

*Australian Journal of Experimental Agriculture*, 2007, **47**, xx-xx (in press)

## **Risk management for mycotoxin contamination of Australian maize**

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Short title: Mycotoxin risks in maize

*Abstract.* Recent incidents of mycotoxin contamination (particularly aflatoxins and fumonisins) have demonstrated a need for an industry-wide management system to ensure Australian maize meets the requirements of all domestic users and export markets. Results of recent surveys are presented, demonstrating overall good conformity with nationally- accepted industry marketing standards but with occasional samples exceeding these levels. This paper describes mycotoxin-related hazards inherent in the Australian maize production system, and a methodology combining Good Agricultural Practices (GAP) and the Hazard Analysis Critical Control Point (HACCP) framework to manage risk.

### **Introduction**

Mycotoxins are toxic products of secondary metabolism produced by a range of fungi on a wide variety of substrates. Past investigations into Australian maize, as well as data collected by millers and manufacturers, have identified aflatoxins, fumonisins, ochratoxin A, trichothecenes (including nivalenol and deoxynivalenol) and zearalenone in Australian maize (Blaney 1981; Connole *et al.* 1981; Blaney *et al.* 1984; Blaney *et al.* 1986; Blaney 1999; 2004; Blaney *et al.* 2006). This is of concern because of the risk they pose to human and animal health (Pitt & Tomaska 2001; 2002; Council for Agricultural Science & Technology 2003; Whitlow Jnr & Hagler Jnr 2003).

The National Agricultural Commodities Marketing Association (NACMA) has formulated trading standards for aflatoxins and fumonisins in maize, shown in Table 1. While these are not standards enforceable by law, they have been widely accepted by industry and it is to be expected that they will be used in most domestic contracts.

In recent years, the Australian maize crop has experienced a number of cases of mycotoxin contamination causing disruption to maize marketing (Blaney *et al.* 2006). Despite only affecting a small proportion of Australian maize, these incidents have indicated a need for an industry-wide system to ensure Australian maize meets the standards of all domestic users and export markets.

This paper provides preliminary data from a survey of Australian maize produced during 2004-2006 and discusses factors associated with contamination. With this as a basis, we describe mycotoxin-related hazards inherent in the Australian maize production system and propose potential controls for these hazards.

### **Mycotoxin occurrence in Australian maize**

In 2003, industry monitoring identified an outbreak of fumonisin and aflatoxin contamination in maize received for milling (Blaney *et al.* 2006). At this time, although members of the manufacturing sector conducted in-house monitoring, there had been no systematic review of the entire Australian maize crop over a number of seasons. In response to industry concern, we conducted an extensive survey of maize across all growing regions of Australia during 2004, 2005 and 2006.

The detailed results of these surveys will be published separately. Five kilogram samples of shelled maize were requested from growers, seed companies and bulk handlers; with samples received ranging between 500g-20kg. Samples were ground in entirety and subsampled in a Romer Mill. Milled maize was assayed using 2-dimensional thin-layer chromatography for aflatoxins, ochratoxin A and zearalenone (Blaney *et al.* 1984). For fumonisins, milled samples were quantified using HPLC (AOAC International 1998; Shephard 1998) .

Preliminary results for aflatoxins and fumonisins are summarised in Table 2, showing that aflatoxin and fumonisin contamination is widespread across Australian maize growing regions. Aflatoxins were detected in 25% of samples (>0.001 mg/kg), and fumonisins were detected in 66% of samples (>0.1 mg/kg). Nevertheless, over 85% of all samples complied with the NACMA standard for milling grade maize. Ochratoxin A was not detected in any of the samples and zearalenone was detected in only a few, almost entirely in maize originating from the Atherton Tableland region in North Queensland – the only part of Australia where this is common (Blaney *et al.* 2006).

These results indicate that geographic region plays an important part in determining the type of mycotoxin contamination that occurs, probably due to climatic differences. Distribution also appears to be related to a combination of other factors including soil type, humidity, availability of inoculum and season; relationships which will be explored in a future paper.

Aflatoxins appear to be the mycotoxins of most concern in Australian maize (Blaney *et al.* 2006), based on their implications to human and animal health (IARC 1993) and widespread occurrence, but our results indicate that this is mainly for companies supplying the human food and pet food markets that are aiming to meet the

NACMA milling standard of 0.005mg/kg. Fumonisin is of secondary concern, but do require regular monitoring and management owing to their potential carcinogenicity and proven negative health effects in animals (Gelderblom et al. 1988; Diaz & Boermans 1994; IARC 2002; Gelderblom et al. 2004).

### **Mycotoxin- associated risk factors in Australian maize**

Fungi on crops can produce mycotoxins in the field, during handling and in storage. The conditions required for the production of mycotoxins are complex and involve a combination of those favourable to fungal infection, growth and mycotoxin formation. Not all mycotoxins require the same combination of conditions.

