GM CO-EXISTENCE FOR MAIZE

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Abstract

With the introduction of GM varieties into a crop production and supply system comes the risk that, through gene flow or co-mingling, that there is potential for some products in that supply chain to be disadvantaged in the market place if there is price or market access discrimination. If an industry such as the Australian maize industry is to access the benefits of new plant technologies and at the same time avoid losses due to compromised markets, then strict programs need to be implemented to manage product integrity.

In Australia, the maize industry is entirely based on non-GM cultivars, but overseas there is a range of new traits being introduced into maize and other crop species that potentially represent new market opportunities – insect tolerance traits, herbicide tolerance traits, nutritional traits and traits that can act as the basis for new industrial and pharmaceutical industries.

If Australia is to avail itself of these new technologies then it faces, for each crop, a formidable task to achieve the regulatory freedom to operate, a freedom that will be based upon the industry’s capacity to implement practical and economic co-existing production strategies that enable segregated supply chains to operate within defined tolerances for adventitious presence of specified materials such as genetically modified products.

Introduction

In the context of this discussion co-existence is usefully defined in a recent policy document issued by the Queensland Government (2005) as “…the ability to grow and manage along the supply chain both genetically modified (GM) and traditional (grown either conventionally or organically) crops in a way that avoids unwanted mixing and delivers products at predetermined market specifications”. Seed producers and others will be familiar with the broad requirements of coexisting supply chains that manage other issues such as varietal seed purity and specialist crops.

Before examining the detailed processes of co-existence management, it is necessary to take a wider view of issues that would need to be addressed if the commercial cultivation of genetically modified maize is to occur at some point in this country. There are four parallel paths of development that are critical if the Australian maize industry is to achieve freedom to operate status with genetically modified maize.

Involvement of a commercial seed supplier

Any move to establish genetically modified maize as a commercial crop will require one or more seed companies to be willing to invest in variety development and distribution. This would appear to pose an immediate and significant limitation, as without a supply of suitable genetically modified cultivars for use in at least the major production environments the industry cannot progress down this path.

It is noted that no work is currently underway to introduce genetic events appropriate for our maize production systems into local high performing germplasm. Since 2000 there have not been, according to the Office of the Gene Technology Regulator (2005), any applications for restricted or unrestricted releases of genetically modified maize into the environment in Australia.

Given that it is highly unlikely that cultivars with desired GM traits and capable of high performance in our environment would be identified off shore, this only leaves the industry with one way ahead - seeking to convince a seed company that has access to GM events wanted by the local maize industry to invest in introducing them into a range of elite local germplasm. Should this occur, it would then commence a process that required significant investment in research and development to incorporate these events and to undertake the field testing to identify the elite parents that would then form the basis of that new GM cultivar in Australia.
This process will take time – probably in excess of four years. The strategic weakness in this critical path is the size of the Australian maize seed industry, which may not be of a scale that warrants the level of investment needed.

**Commonwealth licensing**

*Licence for intended release into the environment*

All dealings with genetically modified organisms in Australia come under the jurisdiction of the Gene Technology Regulator, who administers the Gene Technology Act 2000 (Cmth). Within that regulatory environment is the requirement that genetically modified organisms may only be legally released into the environment if licensed for that purpose by the Regulator. Licensing involves a stringent assessment process that evaluates whether the release of the genetically modified organism poses any greater risk to human health and to the environment than conventional cultivars.

Such applications for a licence are made by the technology owner, typically a seed company or plant breeding institute, and require the provision of an extensive technical case for the Regulator’s critical examination.

The assessment of a licence application requires mandated public consultation and may take nine months or more for a restricted licence, longer for an unrestricted licence.

*Licence for use in food*

The previous licensing process does not extend to approval to use the organism or its products for human consumption - that is a separate approval, granted after appropriate assessment, by Food Standards Australia New Zealand under sec 1.5.2 of the Food Standards Code. In the case of maize, the major GM events in use overseas have already been granted approval.

To seek freedom to operate with maize cultivars containing any other genetic events than those listed in the Food Standards Code would require an application to be made to FSANZ seeking approval. This assessment process also has several mandated stages and may take in excess of a year.

