

## 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 marketing. Low to moderate levels of aflatoxins and fumonisins can be widespread in some seasons, but zearalenone, nivalenol and deoxynivalenol are usually confined to small growing localities. Possible approaches to such situations were tested by an analysis of several case studies. It is concluded that communication and coordination across the industry, prediction and prevention of contamination, rapid detection and assessment of contamination, effective use of contaminated maize and breeding for resistance comprise a useful set of strategies for managing mycotoxins in maize.

### 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 uses, whether that is milling for human food, manufacturing purposes such as gluten extraction, or incorporation into pet foods and stock foods. This paper examines the problem of mycotoxins in Australian maize to clarify the underlying causes of failure to meet market specification through an analysis of several case studies and provides suggestions to assist industry to find solutions.

### Mycotoxin occurrence in Australian maize

Information about mycotoxin contamination of maize has been obtained from some targeted mycotoxin surveys in certain regions, from industry quality testing programs, and from investigations into occasional episodes of livestock poisoning by animal health laboratories. Plant disease control and maize breeding programs also provide information on the prevalence of mycotoxigenic fungi.

#### Aflatoxins

Aflatoxins are usually present at low frequency and concentration (0.001–0.005 mg/kg) in maize grown in subtropical and temperate regions of Queensland (Qld) and New South Wales (NSW), but occasional samples can contain higher concentrations, up to 0.2 mg/kg (Blaney 1981). Invasion of maize by the fungi *Aspergillus flavus* and *A. parasiticus* is favoured 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 at 16–20% moisture (Blaney and Williams 1991). Preharvest contamination can involve a very small number of kernels, yet

provide enough aflatoxin to significantly contaminate an entire crop. In moist, hot 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 (35–40°C); control of insects; and harvest and storage at recommended moisture contents (<14%).

#### Ochratoxins

Ochratoxin is quite uncommon in Australian maize, although traces are occasionally detected (0.001–0.003 mg/kg). The causative fungus in maize is generally considered to be *A. ochraceus* although identification of other ochratoxin-producing fungi that used to be grouped with *A. ochraceus* (Frisvad *et al.* 2004), and production of ochratoxin by some isolates of *A. niger* has raised some uncertainty about the point. Ochratoxin production by Qld isolates of *A. ochraceus* was reported by Connole *et al.* (1981). This fungus 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 stored maize. Control steps are similar to those for aflatoxin.

#### Fumonisin

Fumonisin are produced by *Fusarium verticillioides*, *F. proliferatum*, *F. thapsinum* and *F. nygamai*. These fungi all occur in Australian maize, but *F. verticillioides* appears to be the main source of fumonisins. *F. verticillioides* was previously called *F. moniliforme*, but the latter is now considered to include several related fungi (Seifert *et al.* 2003). *F. verticillioides* causes kernel rot, but is now considered an endophyte that is present in apparently sound grain (Williams *et al.* 1992). Low concentrations of fumonisin (0.2–1 mg/kg) are consequently very common (Bryden *et al.* 1995). Increased stress due to water restrictions and insect attack has been associated with increased

ear rot in NSW (Watson *et al.* 2006). Occasionally, very high concentrations (>100 mg/kg) of fumonisin can be produced, albeit in visually rotted kernels (Shanks *et al.* 1995). The cause is not clear, although hybrid susceptibility and climate are involved. Until these factors are explored, control measures cannot be fine tuned, but selecting suitable hybrids for each region and not restricting water during grain maturation will certainly help.

#### *Zearalenone*

Zearalenone can be produced by several *Fusarium* spp., but the main producer in maize is the ear- and stalk-rot pathogen *F. graminearum*, often associated with a deep purple colouration of infected kernels (Blaney *et al.* 1984b). 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 North Qld tablelands and the northern rivers region of NSW. Even in these minor growing regions, samples do not often exceed 1 mg/kg (Blaney *et al.* 1986), the level that can affect pigs (Blaney *et al.* 1984a). Zearalenone contamination can be limited through the use of hybrids resistant to *F. graminearum*.

#### *Nivalenol and deoxynivalenol*

The trichothecene mycotoxins, nivalenol (NIV) and deoxynivalenol (DON), are produced in maize by *F. graminearum*, which can also produce 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 NIV. In southern Qld and in NSW, the fungus produces mainly DON, also called vomitoxin (Blaney and Dodman 2002). It is very unusual for NIV and DON to exceed 1 mg/kg, a level reducing feed intake by pigs (Williams and Blaney 1994). Control of NIV and DON 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.