#### *Aflatoxins*

In Australian maize, aflatoxins are most often produced by *Aspergillus flavus*. *A. flavus* is able to grow in maize of lower moisture content (16% at 35°C; water activity ~0.8) and at higher temperatures (12 – 43°C; optimum 30°C) than many other fungi found on field crops (Diener & Davis 1987), and for this reason it was originally classified as a ‘storage fungus’. The combination of drought stress and high ambient temperatures has been well established as the primary environmental factor leading to aflatoxin contamination in the growing crop (Trenk & Hartman 1970; Bruns 2003; Munkvold 2003). Although aflatoxin research in maize has mostly been conducted in the USA, our results support similar principles. The critical period for aflatoxin production begins approximately twenty (20) days after anthesis (Bruns 2003) and, if average day/night temperatures exceed 27°C, two conditions are met. Firstly, the natural resistance of the maize plant to fungi in general is compromised; and secondly, the relatively heat-tolerant *A. flavus* has the advantage over other fungi present. At this stage, windblown fungal spores can enter through the silks. These temperatures also fall within the optimum conditions for aflatoxin production (Diener & Davis 1987).

Physical damage to the ear from insects (especially boring insects) or birds also is a critical factor in aflatoxin contamination, since it exposes the endosperm to premature drying and *A. flavus* invasion. Once fungal growth has begun, it can continue until the moisture content of the grain reduces below 14% and so, if environmental conditions do not ensure rapid drying, delaying harvest can increase contamination (Munkvold 2003; Kaaya et al. 2005).

Good agricultural practice (GAP) for managing aflatoxin in growing maize involves selection of planting times to avoid extreme temperatures during the critical period of kernel formation, maintaining irrigation evenly across fields, good nutrition, insect control, early harvest, minimising light-weight material at harvest, and drying to <14% moisture before storage by either pre-harvest natural or post-harvest mechanical means.

Aflatoxins can be an even greater problem in stored maize. At moisture contents even slightly above 14%, temperature fluctuations will cause the smaller amount of

‘available moisture’ to migrate into pockets. If these pockets reach 16% with average temperatures around 35°C, the ‘water activity’ ( $a_w$ ) of maize reaches the minimum of 0.80 at which *A. flavus* can start to grow (Sanchis & Magan 2004). Initially, the fungus will grow in the very small proportion of infected kernels, but this growth releases more moisture from the maize and eventually the fungus will rapidly spread into adjacent sound kernels. This process is accelerated by storage insects. Good agricultural practice for aflatoxin management in stored maize include minimising damaged kernels before storage, either during harvest or gravity grading; using appropriate types of storage – shape of container and grain depth must not restrict air flows; managing night-day air flows as appropriate for ambient temperatures to avoid moisture condensation; and controlling insects with appropriate chemicals.

### *Fumonisin*

Many *Fusarium* species are associated with ear rot and stalk rot in maize. The most common species in Australian maize is *Fusarium verticillioides* (previously called *F. moniliforme*) which is presumed to be the main source of fumonisins (Munkvold & Desjardins 1997). However, *F. proliferatum*, *F. thapsinum* and *F. nygamai* have also been isolated from ear-rotted maize, and are on record as capable of producing fumonisins .

*F. verticillioides* is considered ubiquitous in maize and is systemic in the maize plant but seems to grow rapidly and increase fumonisin concentrations only when the plant is stressed (Munkvold & Desjardins 1997; Jackson & Jablonski 2004) . While drought is a significant factor in fumonisin contamination, the association with very high temperatures is not as strong as with aflatoxin.

Irregular water availability (which can occur at the edges of irrigated fields) can produce sudden contraction and expansion of the pericarp, causing a ‘starburst’ pattern of fine cracks which appears to be associated with increased growth of *F. verticillioides* and production of fumonisins (Munkvold 2003; Jackson & Jablonski 2004). Physical and insect damage to the kernel can also increase fumonisin contamination (Munkvold & Desjardins 1997; Bruns 2003) increasing access to the endosperm. Different maize hybrids could vary in susceptibility to fumonisin, but more research is needed in this area (Jackson & Jablonski 2004). When serious fumonisin contamination does occur, our *ad hoc* analysis has shown that more than 90% can occur in the lightweight fraction and is thus removable by gravity grading. This is supported by Johansson et al. (2006) and Munkvold & Desjardins (1997) although the latter qualify that the method is not completely effective. Because *F. verticillioides* requires a minimum moisture content of 18% and relative humidity of ~95%, fumonisins are unlikely to increase in maize post-harvest.

### *Zearalenone, deoxynivalenol and nivalenol*

In maize, zearalenone, deoxynivalenol (DON) and nivalenol (NIV) are primarily produced by *F. graminearum*, a fungus responsible for causing ear and stalk rots. *F. graminearum* also causes head blight of wheat, and rotating wheat and maize is a common cause of increased infection in both crops if climatic factors suit (Codex Alimentarius Commission 2003). Provided that inoculum is present on crop residues in soil, infection of maize occurs at flowering and is facilitated by cool, wet weather at this time (Blaney 2001). These conditions are uncommon in the main Australian maize-growing regions, although exceptions include parts of the Atherton Tableland area in North Queensland and wet coastal areas like the Northern Rivers district of NSW (Blaney *et al.* 1984; Blaney *et al.* 1986; Blaney *et al.* 2006).

In the main Australian maize production areas, these mycotoxins do not appear to warrant specific controls, but if necessary this could involve reduced stubble retention and avoiding maize-wheat rotation. On the Atherton Tableland in far-north Queensland, effective management involves use of the hybrids specifically developed for disease resistance in that region.