**Compliance with state regulations and policies**

*New South Wales legislation*

NSW has, under the Gene Technology (GM Crop Moratorium) Act 2003, the capacity to declare a moratorium on the cultivation of any genetically modified food plant, but to date only genetically modified canola has been the subject of such an order, an arrangement that currently stands until March 2008.

While research and development into the incorporation of the GM events into local elite maize germplasm could proceed at laboratory level with only Commonwealth approval, the subsequent establishment of research and development field trials would appear to require a case to be successfully made to the NSW Minister for Agriculture to not implement a Moratorium Order for genetically modified maize under the Gene Technology (GM Crop Moratorium) Act 2003 (NSW).

*Queensland co-existence policy*

Queensland does not have legislation regulating the cultivation of GM crops, but the Queensland Government (2005) policy document on co-existence stands as a clear statement of how that Government expects the development of GM cropping industries to proceed. This framework is established on six principles that:

- Offer freedom of choice to farmers, supply chain participants and consumers;
- Are transparent, and enable consultation;
- Are based on science and practical process management;
- Minimise impacts on others;
- Can be assessed on a case-by-case basis; and
- Can be monitored and reviewed.
With appropriate isolation precautions, field trials of GM maize would appear able to proceed in Queensland if a Commonwealth licence were to be granted for release into the environment.

**Supply chain agreement on co-existence arrangements**

*General principles of co-existence management*

The introduction of a GM production stream into an industry such as the maize industry would see the development of three categories of supply chain:

- Undifferentiated markets that do not discriminate between GM and non-GM product, and which accept unrestricted co-mingling of these products;
- Non-GM markets which accept co-mingling with GM product up to a specified threshold. This is frequently the legislated GM threshold above which labeling of food will be required to reflect its GM content; and
- Non-GM markets wanting a near zero level of GM product, guaranteed through identity preservation systems. Maize produced by organic systems is included in this category as well. Due to sampling variability, it is not technically possible to give a 100% assurance that a grain lot is 100% non-GM. Instead, a standard is used that gives a high (but not absolute) confidence that no GM was detected by a nominated analytical process. Current PCR analytical technology can detect the presence of known GM events down to levels of approximately 0.01% in grains.

Co-existence will require the development of agreed thresholds for the adventitious presence of GM traits at a few key points along non-GM supply chains, and the development of risk management strategies to enable these thresholds to be routinely met. These adventitious thresholds may be set by regulation, by industry standard or by contract. The risk management strategies, whose primary purpose is to keep adventitious presence of GM material in non-GM supply lines below the defined threshold levels, become translated into management protocols that are communicated to those in the supply chain that need to know and apply them.

At the farm level, adventitious GM levels in non-GM production arise through:

- GM seeds in sowing seed arising from cross-pollination or physical admixture in the seed supply;
- GM seeds present in seeding equipment;
- GM volunteer plants from previous crop rotations germinating in the non-GM crop;
- GM pollen ingress from neighboring GM crops;
- GM pollen ingress from feral GM plants in the neighborhood;
- GM seed present in harvesting, transport and storage equipment.

*Issues relating to maize*

Maize is a strongly out-crossing (circa 95%), wind-pollinated species (Glover 2002). It has relatively heavy pollen (Brookes et al. 2004) that, while dispersed by wind, tends to fall to ground rapidly over a relatively limited area. Separation of synchronously flowering GM and non-GM maize fields by relatively short distances of 20 to 50 meters typically results in very little pollen mediated gene flow, and the penetration of pollen into neighboring maize crops at those distances is largely limited to the near-edge rows, so that the average GM content across the whole non-GM field is usually well below 1%.

Maize does not shed seed naturally and has a limited dormancy period, so that the presence of volunteer GM maize plants after seed bed preparation for a subsequent non-GM maize crop is minimal.

Despite the commercial and regulatory challenges that face the introduction of any genetically modified crop, particularly food crops, it is encouraging that the management of coexisting GM and non-GM maize has already been addressed elsewhere with very encouraging results that would appear to be at least partly transferable to Australia.
Co-existence of GM maize in Spain

Maize is a major summer crop in Spain (April/May sowing, October harvest), where nearly 500 000ha are sown annually, mostly under irrigation. About 20% of this is ensiled, with the remainder producing about 4.2 Mt of grain. An additional 3 Mt of maize is also imported annually from France, USA and Argentina. About 90% of locally produced maize is used for animal feed. Wet and dry milling occurs near major ports, and uses a significant quantity of imported maize. The regions of Catalunya and neighboring Aragon produce together about 26% of the Spanish maize crop. For largely climatic reasons, these two regions are the areas most infested by two species of the corn borer caterpillar, making the use of Bt cultivars attractive to the local industry.

