grower declined to feed his stock but accepted an offer of $115/tonne for the material (cf $195/tonne for sound maize) which was incorporated into mineral supplement blocks. Such blocks are used mainly for cattle and sheep, which have some resistance to fumonisins and aflatoxins, and the formulation is usually designed to limit intake to <0.2 kg/day (maybe a 50-fold dilution of many mycotoxins present). This appears a reasonable decision in the circumstance.

Other options explored included the use of ‘mycotoxin-binding’ agents, but we were unable to find any scientific evidence that these were effective with fumonisin, and the cost/benefit is usually too high. Directing grain to ethanol production plants is another avenue, but the by-product of distillers grain retains much of any mycotoxins present in the original grain, so the hazard remains. The take-home message is that effective use of contaminated grain means to get the best economic outcome, and despite adding a cost, accurate mycotoxin assay can minimise the risk of an adverse outcome.

**Case study F: Breeding for resistance to mycotoxin-producing fungi**

Almost 40 years ago, a maize breeding program was set up in tropical north Queensland by DPI to develop hybrids suitable for the particular climate of the northern Tablelands, which features a persistently wet and often cool growing and maturation period. This climate was conducive to many diseases affecting yields and quality, and the breeding program led by Ian Martin at Kairi Research Station has gradually eliminated many of these. *Fusarium graminearum*, *F. verticillioides* and other *Fusarium* species were common causes of stalk and ear rots of maize in the early 1980s, and zearalenone contamination was very common in surveys conducted at the time (Blaney et al. 1986). Since that time, the breeding program has greatly reduced the extent of *F. graminearum* ear rots, and also zearalenone contamination, judging by our recent surveys. The hybrids might be resistant to fumonisin contamination as well, but this hasn’t been fully investigated. The message is clear – breeding for resistance to certain fungi is a vital strategy in managing mycotoxins, and this characteristic should be as important in breeding targets as yields and other agronomic values.
The major breeding companies are aware of these issues, but the demand for mycotoxin resistance needs to come from the market place. Rightly or wrongly, some hybrids are being blamed for increased fumonisin contamination, and this needs further investigation. It is noted that Bt hybrids have been reported to have some resistance to fumonisin contamination in the USA through increased resistance to boring insects (Munkvold and Butzen 2004). The message to growers from this case study is to choose hybrids appropriate for each region, and to take account of the potential impact of a stressful season on mycotoxin contamination and eventual market suitability.

Conclusions

An examination of the case studies above indicates that our strategies are generally appropriate, providing we (ie, the whole industry) tackle the issues well and achieve the objectives set. The more we discuss these issues and the problems they cause, the more those solutions will present, but pragmatically, any particular industry participant is more likely to retain the necessary information once they have had to deal with the problems these situations create, or at least to adopt and routinely apply the necessary mycotoxin management processes. How can we facilitate this process? At this conference, my colleague Lisa Bricknell will present a plan for using the HACCP framework for the purpose of managing mycotoxins in Australian maize (Bricknell et al. 2006). While many of the solutions to mycotoxin problems are already within our hands, some issues such as the impact of variable weather patterns on mycotoxins require more research, and at this conference our colleague Yash Chauhan will present progress on climatic modelling to predict aflatoxin contamination in maize (Chahuan et al. 2006). Also at this conference, other presenters such as Andrew Watson will address issues of disease control with relevance to mycotoxin control (Watson et al. 2006). In conclusion, I hope that this presentation has shown that mycotoxin problems can and do affect the entire maize industry, and all industry participants have an important role to play - managing mycotoxins in maize is too serious an issue to be ignored.

References