### **Hazards inherent in the Australian maize supply chain**

Some factors increasing risk of contamination such as weather variables are not entirely controllable, although there are good agricultural practices (GAP) that will assist. Other factors such as insect pressure and storage conditions can be controlled. One framework for risk management is the Hazard Analysis Critical Control Point (HACCP) system. Codex Alimentarius, in its *Code of Practice for the Prevention and Reduction of Mycotoxins in Cereals*, identifies mycotoxin related hazards at each stage of cereal production in line with GAP and HACCP principles. A similar framework is used below, describing generic hazards as well as those specific to different Australian regions.

#### *Pre-planting*

Pre-planting planning should include attention to several critical steps in minimising mycotoxin contamination. The first step lies in reducing exposure to infection through reducing the available fungal inoculum. Fungal spores remain dormant in soil from crop to crop and from year to year, present in layers of infected crop residues. Increasing adherence to no-till cultivation aimed at preserving topsoil, can increase soil contamination with fungal spores, requiring a trade off between mycotoxin control and soil conservation.

Rotating crops that share susceptibility to specific fungi increases the availability of inoculum in shared fields. Wheat and maize share a susceptibility to some *Fusarium spp.*, particularly *F. graminearum*. Rotating these two crops increases the availability of inoculum and subsequent zearalenone, NIV and/or DON contamination in these crops, particularly if there is rainfall during anthesis and persistently moist conditions during maturation (Blaney 2001). Such conditions rarely occur in the main grain production

regions of Australia, although they did occur in 1999-2001 at a few localities on the Liverpool Plains of NSW (Southwell *et al.* 2003).

While GAP can reduce the availability of inoculum, it is impossible to eliminate it altogether. Selection of a hybrid adapted for local conditions and suitable for the proposed end-use is a key decision. For example, the Queensland Department of Primary Industries and Fisheries has had a long-term breeding program in North Queensland to develop hybrids tolerant to *Fusarium spp.* infection, and in this region selection of appropriate hybrids may prove to be the most effective way to minimise zearalenone and NIV contamination. While no hybrids are currently available specifically for aflatoxin and fumonisin resistance, hybrids with increased resistance to insect attack and increased drought tolerance could be less susceptible. It has been known for many years that hybrids with long cobs with tight husk cover are more resistant to insect attack than other hybrids and experience less aflatoxin contamination (Bruns 2003). Other varieties are more tolerant to drought and thus experience less stress in dry conditions. In the United States there has been some success in identifying inbred genotypes for aflatoxin resistance, although the majority of these lack traits that make them suitable for commercial purposes (Betrán *et al.* 2002; Betrán & Isakeit 2004). Early maturing hybrids common in the Midwestern corn belt of the USA were trialled in Mississippi to avoid the high temperatures commonly occurring in the grain filling stage in that state, however, these early maturing varieties had looser husks that made cobs susceptible to insect attack and subsequent aflatoxin contamination and the trial was not successful (Betrán & Isakeit 2004).

New techniques in genetic engineering are aimed at improving resistance to toxigenic fungi and their toxins. The first commercially available transgenic variety is Bt (*Bacillus thuringiensis*) corn which has proven partly resistant to aflatoxin contamination through resistance to certain boring insects (Hammond *et al.* 2004; Munkvold & Muntzen 2004; Williams *et al.* 2005). The Australian maize industry's voluntary genetically modified organism (GMO) - free policy means that genetically engineered hybrids are not currently available to Australian producers and, given that early maturing hybrids have proven ineffective in climatic conditions similar to Australia's in the USA, GAP will remain the only option to minimise aflatoxin contamination in the near future.

### *Planting*

Timing planting dates to avoid high temperatures and/or drought stress during the period of kernel development and maturation could be an important precaution in the prevention of both aflatoxin and fumonisin contamination. The Queensland Department of Primary Industries & Fisheries is using computer modelling to assist growers to schedule planting and harvesting dates by predicting potential aflatoxin contamination in maize based on existing and historical climatic conditions (Chauhan *et al.* 2006).

### *Pre-harvest/ growing*

Australia's climate poses specific challenges in terms of mycotoxin control. Many maize growing areas of Australia, including the Murrumbidgee Irrigation Area (MIA), central west of NSW and Central Queensland can experience high temperatures and low precipitation during the maize growing season. Maize crops in these areas are irrigated but aflatoxin problems still occur occasionally in parts of crops if irrigation is uneven or if soil is shallow in spots due to field levelling for flood irrigation. The risk increases if crops are planted in December, when the developing ear can be exposed to very high January/February temperatures, often exceeding 35°C.

Although less often subject to such high temperatures, crops in the Central Burnett, South Burnett and Darling Downs in Queensland are often rain-fed (Robertson *et al.* 2003) and have regularly suffered stress over the last ten seasons. Surveys indicate more frequent aflatoxin contamination in these areas, particularly in the central Burnett. Our data indicate aflatoxin contamination of grain produced in these areas is more common than elsewhere. Data from modelling also show that in some regions during summer, even full irrigation may not provide sufficient water to the growing ear to combat the extreme evaporation rates from high temperature and dry winds (Chauhan *et al.* 2006). When sufficient irrigation is not available and long term climate predictions indicate below average rainfall, maize may not be an appropriate crop and producers should consider alternatives.