Following EU approval for a limited range of GM maize varieties in 1998 (prior to the moratorium on granting approvals for new GM crops established by the EU Council of Ministers in 1999), Bt varieties have been made available in limited quantities to be grown commercially under industry-based co-existence arrangements in Spain, and now comprise nearly 15% of the area sown to maize. Spain, as an EU member, complies with EC Regulations 1829/2003 and 1830/2003 which prescribe that food and feed products that contain more than 0.9% genetically modified organisms must be labeled accordingly.

During this period it has been demonstrated that co-existence is possible under Spanish circumstances (Brookes and Barfoot 2004), and this has been increasingly substantiated by monitoring programs (Alcalde and Bachmann 2005), by field research into gene flow (Melé et al. 2005), and by modeling of gene flow at landscape level using tools such as MAPOD® (Angevin et al. 2001). It is now accepted in the EU that it is technically possible for GM, non-GM and organic maize to be produced in co-existence as long as appropriate practical management measures are in place (Messéan et al. 2005).

In Spain during this period all GM maize was sold through normal marketing channels to the livestock feed industry, an industry that was already an importer/user of GM soybean. There was no segregation of GM and non-GM maize, as the food and starch industry (and the small sector of the feed industry) that used non-GM maize was able to source this readily from those regions of Spain not growing GM maize. The food industry appears to arrange contract supply from areas where local cooperative arrangements are made with the end-user for a small price margin (a margin of approximately 3%, (Mariné 2005)). Production and supply are carried out to meet end-user quality requirements, including pest control, which is undertaken by the end-user at their cost to ensure appropriate choice of chemical and timing of application.

GM maize growers were provided with crop management protocols as part of their user agreement with the seed supplier (Mariné 2005). These included guidelines for separation distances to non-GM crops, the incorporation of buffer rows of non-GM maize, the management of insect resistance to the Bt trait, and above all the need to communicate with neighboring growers.

In mid 2005 the Spanish Ministry of Agriculture issued a decree that will formally regulate the handling, planting, isolation, harvest, storage, inspection and record keeping in relation to GM crops up to first-buyer stage (Ferrer 2005). This new legislation is intended to add robustness to the current co-existence arrangements, and is to be in place in time for the sowing of the 2006 maize crop.

This will dictate that farmers intending to grow GM maize:

- Notify local agricultural authorities in advance of the variety, area to be sown and site location for inclusion in a public register.
- Establish a 50 m isolation zone from any non-GM maize. Where such a separation cannot be established, the GM crop is required to have a buffer of non-GM maize, which is to be harvested and labeled as GM maize. The dimensions of this buffer are not yet finalized, but may be defined as two passes with the harvester.
- Must follow strict procedures for planting, harvesting, drying, storage and delivery. This will include the dedication of specific harvesters to GM maize, with those harvesters not to be used for the harvesting of non-GM maize.
For resistance management reasons, where insect resistant maize varieties are sown, 20% of the area is to be sown to non-GM varieties.

Must keep all seed labels, together with any other evidence of complying with the decree, for a period of five years.

A minimum of 5% of the area sown to GM crops will be inspected annually for compliance. During the period June to September local agricultural authorities will monitor compliance through an extensive program that will involve sampling and testing crops in the neighborhood of registered sites. At this stage it appears that the costs of compliance will be borne by the national and regional governments.

Seed for sowing non-GM crops will be required to meet a minimum threshold for adventitious GM presence. To date the EU has not set such a standard, and there is considerable speculation as to what this threshold might be.

The production of maize in organic systems is not common in Spain, and amounts to less than 0.2% of maize production (Mariné 2005). Only two claims appear to have been reported relating to the adventitious presence of GM material (Alcalde 2005).

**Key issues for introducing GM maize into Australia**

The introduction of GM maize into Australia will require a coordinated program involving several parallel streams of development. As mentioned above, the introduction of desirable GM traits into local elite germplasm has to be undertaken, and licences gained for field evaluation and subsequently for commercial use. State governments need to be fully satisfied that the industry as a whole has, and is committed to, a plan for coexistence, and this is a matter that the industry’s leadership will needs to give serious attention to if it wishes to proceed towards accessing GM crop technology.