### **Overview of current mycotoxin surveillance**

Mycotoxin testing is regularly carried out by organisations in the milling and pet food industries, and by some stock food manufacturers if a problem is suspected. Data provided to the authors of testing results over the last 5–10 years by some of these organisations, are consistent with conclusions from surveys (Bricknell *et al.* 2008) that the major proportion of Australian maize meets the most stringent milling standards, and that all but a very few of the remaining crops are suitable as stock food. Aflatoxins are of most concern, particularly for companies supplying the human food (millers) and pet food markets, who are using a standard of 0.005 mg/kg. Increasing drought and high temperatures associated with global warming are increasing the risks. Less data have been collected on fumonisins, but these also require regular monitoring. There are some localities where the risk of contamination with certain mycotoxins is always higher (such as zearalenone and NIV on wetter parts of the Atherton Tableland), and seasons where the

aflatoxin risk increases (such as the impact of drought on rainfed crops in hotter localities in central Qld).

Despite these localised and seasonal risks, there are no indications over the last 30 years that mycotoxin contamination has ever been so excessive that it could not be managed, at least potentially, in a way that achieved satisfactory outcomes for both the producer and the end-user of maize. Problems in managing situations that have arisen in the past appear to be due to several factors. These are:

- (1) 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.
- (2) The sporadic seasonal nature of contamination, and inability to predict situations where the risk of contamination increases. Sometimes, this is compounded by failure to use good storage and transport practices to avoid increases in mycotoxin contamination.
- (3) The current inability to test maize for contamination within the current truck turn-around times for grain deliveries to end-user, and the inappropriateness of general grain quality standards for assessing mycotoxin contamination. Related to this is the availability of cost-effective mycotoxin testing methods.
- (4) 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 livestock to mycotoxins, and internationally accepted limits for maize used as human food. Related to this is lack of awareness of, and failure to meet, the expectation of international trading partners in respect to mycotoxin levels.
- (5) Use of maize hybrids with innate susceptibility to certain fungi in high risk localities.

### **Proposed management strategies**

From 2003–06, the Grains Research and Development Corporation (GRDC) supported a project on managing mycotoxins in maize, conducted by the authors and other officers of the Qld and NSW Departments of Primary Industries (DPI) and the Universities of Qld and Sydney. This project 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 engaged in various activities aimed at providing the tools to help industry address these strategies, as listed below:

#### *Strategy 1 – communication and coordination across the industry*

Activities included: 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; adapting the guidelines for good agricultural practice for managing mycotoxins in grain

published by the Codex Alimentarius Commission (2003) to the specifics of mycotoxins in Australian maize; and developing information packages on managing mycotoxins in maize.

Success criteria for this strategy were that the project team and steering group worked effectively, that a national strategy was endorsed by stakeholders, and that information on managing mycotoxins in maize was distributed and adopted across the industry.

#### *Strategy 2 – prediction and prevention of contamination outbreaks*

Activities included: investigating outbreaks of contamination to determine key contributing factors; identifying the fungi involved in diseases of maize that give rise to mycotoxin contamination; and developing a model to predict mycotoxin contamination of maize from climatic variables, starting with an approach similar to that used for aflatoxin in peanuts (Rachaputi *et al.* 2002).

Success criteria for this strategy were that the epidemiology and aetiology of the plant pathogens producing mycotoxins were well understood, that control measures were available, and that maize growers and other industry participants were able to predict seasons with a high risk of contamination, and took measures to minimise the impact of this on their operation.

#### *Strategy 3 – rapid detection and assessment of contamination*

Activities included: developing sampling protocols appropriate to Australian maize; compiling and promulgating information on physical indicators of contamination; investigating near infrared analyser technology for rapid assessment of contamination (Dowell *et al.* 2002); validating sampling plans and analytical methods for mycotoxins of interest; maintaining a list of Australian laboratories that were accredited for performing mycotoxin assays; and assaying maize from all major production regions during the project (three to four seasons).

Success criteria for this strategy were that a suite of sensitive, specific and rapid assay methods and sampling protocols were available to industry for testing maize; and that detailed information was obtained on mycotoxin contamination of the Australian maize crop over four seasons.

#### *Strategy 4 – effective use of contaminated maize*

Activities included: 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; and helping to establish industry and regulatory standards for mycotoxins in maize, based on good science, which balanced the ability of growers to produce quality grain with the requirements of end-users.