The conditions in north-eastern NSW and the southern Darling Downs in south-east Qld are more moderate in terms of temperature and rainfall, and aflatoxin contamination is rarely a problem. Less data exist for fumonisins in these areas but our surveys show no more contamination than in other regions. As the climate becomes cooler and moister, for example in proximity to the QLD-NSW border ranges, conditions become more conducive for growth of the mould that produces zearalenone, nivalenol and deoxynivalenol, *F. graminearum*, but even so, significant contamination of crops is quite unusual.

As previously noted, parts of the north Queensland tablelands feature a cool, persistently wet climate during maize silking and maturation, and zearalenone and nivalenol contamination can be common. Genetic variations and distribution of *F. graminearum* isolates mean that while both areas experience zearalenone contamination, nivalenol tends to occur in northern Queensland and deoxynivalenol in southern Queensland. In this region, aflatoxin occurs only rarely in maize, and is limited to the hotter, drier parts, such as the Mareeba tableland, although further study is warranted as maize production is extending into the hot, wet lowlands of this region.

Australian maize does not seem to experience the amount of insect damage common in parts of the USA. The predominant insect pest in Australian pre-harvest maize is the ear worm, *Helicoverpa armigera* (Hübner) (Murray & Miles 2003). Eggs of this species are common on maize during silking and the larvae develop in the cob, leaving the kernels susceptible to fungal invasion. Treating infestations of this species in growing maize is difficult owing to the difficulty in reaching the target through large canopies (O'Keeffe 2006). Another pest known to affect Australian maize is common armyworm, *Mythimna convecta* Walker (Lepidoptera: Noctuidae) (Hardwick 2006). In Australia, mycotoxin contamination appears to be more related to climate than to insect

attack, with incidents of medium to high contamination occurring in undamaged grain, but more investigation is certainly warranted. One study in northern Queensland did not indicate increased zearalenone in maize infected with *F. graminearum* as a result of severe insect damage (*Spodoptera* sp.) (Blaney *et al.* 1986). Control of insect pests should be approached using Integrated Pest Management (IPM) programs which are available from local agricultural advisors.

### *Harvest*

Mycotoxin production during the actual harvest operation is unlikely, unless the process is interrupted and prolonged by rain, but mechanical harvesters can cause damage to kernels and leave them more vulnerable to fungal invasion. Contamination with soil-borne spores and damage to kernels may make mycotoxin formation more likely during storage.

Mechanical damage is more likely to occur when grain is insufficiently dried before harvest, an uncommon situation in Australia, where it is more common to allow grain to dry to storage conditions before harvest. However, over-drying maize can lead to the kernel becoming brittle and susceptible to damage (Munkvold 2003).

Another hazard is unexpected precipitation or high humidity during harvest. If these conditions are forecast or expected to occur around harvest, early harvest should be considered. The most critical factor during harvest is accurate determination of moisture content, and ensuring that the entire crop meets desired moisture targets. Removal of trash and weeds is also very important, as admixture will compromise air flows in storage.

### *Storage*

The factors conducive to fungal growth during storage are primarily related to the amount of inoculum present, temperature, relative humidity, moisture content and insect activity. Fungal infection usually occurs prior to harvest, but can also occur from dormant fungal spores present in grain dust residues in storage silos, which can also be transported through grain by insects or rodents.

Mycotoxin production in storage is also governed by moisture content and temperature. While fumonisin, zearalenone, DON and nivalenol are predominantly pre-harvest problems in Australia, aflatoxin can be both a pre-harvest and post-harvest problem. Avoiding aflatoxin production in storage involves ensuring that the water activity of the maize is kept below 0.70, which corresponds to 14% moisture at 30°C (DPI&F 2005a).

The climate in major Australian grain production regions means that elevated temperatures (>30°C) in storage are routinely experienced, making the moisture content of stored grain critical. Even if the moisture content is in the range of 14-15%, at 30°C moisture migration and accumulation due to temperature differentials at the grain surface can easily provide pockets of maize with 16-18% moisture, favouring rapid growth of



*Aspergillus* species and aflatoxin (and ochratoxin) production. Conversely, maize stored (and maintained) at 10 - 20°C is very unlikely to support significant aflatoxin production. Good aeration is essential when ambient temperatures are high, but is only effective when the external air has a relative humidity <80% and temperature of <20°C (Shapira 2004). For this reason aeration is usually best carried out at night.

Insects also play a role in rendering stored maize susceptible to fungal invasion. There are five major insect pests of stored cereal grain in Australia; moths (Angoumois, Tropical warehouse and Indian moths), weevils (*Sitophilus spp.*), the lesser grain borer (*Rhyzopertha dominica*), flour beetles (*Tribolium castaneum*), the saw-toothed grain beetle (*Oryzaephilus surinamensis*) and flat grain beetles (*Cryptolestes spp.*) (DPI&F 2004). Moths and the sawtooth grain beetle multiply rapidly at temperatures between 30-35°C and humidities between 75-80% (DPI&F 2004). Controlling temperature and humidity with aeration not only reduces mould growth, and thus mycotoxin production, but also insect populations.