The regulatory requirements in Australia are somewhat less stringent than in the EU. In Australia, the labeling threshold for GM content is 1.0%, compared to 0.9% in the EU, and the labeling requirement in Australia only applies to food materials, and does not include a requirement to label products destined for the livestock feed industry.

The development of a coexistence plan will require the involvement of all stakeholders in the supply chain, and the Queensland Government’s framework for co-existence provides a ready made structure.

Lessons may be learnt from the Spanish experience and the extensive EU research and modeling into gene flow already undertaken, although we must also understand that in Spain only about 10% of the GM maize that is grown is done so in a co-existence situation. The remaining 90% of GM maize is grown in areas where all maize is sold to the livestock feed industry that is already a heavy user of imported GM soybeans, and where buyers do not differentiate on GM status (Brookes 2004).

There are also developments in Germany in preparation for an expansion of GM maize growing in that country. In the difficult German legislative environment that ascribes strict liability for any economic loss arising from the presence of GM material to growers of non-GM crops, the German grain trading company Maerka is implementing a pilot scheme in a region of Germany to purchase GM maize as well as non-GM maize from fields adjacent to the GM crops if produced under Maerka’s special quality assurance system. (Pohl 2005).

This system is heavily based on the adoption of defined Good Agricultural Practice, good documentation (including information about non-GM fields neighboring each GM field), segregation through separate machinery, storage and handling, and sampling/testing to identify non-conforming product.

A critical element of a coexistence plan will be the determination and agreement with end-users of practical and achievable thresholds for adventitious GM presence in non-GM maize, and how these will be formalized. Without these, co-existence cannot operate.
Other critical elements will need to ensure that:

- non-GM seed meets an agreed threshold for adventitious GM levels;
- farmers using GM understand and comply with the coexistence rules and the management protocols required to minimise gene flow to neighboring non-GM crops. This may include a variety of measures involving isolation zones, buffer rows, windbreaks, and non-synchronous flowering;
- some dedication of seeding, harvesting, transport and storage may be needed, or at least consideration given to sequencing machinery use so that non-GM applications are concluded before commencing work in GM crops. There may be a need for increased on-farm storage to increase the capacity for segregation;
- sampling and testing will be needed to ensure that co-existence is working. This represents potential added cost.

Part of any strategy for coexistence might well seek to maximize the opportunity for spatial solutions that reduce the overall risk of co-mingling in supply chains over large grain collection areas.

Research and Development organizations might well consider the merit of giving funding assistance to the modeling of pollen flow with the view to validating existing EU work against the Australian landscape and environment and to provide an improved technical basis on which to manage gene flow through good agricultural practice.

Conclusions

Australia may well be able to adapt co-existence management strategies in place in Spain and other parts of the EU.

The path to the commercial use of GM corn in Australia, however, will not be politically forward. It will be dependant on the willingness of at least one seed company to invest in Australian germplasm development, and will require industry leadership to articulate the vision, engage the stakeholders, and lead the supply chain consultative process developing practices, thresholds and supply chain arrangements.

References


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 °C); 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](http://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 subsampling, 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 fumonisn, 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 DPIandF, 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 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 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


INTRODUCING HACCP- BASED RISK MANAGEMENT FOR MYCOTOXIN CONTAMINATION IN AUSTRALIAN MAIZE

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Abstract

Recent incidents of mycotoxin contamination have highlighted the need for an industry wide management system to ensure Australian maize meets the standards of all domestic users and export markets. One potential framework is the HACCP (Hazard Analysis Critical Control Point) system, developed to ensure “absolute food safety” and used internationally for quality control in the food industry.

HACCP is a logical process which analyses each step in production and identifies controls critical in minimising contamination. Applying these controls ensures that risk is managed throughout the entire production chain, not just in the end product. Documented monitoring of critical control points contributes to quality assurance and allows purchasers to select product from agents who have followed appropriate management procedures.

The international body Codex Alimentarius has formulated a code of practice for minimising mycotoxins in cereals. We have prepared a step-by-step guide to developing a management plan that applies the principles in the Codex document combined with Good Agricultural Practice (GAP) to the Australian maize production chain within a HACCP framework. The guide uses simple, clear examples to guide growers, bulk handlers, millers and other industry sectors in identifying critical control points in their individual situations.