Success criteria for this strategy were that standards for acceptable levels of mycotoxins in maize were established and incorporated into livestock feeding practices, and that markets accepted these standards and responded in an economically rational manner.

#### *Strategy 5 – breeding maize for mycotoxin resistance*

Activities included: collecting data that might indicate variable susceptibility of maize cultivars to mycotoxin contamination;

and developing germplasm combining resistance to certain mycotoxigenic fungi with other desirable characteristics, for incorporation into commercial cultivars.

Success criteria for this strategy were that mycotoxin minimisation was incorporated into objectives of maize breeding programs, and that cultivars with appropriate resistance to mycotoxins were planted in higher risk situations.

#### **Testing the strategies: case studies**

The appropriateness of these management strategies was tested via case studies of contamination incidents that arose over the previous few years. These cases provide examples of the problems that can arise and lessons for their effective resolution.

##### *Case study A – aflatoxins in central NSW*

In 2001, levels of aflatoxin described as ‘extremely high’ (0.2–0.3 mg/kg) were detected in some maize grown in ‘central NSW’ by member companies of the Australian Food and Grocery Council (AFGC). 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. With hindsight, the reaction appeared excessive, as the problem was confined to a very small locality, affected by crop flooding, and where high moisture storage was involved.

In examining the case response, it is clear that the problem was identified and appropriately communicated across those industry participants in the AFGC. What was not done was predicting the problem in the first place, quickly defining the extent of contamination within the overall picture of good quality grain, 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. A strong case can be made that Australia is in a good position in regard to mycotoxins compared with many other countries – mainly because of climatic patterns, dry harvests and fewer storage problems – and stands to benefit from a full and open scrutiny of grain quality. There is natural concern that instances of contamination are not blown out of proportion, but this should not occur if the industry can produce evidence of responsible testing, and managing incidents as they arise.

##### *Case study B – fumonisins in the Murrumbidgee irrigation area*

In April 2003, 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 (t), 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 from growers with quality concerns, and submitted for fumonisin testing at a commercial laboratory in order to assess the problem and check on tolerances. About 40 samples had detectable fumonisin, 20 exceeded 5 mg/kg, and a few samples contained 10–50 mg/kg. Gravity grading was demonstrated to remove 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 quickly provided to growers, and this was aided by the timely release of a farmers' newsletter that provided management information. There was less focus on aflatoxin than fumonisin, 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). *The Cob* is the magazine of the Maize Association of Australia (MAA), and 4000 copies of this magazine are regularly circulated to maize industry participants across Australia. Also involved were radio interviews, addresses to farmer groups, and presentations to district agronomists who extended the message. Detailed information on tolerances of livestock to mycotoxins and the impact of nutritional changes in infected grain on livestock production was also provided (Blaney and Williams 1991; Williams *et al.* 1992).

The cause of the outbreak was not clear. After severe heat in December 2002, 32 mm of storm rain fell at the start of January 2003, and crops received ~40 mm rain on 21 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 21 February provided perfect conditions for fungal growth and fumonisin production (18% is the minimum moisture content for growth of *F. verticillioides*). Current recommendations are to plant on time (to sow late September), to adjust irrigation intervals (but not extend them), to manage nitrogen application (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 2003). These standards are shown in Table 1. 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 aflatoxin B1/kg. This level exceeded the Qld stock food regulation limit of 0.02 mg aflatoxin B1/kg for 'grain, crushed grain and seeds' (Anon. 2003). The average level would meet the limit of 0.05 mg/kg for 'stock food for beef cattle, horses or sheep', but the regulation did not specify a process whereby grain became stock food for beef cattle, horses or sheep.

It was recognised that the samples tested were 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), a minimum of 2 pounds (908 g) should be taken per truckload (USDA 2003). 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, a 1-kg sample might be 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 subsampling, and certain mills like the Romer mill are available for this purpose. The logistics of testing such large samples have been addressed by certain milling companies in Australia, but not by many other maize end-users.

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 stock food standard for grain 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 feedlot, and this appeared to have been an appropriate course of action.