The most effective and widely accepted method of control of insect invasion is prevention, through using airtight storage, hygiene, aeration, controlled atmosphere and drying. Market restrictions and grain-specific chemical registrations limit other pest control options. Carbaryl can be used a protective treatment for grain to be used on-farm or in feed grain but residues are not accepted in grain intended for human consumption. Phosphine fumigation is accepted in cereals by all markets; dichlorvos and other residual pesticides are only acceptable to non-restricted markets. With pest species becoming resistant to commonly used organophosphate chemicals, alternative chemical registrations for use in grain are expected in the future (DPI&F 2005b).

### *Transport and export*

The hazards associated with mycotoxin production during transport and export are effectively the same as those occurring in stored grain. Maize should be sound and as free as possible of lightweight grain, cracked grain and contaminants. Ensure that only food grade containers are used, and that they are clean and free of grain residues and dust, which can be heavily contaminated with fungal spores. Once these prior conditions are met, the primary reason for fungal growth and mycotoxin production during transport is moisture migration and accumulation within sealed containers. These containers are often held at tropical summer temperatures for several weeks, which can cause condensation to form on the grain.

Acceptable moisture content for maize decreases as ambient temperature increases. At 40°C, the water activity ( $A_w$ ) of maize with 14% moisture rises to 0.75, and at 50°C to 0.8 (the minimum for *A. flavus* growth), so maize that might be subject to such temperatures during transport should be dried to 12 – 13% moisture. During export, the risks can be minimised by ensuring shipping containers are placed on lower decks to avoid temperature fluctuations and including moisture absorbing materials in containers during transport. Commercial products are available for this purpose, based on silica gel or diatomaceous earths.

In response to this issue, a protocol for managing mycotoxins in maize intended for export has been compiled and is being promoted by the Maize Association of Australia for wide adoption across the industry (Blaney et al. 2007).

### **An Australian risk-based mycotoxin management system**

Mycotoxins cannot be easily eliminated from grain once contamination has occurred. It can be difficult to predict when contamination will occur and when it does, mycotoxins can be distributed extremely irregularly, both in maize growing in the field and in stored maize. If not detected before reaching the end-use, the costs can be very high in terms of rejected product, trade embargos and product recalls.

There are two approaches to deal with this problem. Firstly, we can assume that contamination is beyond our control and perform multiple mycotoxin tests on each load of maize at harvest, each load sold from storage, and in each batch of final product. Alternatively, we can apply a quality control system at all stages of production, transport and storage, to minimise contamination, and limit mycotoxin tests to the occasional confirmatory assay.

A quality control system incorporates many of the specific measures already in place in most well-run maize growing, processing, transport, storage and marketing operations, particularly with respect to moisture control and storage. A formal quality control system includes appropriate documentation assuring that maize has been subject to appropriate care throughout its history. Although most stakeholders try to maintain a good quality product, without documentation there is no way to assure a purchaser that good practice has been followed and that the risk of contamination is therefore low.

The Food and Agriculture Organisation of the United Nations has published a manual on the application of the HACCP system in mycotoxin prevention and control (FAO 2001), but the case studies and examples in that document relevant to maize are for conditions in South East Asia rather than Australia. The risk factors for maize grown under Australian conditions are in many cases different to those described in these examples. Environmental parameters are critical in mycotoxin production and Australian conditions also significantly vary from those in the major maize growing centres of the USA and Canada.

In the northern states of the US and in Canada, maize is often harvested at higher moisture contents. In the lower ambient temperatures of these northern latitudes this does not present a significant problem (Abbas *et al.* 2002), but in Australia this would lead to a high risk of aflatoxin contamination occurring during storage owing to high ambient temperatures in storage. In South East Asia, high relative humidity means maize is harvested at high moisture content and dried post-harvest prior to storage. The major Australian maize growing areas are more subject to low relative humidities, making pre-harvest drying the normal procedure.

In response to the identified hazard of mycotoxins in Australian maize and the lack of a suitable management tool adapted to Australian conditions, we have developed

a guide book for Australian maize producers applying the principles in the Codex Alimentarius Code of Practice for minimising mycotoxins in cereals of GAP and combine them with HACCP principles of quality control. The guide acknowledges the fact that the grower has the best understanding of their own process/production line. Consequently, we have not prescribed a specific detailed plan, but instead a process to assist operators to develop their own plan, using examples specific to Australian conditions and the maize industry. An example of hazards identified in a fictional Australian maize producing operation is provided in Table 3.

In the guidebook, once the grower has identified hazards in their operation, they are guided through the process of identifying appropriate control measures. These control measures are then designated to be either GAPs or HACCP critical controls. Examples of GAPs are given in Table 4. For those controls considered critical, the grower is directed through the process of defining critical limits; and developing a monitoring programme for critical control points. An example of the resultant HACCP plan is shown in Table 5.

## **Conclusion**

Our survey results indicate that, while mycotoxins are often present at low levels, in general Australian maize is of good quality. Aflatoxin is the mycotoxin of greatest concern, primarily to manufacturers of human food products and pet food. Despite this, with the worldwide move toward total quality control and risk management, it is to the maize industry's benefit to manage mycotoxin contamination during production, rather than rely on industry and/or regulatory standards that apply to the end product. While it is not possible to eliminate mycotoxin contamination, it is possible to minimise contamination by using effective risk management strategies.