Introduction

In the last three years the Australian maize crop has experienced a number of cases of mycotoxin contamination including rejection of maize by millers in the MIA in 2003 because of fumonisin and aflatoxin contamination; aflatoxins in bulk maize in Central Queensland in 2004; rejection of a maize shipment to Japan in early 2005 because of aflatoxin contamination; and the effects of severe drought on aflatoxin contamination of maize in southern Queensland in 2005 (Blaney et al. 2006). Despite only affecting a small percentage of Australian maize, these recent incidents have highlighted the need for an industry wide management system to ensure Australian maize meets the standards of all domestic users and export markets.

Mycotoxins are ubiquitous, environmental pollutants which cannot be easily eliminated once contamination has occurred (Blaney 2004). They are formed as metabolic byproducts by a wide variety of fungi and found in most regions of the world in a wide variety of substrates. Mycotoxins that have been found in maize include aflatoxins, citrinin, cyclopiazonic acid (CPA), fumonisins, ochratoxin A, penicillic acid, tricothecenes (including nivalenol and deoxinivalenol) and zearalenone (Council for Agricultural Science and Technology 2003). Of these, aflatoxins, tricothecenes, fumonisins, zearalenone and ochratoxin A are of concern, because of the risk they pose to human health as food contaminants (Council for Agricultural Science and Technology 2003, Pitt and Tomaska 2001, 2002, Whitlow Jnr and Hagler Jnr 2003). While no Australian regulatory standards are currently in place, Codex Alimentarius supports the ALARA (as low as reasonably achievable) principle. The National Agricultural Commodities Marketing Association (NACMA) has formulated trading standards for aflatoxins and fumonisins and it is to be expected that these industry standards will be used in most cases.

There are two methods by which mycotoxin contamination of foodstuffs or feedstuffs can be minimised. The reactive approach is for loads to be chemically analysed for the presence of specific mycotoxins and accepted or rejected accordingly; the proactive approach is to implement a quality control system applied to production, transport and storage to produce grain that is more likely to meet standards.
Reliance on testing of the final product creates waste both in terms of potentially wasted money and wasted grain should a load be rejected for all potential purposes. Contaminated maize may be diluted with uncontaminated grain, but the sale price for contaminated grain would be substantially reduced. Mycotoxins occur heterogeneously throughout a load and so accurate sampling for mycotoxin analysis is extensive, time consuming and requires substantial quantities of grain. Chemical analysis is complex, requiring trained analysts, costly consumables and significant time to complete each assay. Additionally, a significant number of chemically diverse mycotoxins occur in maize, with a specific chemical assay required for each one. These factors result in considerable expense for the operator.

Conversely, a proactive quality control system incorporates many of the specific measures already in place in most well-run maize production, transport, storage and marketing operations, particularly with respect to moisture control and storage. The major benefit to a quality control system is that specific points and factors conducive to mycotoxin production are controlled and monitored against specified critical limits. Moisture, for example, is significantly easier to control, monitor and rectify- and significantly less costly- than monitoring for mycotoxins in the end product.

Problems have been identified by various sectors because, although they can assure purchasers that grain has been stored correctly whilst in their possession, there are no guarantees on what has happened further up the chain. A formal quality control system includes appropriate documentation and eliminates this uncertainty and guarantees that maize has been subject to appropriate storage throughout its history. Consequently, buyers can be assured that the risk of mycotoxin contamination of the product is low.

Overseas markets are becoming increasingly important in today’s primary industries. The move toward quality control is occurring rapidly and in order to compete successfully in international markets, Australian producers are finding it necessary to embrace quality control locally. Quality control has been successfully practised in other sectors of Australian primary production and the experience is that product marketed as being generated in compliance with an accredited quality control system demands significantly higher prices than that without the “tick of approval”.

One potential quality control framework is the HACCP (Hazard Analysis Critical Control Point) system, developed to ensure “absolute food safety” for US astronauts (NASA 1998). There is a significant amount of research currently supporting the use of HACCP planning in primary production and specifically in the grain industry (Brandt et al.; EUROPA 2000; FAO 2001; Wyss 2005) and it has been endorsed by the WHO for this purpose (Codex Alimentarius Commission 2003).