This case study raises several learning points. First, the industry now 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 the maize industry, via NACMA, has set mycotoxin standards for maize, pressure will increase for suppliers to provide evidence that their product meets those standards! Second, appropriate sampling procedures

**Table 1. Aflatoxin and fumonisin limits for maize sold under National Agricultural Commodities Marketing Association (NACMA) contracts**

NACMA grade	Milling	Prime	Feed 1	Feed 2
Aflatoxins (B1 + B2 + G1 + G2) (mg/kg)	0.005	0.015	0.02	0.08
Fumonisin (B1 + B2 + B3) (mg/kg)	2	5	10	40

for aflatoxin must be used. Third, it is important to debate the question of whether government regulations are still required if industry sets its own standards. If regulations are to be retained, it is important that these be harmonised with industry (NACMA) standards. Another important question for processors (e.g. grit or gluten or stock feed manufacturers) is whether standards should be applied to incoming maize or to the final products, since processing can either reduce or concentrate mycotoxins in different product streams. These questions involve all the maize industry, and cross-industry forums such as was hosted by the MAA in Brisbane in October 2006 (Cogswell 2006) provide the opportunity to resolve these matters.

#### *Case study D – aflatoxins in an export maize 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 total aflatoxins/kg, and the container tested at 0.027 mg/kg. The Australian Department of Agriculture, Fisheries and Forestry 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.

The investigation was a good example of cooperation at the national level, being coordinated by members of the Grains Council, MAA, NSW DPI, Qld Department of Primary Industries and Fisheries (DPI&F), and the GRDC, and revealed the following story. The maize was grown under irrigation in 2003–04 over a particularly hot and dry summer in the MIA – conditions known to favour *A. flavus* invasion. Harvesting took place during unusually cool and showery 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 owner gravity-graded the maize and ~90% of physically damaged grain was 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 (both in Australian and Japan), and on ships at temperatures ranging up to 50°C, before testing was conducted 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 MAA has recommended all exporters test for mycotoxins before export (in addition to existing testing being carried out by milling companies) and to fully document the test results. Another 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 will remove some

condensation (there are commercial products for this purpose). Containers should be carried in the hold of ships, not on deck where it can be hotter. These measures have been implemented by grain exporters, and a large number of shipments have since been accepted. It has become clear that several additional issues need to be negotiated and inserted into contracts between exporter and importer. These should define how containers are to be sampled and tested and which standards will apply, and limit the time between arrival in the importing country and testing to avoid further deterioration.

Even with these precautions in place, some serious risks remain: first, that some occasional or first-try exporter might send untested maize overseas, either through ignorance or overconfidence, and put all Australian grain markets at risk; and second, 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. All of these recommendations have been incorporated into supply chain and export protocols for maize, which the MAA is proposing for wide adoption across the industry (Cogswell 2006).

#### *Case study E – effective use of contaminated maize screenings*

In mid 2004, a sample of maize screenings was submitted to the authors by a grower in mid-west NSW. Alert to visible damage and the possibility of mycotoxin contamination, his agent had gravity graded several hundred tonnes of lightweight material out of a 30000 t crop. We detected 0.06 mg aflatoxins/kg and well over 200 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 fumonisin.

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 tolerated by adult ruminants, but the fumonisin content was too high. Ruminants are tolerant to fumonisins compared with horses and pigs, but reduced production has been reported in dairy cows fed 75 mg fumonisin B1/kg for 14 days (Richard *et al.* 1996), and evidence of liver damage in feeder calves given 148 mg total fumonisins/kg for 30 days (Osweiler *et al.* 1993). Consequently, it would seem best to feed no more than 1–2 kg of these screenings/animal.day to cattle.

The grower was warned that the material must not be fed to horses, which are very susceptible to fumonisin (EU 2005), nor to pet species of unknown susceptibility. Given this information, the grower declined to feed his own stock but accepted an offer of \$115/t for the material (cf. \$195/t for sound maize), which was incorporated into mineral supplement blocks. Such blocks are used mainly for cattle and sheep, which are relatively resistant 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 appeared a reasonable, low-risk decision in the circumstance. A set of guidelines for maximum aflatoxin and fumonisin content of food for various livestock and pet species was published in *The Cob* (Kopinski and Blaney 2006).

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, so the benefit to cost ratio was doubtful. 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. In summary, effective use of contaminated grain means to get the best economic dividend (Blaney and Williams 1991) 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 Qld by DPI&F 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. *F. 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 linked to increased fumonisin contamination, and this needs further investigation. Research into sources of fumonisin resistance is well underway in other countries (Clements *et al.* 2004; Butron *et al.* 2006). 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). There is a possibility that breeding for drought resistance might have a positive impact on aflatoxin susceptibility.