## **Acknowledgements**

We thank Stephen Were; Warwick Turner; Dennis Webber; Kerrin Morrissy; Madeleine Modina; the project team and steering group- Wayne Bryden, Lester Burgess, Yash Chauhan, Glen Fox, Neil Gannon, Matthew George, John Kopinski, David Lobwein, Ian Martin, Nick Maynard, Rod McNab, Sally-ann Murray, RCN Rachaputi, Robin Reid, Malcolm Ryley, Graeme Smith, Brett Summerell, Mike Taverner, Andrew Watson, Stephen Wilson, Stephen Were and Graeme Wright and Teresa Miklaszewicz. Thanks must also go to the growers, agents, seed companies and millers who provided samples for analysis. The Grains Research & Development Agency provided financial support.

## **References**

Abbas HK, Williams WP, Windham GL, Pringle HC, Xie W, Shier WT (2002) Aflatoxin and fumonisin contamination of commercial corn (*Zea mays*) hybrids in Mississippi. *Journal of Agricultural and Food Chemistry* **50**, 5246-5254.

- AOAC International (1998) 'Official Methods of Analysis of AOAC International Volume II.' (AOAC International: Gaithersburg, USA)
- Betrán FJ, Isakeit T (2004) Aflatoxin accumulation in maize hybrids of different maturities. *Agromony Journal* **96**, 565-570.
- Betrán FJ, Isakeit T, Odvody G (2002) Aflatoxin accumulation of white and yellow maize inbreds in diallel crosses. *Crop Science* **42**, 1894–1901.
- 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 (1999) 'Report of a limited survey of maize, grown in southern Queensland during 1997 and 1998, for fumonisin and other mycotoxins (unpublished report).' Department of Primary Industries.
- Blaney BJ (2001) Recent research on mycotoxins in Australia: chemistry, analytical methods and prevalence. Paper presented to '18th Conference of Residue Chemists'. Canberra, Australia
- Blaney BJ (2004) Managing mycotoxins in maize. In 'The Cob'.
- Blaney BJ, Bricknell LK, O'Keeffe K (2006) Managing mycotoxins in maize: case studies. In "'Water to Gold"- 6th triennial conference of the Maize Association of Australia'. Griffith. (Maize Association of Australia)
- Blaney BJ, Moore CJ, Tyler AL (1984) Mycotoxins and fungal damage in maize harvested during 1982 in far north Queensland. *Australian Journal of Agricultural Research* **35**, 463-471.
- 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**, 235-44.
- Bruns HA (2003) Controlling Aflatoxin and Fumonisin in Maize by Crop Management. *Toxin Reviews* **22**, 153- 173.
- Chauhan Y, Wright G, Rachaputi N (2006) Modelling mycotoxin contamination in maize. In "'Water to Gold" 6th triennial conference of the Maize Association of Australia". Griffith, Australia. (Maize Association of Australia)
- Codex Alimentarius Commission 2003, *Code of practice for the prevention and reduction of mycotoxin contamination in cereals, including annexes on ochratoxin A, zearalenone, fumonisins and tricothecenes*, FAO/ WHO Food Standards, viewed 6 January 2005, <[http://www.codexalimentarius.net/download/standards/406/CXC\\_051e.pdf](http://www.codexalimentarius.net/download/standards/406/CXC_051e.pdf)>.
- Connole MD, Blaney BJ, McEwan T (1981) Mycotoxins in animal feeds and toxic fungi in Queensland 1971-80. *Australian Veterinary Journal* **57**, 314-318.

- Council for Agricultural Science & Technology (2003) 'Mycotoxins: Risks in plant, animal, and human systems.' Council for Agricultural Science & Technology, 139, Ames, Iowa, USA.
- Diaz GJ, Boermans HJ (1994) Fumonisin toxicosis in domestic animals: a review. *Vet. Human Toxicol.* **36**, 548-555.
- Diener UL, Davis ND (1987) Biology of *Aspergillus flavus* and *A. parasiticus*. In 'Aflatoxins in Maize'. (Eds MS Zuber, EB Lillehoj and BL Renfro) pp. 33-40. (CIMMYT: Mexico)
- DPI&F 2004, *Grain insect pests*, The State of Queensland, viewed 2 April 2006, <<http://www2.dpi.qld.gov.au/fieldcrops/16418.html>>.
- DPI&F 2005a, *Grain storage- basic design principles*, viewed 2 April 2006, <<http://www2.dpi.qld.gov.au/fieldcrops/15052.html>>.
- DPI&F 2005b, *Grain storage - insect control in stored grain*, The State of Queensland (Department of Primary Industries & Fisheries), viewed 10 April 2006, <<http://www2.dpi.qld.gov.au/fieldcrops/3448.html>>.
- FAO (2001) 'Manual on the Application of the HACCP System in Mycotoxin Prevention and Control.' (FAO/IAEA Training and Reference Centre for Food and Pesticide Control.: Rome)
- Gelderblom WCA, Jaskiewicz K, Marasas WFO, Thiel PG, Horak RM, Vlegaar R, Kreik NPJ (1988) Fumonisin: novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl Environ Microbiology* **54**, 1806-1811.
- Gelderblom WCA, Rhadeer JP, Leggott N, Stockenstrom S, Humphreys J, Shephard GS, Marasas WFO (2004) Fumonisin contamination of a corn sample associated with the induction of hepatocarcinogenesis in rats—role of dietary deficiencies. *Food and Chemical Toxicology* **42**, 471-479.
- Hammond BG, Campbell KW, *et al.* (2004) Lower fumonisin mycotoxin levels in the grain of Bt corn grown in the United States in 2000-2002. *Journal of Agricultural and Food Chemistry* **52**, 1390-1397.
- Hardwick S (2006) Parasitoids and hyperparasitoids of *Mythimna convecta* (Walker) (Lepidoptera: Noctuidae) larvae infesting late-maturing maize in southern New South Wales. *Australian Journal of Entomology* **45**, 96-100.
- IARC 1993, *Aflatoxins (IARC Summary & Evaluation, Volume 56, 1993)*, IPCS INCHEM, viewed 23 December 2004, <<http://www.inchem.org/documents/iarc/vol56/09-afl.html>>.
- IARC 2002, *Fumonisin B1 (IARC Summary & Evaluation, Volume 82, 2002)*, IPCS INCHEM, viewed 23 December 2004, <<http://www.inchem.org/documents/iarc/vol82/82-05.html>>.