The HACCP framework

HACCP is a logical process which analyses each step in production and identifies controls critical in minimising contamination. Applying these controls ensures that risk is managed throughout the entire production chain, not just in the end product. Documented monitoring of critical control points contributes to quality assurance and allows purchasers to select product from agents who have followed appropriate management procedures. Each of these critical control points is assigned an acceptable limit and a method for testing against that limit. Test results are recorded for quality assurance purposes and the HACCP plan is documented and, ideally, certified by an appropriate body.

HACCP has seven basic principles (EMAN 2003), outlined in Table 1.
Table 1: Principles of HACCP planning

<table>
<thead>
<tr>
<th>HACCP Principle</th>
<th>Description</th>
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<tbody>
<tr>
<td>Conduct a hazard analysis.</td>
<td>A detailed step by step diagram of the process is prepared, identifying where significant hazards occur.</td>
</tr>
<tr>
<td>Determine critical control points</td>
<td>Critical Control Points (CCPs), points at which the hazards can be controlled, are identified throughout the process.</td>
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<tr>
<td>Establish critical limits.</td>
<td>These are limits that must be adhered to if risk is to be minimised.</td>
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<tr>
<td>Devise a monitoring programme.</td>
<td>Monitoring is critical in any HACCP programme to ensure control points remain under control.</td>
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<tr>
<td>Define corrective actions.</td>
<td>If a control point is shown to be out of range, corrective measures must be implemented.</td>
</tr>
<tr>
<td>Establish verification procedures.</td>
<td>Verification that the HACCP plan is successfully controlling mycotoxin contamination is necessary. At this point, some chemical analysis of the product is required. If contamination is found to exceed limits, immediate action is necessary to identify the step or steps at which failure has occurred. This may mean new CCPs are identified, critical limits are adjusted or the monitoring programme is altered.</td>
</tr>
<tr>
<td>Develop documentation and record keeping.</td>
<td>A successful HACCP programme relies on comprehensive documentation of procedures and records. This will usually involve a flow diagram of the process; the hazard and risk assessment; and a list of CCPs, critical limits and monitoring programmes. Ongoing records of monitoring and corrective action must be kept for consultation as well as the results of verification. Operation requirements for staff and records of staff training should also clearly documented and available. An audit of a HACCP system will include an examination of all this documentation and must be satisfactory should accreditation be desired.</td>
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Mycotoxin- related hazards in the Australian maize industry

Factors conducive to mycotoxin contamination occur throughout the maize production and marketing process but fall into a number of common categories. These include exposure to infection, plant stress, kernel damage and excess moisture, all of which promote fungal growth. Mycotoxin contamination is cumulative, with no simple step to eliminate contamination after the fact, making continuous quality management the only method for minimising contamination in the end product.

Planting

Exposure to infection

Preventing exposure to infection begins with reducing the available inoculum. Fungal spores remain dormant in soil from crop to crop and from year to year, increased by yearly layers of infected trash material. Soil contamination can be controlled by removing old seed heads, stalks, and other debris that may serve as substrates for the growth of mycotoxin-producing fungi. In recent years, increasing adherence to no-till cultivation, aimed at preserving topsoil, has led to the potential for an increase in soil contamination with fungal spores, requiring a trade off between mycotoxin control and soil conservation.

Research overseas has shown that 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 sp., particularly F. graminearum (Codex Alimentarius Commission 2003). Rotating these two crops in areas of Australia subject to infection with this fungus, such as the Liverpool Plains of NSW, potentially increases the availability of inoculum to these crops and should be avoided where possible.

In most cases, while good practice can reduce the availability of inoculum, it is impossible to eliminate it altogether. Over many years, there has been much research aimed at breeding maize hybrids resistant to fungal infection. Ian Martin of the Queensland Department of Primary Industries and Fisheries (QDPIF) in North Queensland has particularly worked on developing hybrids resistant to Fusarium sp. infection. In the future, selection of infection resistant hybrids may become an effective way to eliminate mycotoxin contamination in the field.
**Plant stress**

Plants suffering from stress caused by poor nutrition or water deficit have greater susceptibility to fungal infection, because this reduces the plant’s natural defences. This can be addressed at planting by ensuring soil pH and nutrients are at recommended levels. Plant spacing is also important to ensure optimum use of available water and nutrients.