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. Adjusting planting times and plant populations can also reduce stress and decrease risks of mycotoxin contamination (Chauhan *et al.* 2008).

#### Conclusions

An examination of the case studies above indicates that the proposed strategies for managing mycotoxins are generally appropriate, providing all industry participants understand the

issues involved and work together to achieve objectives. The more these issues are discussed, the more likely it is that solutions will present. To be pragmatic, 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. We consider that the Hazard Analysis Critical Control Point framework is most suitable for managing the known risks within industry operations (Bricknell *et al.* 2008). Managing the unknown risks such as the impact of variable weather patterns on mycotoxins does require more research, and climatic modelling to predict aflatoxin contamination in maize is feasible (Chauhan *et al.* 2008). Research also needs to continue on disease control relevant to mycotoxins (Watson *et al.* 2006), and on rapid assessments methods for detecting contamination. All participants in the industry have an important role to play – managing mycotoxins in maize is too serious an issue to be ignored.

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#### References

- Anon. (2003) Agricultural Standards Regulations 1997. Queensland Government. Available at [www.legislation.qld.gov.au/LEGISLTN/CURRENT/A/AgrStandR97.pdf](http://www.legislation.qld.gov.au/LEGISLTN/CURRENT/A/AgrStandR97.pdf) [Verified 25 November 2007]
- Blaney BJ (1981) Aflatoxin survey of maize from the 1978 crop in the South Burnett region of Queensland. *Queensland Journal of Agricultural and Animal Sciences* **38**, 7–12.
- Blaney BJ, Dodman RL (2002) Production of zearalenone, deoxynivalenol, nivalenol, and acetylated derivatives by Australian isolates of *Fusarium graminearum* and *F. pseudograminearum* in relation to source and culturing conditions. *Australian Journal of Agricultural Research* **53**, 1317–1326. doi:10.1071/AR02041
- Blaney BJ, Williams KC (1991) Effective use in livestock feeds of mouldy and weather damaged grain containing mycotoxins – case histories and economic assessments pertaining to pig and poultry industries of Queensland. *Australian Journal of Agricultural Research* **42**, 993–1012. doi:10.1071/AR9910993
- Blaney BJ, Bloomfield RC, Moore CJ (1984a) Zearalenone intoxication of pigs. *Australian Veterinary Journal* **61**, 24–27.
- Blaney BJ, Moore CJ, Tyler AL (1984b) Mycotoxins and fungal damage in maize harvested during 1982 in Far North Queensland. *Australian Journal of Agricultural Research* **35**, 463–471. doi:10.1071/AR9840463
- Blaney BJ, Ramsey MD, Tyler AL (1986) Mycotoxins and toxigenic fungi in insect-damaged maize harvested during 1983 in Far North Queensland. *Australian Journal of Agricultural Research* **37**, 235–244. doi:10.1071/AR9860235
- Bricknell LK, Blaney BJ, Ng J (2008) Risk management for mycotoxin contamination of Australian maize. *Australian Journal of Experimental Agriculture* **48**, 342–350.