- Jackson L, Jablonski J (2004) Fumonisin. In 'Mycotoxins in food: detection and control'. (Eds N Magan and M Olsen) pp. 367-405. (Woodhead Publishing Ltd: Cambridge)
- Kaaya AN, Warren HL, Kyamanywa S, Kyamuhangire W (2005) The effect of delayed harvest on moisture content, insect damage, moulds and aflatoxin contamination of maize in Mayuge district of Uganda. *Journal of the Science of Food and Agriculture* **85**, 2595–2599.
- Munkvold G (2003) Cultural and genetic approaches to managing mycotoxins in maize. *Annual Review of Phytopathology* **41**, 99-116.
- Munkvold G, Desjardins A (1997) Fumonisin in maize: can we reduce their occurrence? *Plant Disease* **81**, 556-565.
- Munkvold G, Muntzen S (2004) Mycotoxins in Corn Grain and Effect of Bt Hybrids. *Crop Insights* **14**.
- Murray D, Miles M (2003) Insect pest management in maize. In "'Versatile Maize-Golden Opportunities", 5th Australian Maize Conference'. Toowoomba, Australia. (Eds C Birch and S Wilson) pp. 94-97. (Maize Association of Australia)
- O'Keeffe K 2006, pers. comm. to LK Bricknell, 23 November 2006.
- Pitt JI, Tomaska L (2001) Are mycotoxins a health hazard in Australia? 1. Aflatoxins and Fusarium toxins. *Food Aust*, **53**, 535-9.
- Pitt JI, Tomaska L (2002) Are mycotoxins a health hazard in Australia? 2. Ochratoxin A. *Food Aust*, **54**, 39-43.
- Robertson M, Cawthray S, Birch C, Bidstrup R, Crawford M, Dalglish N, Hammer G (2003) Managing the risks of growing dryland maize in the northern region. Paper presented to '5th Australian Maize Conference'. Toowoomba, Australia. (Maize Association of Australia)
- Sanchis V, Magan N (2004) Environmental conditions affecting mycotoxins. In 'Mycotoxins in food: detection and control'. (Eds N Magan and M Olsen) pp. 174-189. (Woodhead Publishing Limited: Cambridge)
- Shapira R (2004) Control of mycotoxins in storage and techniques for their decontamination. In 'Mycotoxins in food: detection and control'. (Eds N Magan and M Olsen) pp. 190-223. (Woodhead Publishing Ltd: Cambridge)
- Shephard GS (1998) Chromatographic determination of the fumonisin mycotoxins. *Journal of Chromatography A* **815**, 31-39.
- Southwell R, Moore K, Manning W, Hayman P (2003) An outbreak of Fusarium head blight of durum wheat on the Liverpool Plains in northern New South Wales in 1999. *Australasian Plant Pathology* **32**, 465-471.
- Trenk HL, Hartman PA (1970) Effects of moisture content and temperature in mycotoxin production in corn. *Applied Microbiology* **19**, 781-784.

Whitlow Jnr LW, Hagler Jnr WM (2003) Mycotoxins in feeds. *Feedstuffs* **75**, 70-81.

Williams WP, Windham GL, Buckley PM, Perkins JM (2005) Southwestern corn borer damage and aflatoxin accumulation in conventional and transgenic corn hybrids. *Field Crops Research* **91**, 329-336.

**Table 1 National Association of Commodity Marketing Agencies trading standards for mycotoxins in maize**

Mycotoxin (mg/kg)	Milling	Prime	Feed #1	Feed #2
Total aflatoxins	0.005	0.015	0.02	0.08 (0.02 B <sub>1</sub> )
Total fumonisins	2	5	10	40

**Table 2 Aflatoxins and fumonisins detected in maize samples collected in 2004, 2005 and 2006, and compliance of samples with NACMA standards by region (n)**