Timing planting to avoid high temperatures and drought stress during the period of seed development and maturation is also an important precaution (Codex Alimentarius Commission 2003), particularly in Australian conditions. As part of the GRDC project, Yash Chahuan and colleagues at the QDPIF research station in Kingaroy have been developing a computer based model for prediction of aflatoxin levels based on climatic conditions, time of planting and date of harvest (Chahuan et al. 2006).

**Preharvest**

**Plant stress**

Stress during the growth period is often caused by competition for water and nutrients with pest species. To combat this, control weeds in the crop by use of mechanical methods or by use of registered herbicides or other safe and suitable weed eradication practices.

Another common cause of plant stress is insufficient available water. Plants affected by drought stress are not only more susceptible to fungal attack but the low moisture also favours *Aspergillus flavus* and *A. parasiticus*, the fungi responsible for aflatoxin, over other, more common fungi that normally out compete these toxin producers. It is essential to ensure sufficient available moisture throughout the growth period (Codex Alimentarius Commission 2003). Monitoring soil moisture is particularly important in areas of Southern Queensland, where crops are predominantly rain fed. When irrigation is used, ensure even irrigation and time irrigation according to the predominant mycotoxin threat; for example, in areas subject to aflatoxin contamination, soil moisture is critical during kernel development while humidity should be low at harvesting.

**Kernel damage**

Damage to kernels caused by insect attack, mechanical cultivation equipment or drought stress allows the fungi to penetrate the husk and infect the kernel. To address the issue of insect attack, minimize insect damage and fungal infection by using appropriate pesticides and fungicides and other appropriate practices within an integrated pest management program as well as attempting to minimize mechanical damage to plants during cultivation. Ensuring sufficient water is available through the growing process as recommended above will prevent kernels from cracking under drought stress.

**Harvest**

**Exposure to infection**

While mycotoxin contamination occurs frequently in the field, fungal growth causing serious contamination most commonly occurs in storage. Fungi in soil and infected plant material contaminating grain during harvest can introduce inoculum to the stored maize and, if conditions are conducive to colony growth, significant contamination can result. To avoid infection, avoid contact with soil during the harvesting operation; minimize the spread of infected seed heads, chaff, stalks, and debris; and freshly harvested cereals should be cleaned to remove damaged kernels and other foreign matter.

**Kernel damage**

Mechanical harvesters can cause significant damage to the kernel, leaving it open to in-storage infection. To prevent this, harvest grain at low moisture levels (when grain is hard and dry).
Excess moisture

Moisture in storage is the most significant cause of in-storage fungal growth and mycotoxin contamination. To prevent this, grain should be harvested when cobs are at full maturity and after kernels have dried to a moisture content of less than 14%. In situations where the mature cob is expected to be exposed to high rainfall conditions prior to harvest, there may need to be a trade off between an early, less mature harvest and good quality, low moisture maize.

Storage

Prevent infection

Infection occurring during storage, as opposed to prior to storage is most likely to occur through cross contamination from insect and rodent pests or from contaminated storage vessels and areas. Controls include protecting maize from contamination by pests using vermin proof construction and an appropriate pest control program as well as ensuring storage silos and other containers are clean and disinfected before grain is unloaded.

Excess moisture

Excess moisture in storage is the primary cause of fungal growth; keeping grain cool and dry is imperative in controlling mycotoxin contamination in storage. The most important strategy is ensuring moisture content of maize destined for storage is below 14%. Maize that does not meet this criteria may need further drying before storage.

An important method for moisture and temperature control is the aeration of grain by circulation of air through the storage area. Vertical silos can be problematic, even when mechanical aeration is used, with “dead” zones occurring in the central areas of the silo. Appropriate monitoring of airflow is essential.

Other strategies include cooling grain as quickly as possible after harvest; maintaining proper and uniform temperature levels throughout the storage area.

Transport from storage

Exposure to infection

As discussed previously, infection during transport may occur through cross contamination from insect and rodent pests or from contaminated storage vessels and areas. To prevent this, transport vehicles must be kept clean and free from infection. Avoid insect, bird and rodent infestation during transport by the use of insect- and rodent proof containers or insect and rodent repellent chemical treatments if they are approved for the intended end use of the grain.