- Bryden WL, Ravindran G, Salahifar H, Gill RJ, Burgess LW (1995) Fumonisin content of Australian maize. *Proceedings of the Nutrition Society of Australia* **19**, 46.
- Butron A, Santiago R, Mansilla P, Pintos-Varela C, Ordas A, Malver R (2006) Maize (*Zea mays* L.) genetic factors for preventing fumonisin contamination. *Journal of Agricultural and Food Chemistry* **54**, 6113–6117. doi:10.1021/jf0611163
- Chauhan YS, Wright GC, Rachaputi NC (2008) Modelling climatic risks of aflatoxin contamination in maize. *Australian Journal of Experimental Agriculture* **48**, 358–366.
- Clements MJ, Maragos CA, Pataky JK, White DG (2004) Sources of resistance to fumonisin accumulation in grain and *Fusarium* ear and kernel rot of corn. *Phytopathology* **94**, 251–260. doi:10.1094/PHYTO.2004.94.3.251
- Codex Alimentarius Commission (2003) Code of practice for the prevention and reduction of mycotoxin contamination of cereals, including annexes on ochratoxin A, zearalenone, fumonisins and trichothecenes. Available at [http://www.codexalimentarius.net/download/standards/406/CXC\\_051e.pdf](http://www.codexalimentarius.net/download/standards/406/CXC_051e.pdf) [Verified 25 November 2007]
- Cogswell T (2006) MAA president's report. *The Cob* **4**(5), 3. Available at [www.maizeaustralia.com.au](http://www.maizeaustralia.com.au) [Verified 25 November 2007]
- Connole MD, Blaney BJ, McEwan T (1981) Mycotoxins in animal feeds and toxic fungi in Queensland 1971–80. *Australian Veterinary Journal* **57**, 314–318.
- Dowell FE, Pearson TC, Maghirang EB, Xie F, Wicklow DT (2002) Reflectance and transmittance spectroscopy applied to detecting fumonisin in single corn kernels infected with *Fusarium verticillioides*. *Cereal Chemistry* **79**, 222–226. doi:10.1094/CCHEM.2002.79.2.222
- EU (2005) Opinion of the scientific panel on contaminants in food chain on a request from the commission related to fumonisins as undesirable substances in animal feed. *The EFSA Journal* **235**, 1–32. Available at <http://www.efsa.eu.int> [Verified 25 November 2007]
- Frisvad JC, Frank M, Houbraken J, Kuijpers A, Samson RA (2004) New ochratoxin A producing species of *Aspergillus* section Circumdati. *Studies in Mycology* **50**, 23–43.
- Kopinski J, Blaney B (2006) Managing mycotoxins in maize – options when feeding animals. *The Cob* **4**(5), 8. Available at [www.maizeaustralia.com.au](http://www.maizeaustralia.com.au) [Verified 25 November 2007]
- Munkvold G, Butzen S (2004) Mycotoxins in corn grain and effect of Bt hybrids *Crop Insight* **14**(8), 1–5.
- NACMA (2003) 'Agricultural commodity standards manual.' (National Agricultural Commodities Marketing Association: Wilberforce, NSW)
- O'Keeffe K (2003) *Fusarium* in the Riverina. *The Cob* **3**(5), 11. Available at [www.maizeaustralia.com.au](http://www.maizeaustralia.com.au) [Verified 25 November 2007]
- Osweiler GD, Kehrl ME, Stabel JR, Thurston JR, Ross PF, Wilson TM (1993) Effects of fumonisin-contaminated corn screenings on growth and health of feeder calves. *Journal of Animal Science* **71**, 459–466.
- Rachaputi N, Wright GC, Krosch S (2002) Management practices to minimise pre-harvest aflatoxin contamination in Australian peanuts. *Australian Journal of Experimental Agriculture* **42**, 595–605. doi:10.1071/EA01139
- Richard JL, Meerdink G, Maragos CM, Tumbleson M, Bordson G, Rice LG, Ross PF (1996) Absence of detectable fumonisins in the milk of cows fed *Fusarium proliferatum* (Matsushima) Nirenberg culture material. *Mycopathologia* **133**, 123–126. doi:10.1007/BF00439124
- Seifert KA, Aoki T, Baayen RP, Brayford D, Burgess L, et al. (2003) The name *Fusarium moniliforme* should no longer be used. *Mycological Research* **107**, 643–644. doi:10.1017/S095375620323820X
- Shanks G, Tabak P, Begg AP, Bryden WL (1995) An outbreak of acute leukoencephalomalacia associated with fumonisin intoxication in three horses. *Australian Equine Veterinarian* **13**, 17–18.
- USDA (2003) Aflatoxin handbook. Available at <http://www.gipsa.usda.gov> [Verified 25 November 2007]
- Watson A, Burgess LW, Summerell BA, O'Keeffe K (2006) *Fusarium* spp. associated with ear rot of maize in the Murrumbidgee irrigation area of New South Wales. In 'Water to gold: proceedings of the Maize Association of Australia 6th triennial conference, Griffiths, NSW'. (Eds E Humphreys, K O'Keeffe, N Hutchins, R Gill) pp. 127–130. (Maize Association of Australia: Darlington Point, NSW)
- Williams KC, Blaney BJ (1994) Effect of the mycotoxins, nivalenol and zearalenone, in maize naturally infected with *Fusarium graminearum* on the performance of growing and pregnant pigs. *Australian Journal of Agricultural Research* **45**, 1265–1279. doi:10.1071/AR9941265
- Williams KC, Blaney BJ, Dodman RL, Palmer CL (1992) Assessment for animal feed of maize kernels naturally-infected predominantly with *Fusarium moniliforme* and *Diplodia maydis*. I. Fungal isolations and changes in chemical composition. *Australian Journal of Agricultural Research* **43**, 773–782. doi:10.1071/AR9920773

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