Region	n	Fumonisin							Aflatoxins				
		+ve <sup>1</sup>	Milling	Prime	Feed#1	Feed#2	Exceeds	+ve <sup>2</sup>	Milling	Prime	Feed#1	Feed#2	Exceeds
Far North Qld	41	28	37	3	0	1	0	1	41	0	0	0	0
Central Highlands, Qld	50	25	50	0	0	0	0	46	8	15	0	3	24
Burnett, Qld	168	71	161	5	2	0	0	60	141	13	1	4	9
Darling Downs, Qld	146	117	139	7	0	0	0	11	143	3	0	0	0
Mid-NSW	79	68	66	6	1	5	1	8	75	2	0	2	0
MIA, NSW	73	62	57	8	1	5	2	15	68	3	0	0	2
Victoria	5	4	5	0	0	0	0	0	5	0	0	0	0
Western Aust	5	2	2	0	0	0	0	1	2	0	0	0	0

<sup>1</sup>Level of reporting >0.1mg/kg

<sup>2</sup>Level of reporting >0.001mg/kg



**Table 3 Mycotoxin-related hazards in the maize supply chain**

<i>Step</i>	<i>Hazard</i>
Purchase seed grain	Hybrid unsuitable for local conditions
	Hybrid unsuitable for planned market
	Hybrid unsuitable for expected planting window
	Hybrid susceptible to local diseases (eg. hybrid susceptible to <i>F. graminearum</i> for planting on the Atherton Tableland)
Soil preparation	Soil contaminated with excessive <i>F. graminearum</i> inoculum from previous wheat crop
	Soil contaminated with excessive <i>A. flavus</i> inoculum from trash of previous crop
	Soil of uneven depth or moisture holding capacity due to field levelling over different soil types or rocky outcrops.
Planting	Planting time could expose developing kernels to high temperatures & low precipitation at anthesis and the following 20 days
Pre-harvest/ Growing	Low soil moisture leading to plant stress during kernel development
	Insufficient soil nutrients leading to plant stress during kernel development
	Insect attack leading to damaged kernels
Harvest	Damage to ears during mechanical cultivation or from birds
	Damage to kernels from harvester
	Kernels insufficiently dried and susceptible to damage
	Rainfall or high humidity around harvest risks high moisture
Storage	Moisture content of kernels excessive
	Insect attack, allowing fungi to penetrate kernel
	Insufficient aeration, allowing moisture migration and fungal growth
	Storage container contaminated with old grain residues containing high concentrations of fungal spores

**Table 4 Good Agricultural Practices to minimise mycotoxin contamination in maize**

<i>Step in process</i>	<i>Hazard</i>	<i>Good Agricultural Practice</i>
Purchase seed grain	Hybrid unsuitable for local conditions	Select seed in accordance with advice from reputable seed dealer
	Hybrid unsuitable for planned market	
	Hybrid unsuitable for expected planting window	
	Hybrid susceptible to local diseases	
Soil preparation	Soil contaminated with excessive <i>F. graminearum</i> inoculum from previous wheat crop	Avoid rotating wheat and maize crops in susceptible areas
	Soil contaminated with excessive <i>A.flavus</i> inoculum from trash of previous crops	Plough trash into soil
	Soil of uneven depth or moisture holding capacity due to field levelling over different soil types or rocky outcrops	Prepare maps of fields showing shallow areas, that can be monitored for stress using infra-red photography and harvested separately
Planting	Planting time could expose developing kernels to high temperatures & low precipitation during kernel development	Avoid planting times which will lead to the period of anthesis and the following 20 days occurring in periods of very hot weather.
Harvest	Rainfall or high humidity around harvest	Check weather reports and harvest earlier if necessary
	Damage to kernels from harvester	Dry maize in field to 14% moisture before harvest
Storage	Storage container contaminated old grain residues containing high concentrations of fungal spores	Decontaminate container before storage

**Table 5 Example of a possible HACCP plan for minimising mycotoxin contamination in maize**

<i>Step/ CCP</i>	<i>Hazard Analysis</i>			<i>Monitoring</i>		<i>Corrective action</i>
	<i>Hazard</i>	<i>Control</i>	<i>Critical Limit</i>	<i>Monitoring</i>	<i>Frequency</i>	
Pre-harvest/ Growing	Low soil moisture leading to plant stress during kernel development	Available soil moisture	Lower limit of critical $A_w$ (check with local agronomist for an exact value)	Measure soil moisture and record	Weekly	Irrigate; record amounts
	Insufficient soil nutrients leading to plant stress during kernel development	Available soil nutrients	Soil N, P & K as recommended for hybrid by local agronomists (insert the values)	Fertiliser applied (appropriate for soil type and hybrid); amounts and type recorded	As recommended for hybrid	Add fertilizer; record amount
	Insect attack leading to damaged kernels	Integrated pest management (IPM) plan	Insect population within acceptable limits as determined by control program	Inspect for insects and record results	Weekly	Apply pesticide in accordance with IPM
Storage	Moisture content of kernels excessive	Kernel moisture content at point of storage	Moisture content $\leq 14\%$	Measure and record grain moisture	Immediately prior to storage	Dry mechanically
	Insect attack, allowing fungi to penetrate kernel	IPM plan	No evidence of insect or rodent infestation using inspection protocols specified in IPM plan	Inspect for pests and record results	Weekly	Control pests in accordance with IPM
	High ambient humidity and temperature	Aeration	Temperature of air intake $< 20^\circ\text{C}^1$ Humidity of air intake $< 80\%^1$	Measure and record humidity, ambient temperature and airflow	Daily during storage	Adjust aeration- time of day or airflow

<sup>1</sup> Shapira (2004)