Excess moisture

Excess moisture in transported maize caused a serious trade incident in 2005 when maize exported to Japan for milling purposes was contaminated with aflatoxin (Blaney et al. 2006). While there are some questions over whether the initial moisture content of the grain was below 14%, the primary reason for fungal growth and related mycotoxin production during transport was moisture migration and accumulation within shipping containers held at tropical summer temperatures for several weeks. The risks of these can be minimised by ensuring shipping containers are placed on lower decks during transport to avoid temperature fluctuations, measures to remove causes of condensation, and including moisture absorbing materials in containers during transport.

Implementing HACCP

One of the greatest criticisms of HACCP to date has been the complexity and time consuming nature of the paperwork. However, for smaller operations HACCP need not be overly complicated nor include large amounts of paperwork that then require document control.
The most important part of any HACCP plan is the Critical Control Point (CCP). In a simple operation, to avoid unnecessary paperwork and labour, there should be no more than half a dozen CCPs. Not all hazards will be CCPs. CCPs will only rarely involve mycotoxin levels. In most cases a CCP will be a physical variable such as temperature, moisture or damage control.

CCPs must be selected carefully, and must meet the following criteria.

- Do preventative / control measures exist for the identified hazard?
- Is the step specifically designed to eliminate or reduce the likely occurrence of a hazard to an acceptable level?
- Could contamination occur or increase to unacceptable level(s)?

Will a subsequent step or action eliminate or reduce the hazard to an acceptable level?

Other primary components revolve around the Critical Control Points and include a documented monitoring procedure with records of monitoring results and documented corrective action with associated records.

The guidebook

The guide acknowledges the fact that the grower/operator has the best understanding of their own process or production line. As such, the module will not provide a plan ready prepared, but will assist operators to develop their own plan using examples and recommendations specific to Australian conditions and the maize industry. These incorporate the Codex Alimentarius code of practice for minimising mycotoxins in cereals (Codex Alimentarius Commission 2003).

The book is designed to guide growers/operators in identifying critical control points at various stages of their production process, defining critical limits, devising a monitoring programme and documenting their plan. Each concept will be introduced in a separate chapter with background information, step by step instructions, a generic example and a blank worksheet. By completing the tasks in each chapter, a grower or operator will successfully complete a simple HACCP plan for their operation. A sample plan is illustrated in Figure 1.

For the future, a computer program has been proposed that will guide the grower/operator through the chapters and tasks electronically and automatically compile the plan. This program could link to the computer based prediction model previously referred to in this paper. (Refer Figure 1 on next page).

Conclusions

With the worldwide move toward total quality control and risk management it is in the maize industry’s benefit to manage mycotoxin contamination during production, rather than rely on regulatory standards that apply to the end product. While it is accepted that it is not possible to eliminate mycotoxin contamination (Blaney 2004), it is possible, through good agronomic practice, to minimise contamination and limit the negative effects to industry by using effective risk management strategies.
Figure 1. Sample HACCP plan

<table>
<thead>
<tr>
<th>Process step</th>
<th>Hazard Analysis</th>
<th>Monitoring</th>
<th>Corrective action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purchase</td>
<td>Grain carrying spores of mycotoxin producing fungi- will contribute to contamination of soil</td>
<td>Purchase from grower with accredited quality assurance program</td>
<td>Certificate of quality assurance from grower</td>
</tr>
<tr>
<td>Delivery vehicle contaminated with fungal spores</td>
<td></td>
<td></td>
<td>Record check</td>
</tr>
<tr>
<td>Pre-storage check</td>
<td>Moisture content of kernels excessive</td>
<td>Accept/refuse load</td>
<td>Max kernel moisture 14%</td>
</tr>
<tr>
<td>Storage</td>
<td>Excessive environmental moisture in storage</td>
<td>Aerate grain in storage</td>
<td>Airflow….l/sec</td>
</tr>
<tr>
<td>Pest control</td>
<td>Inoculum introduced by rodents and/or insects</td>
<td>Treatment by licensed pest control operator</td>
<td>Certificate from pest control operator</td>
</tr>
<tr>
<td>Transport decontamination</td>
<td>Transport vehicle contaminated with inoculum</td>
<td>Wash with 20% solution of BanMould on walls, floors, ceilings of truck</td>
<td>Record of treatment-amount used, date, person responsible for treatment</td>
</tr>
</tbody>
</table>

References


