CHAPTER 9.

Practical approaches to control mycotoxins

Summary

Control strategies to minimize mycotoxin levels in food comprise several broad categories, including good agricultural practice, good manufacturing practice, and hazard analysis and critical control point principles. In general, intervention strategies include pre-harvest, post-harvest, and dietary approaches, depending on the specific mycotoxins and the food commodity likely to be contaminated. This chapter describes practical interventions, which are arranged by the major groups of mycotoxins and are described according to their stage of development, efficacy, geographical regions in which they have been tested or applied, simplicity or complexity, and breadth of usefulness. Typical pre-harvest interventions include the breeding of resistant plant cultivars, good agricultural practice, and biocontrol using non-toxigenic strains. Post-harvest interventions include the removal of infected and/or insect-damaged food components by sorting, maintaining correct drying and storage conditions, and chemical deactivation such as nixtamalization. Dietary interventions include reducing mycotoxin bioavailability or modulating metabolism in ways that reduce the harmful effects of reactive metabolites. Cost-effective and simple intervention methods, predominantly at the population level, should be emphasized in developing countries, where resources are limited and sophisticated technologies are lacking.

1. Introduction

This chapter provides detailed information about interventions and practices that can help to reduce mycotoxin risks in a variety of settings: before harvest (in the field), after harvest (in storage, transportation, or processing), and in diets. The sections are organized by mycotoxin and the interventions that have been developed to control them, as follows: aflatoxins, fumonisins, ochratoxin A, and deoxynivalenol and zearalenone (treated together because they are produced by the same fungi).

For each intervention, information about each of the following aspects is listed.

Description. A description of the intervention.

Stage of development. How well developed and tested is the intervention in terms of controlling

the mycotoxin? Has it undergone testing under laboratory, field, or epidemiological conditions? How broad is the current adoption?

Efficacy. By how much can the intervention reduce mycotoxin risk, compared with conditions where there is no intervention?

Geographical regions. Where has this intervention been tested or adopted around the world?

Simplicity/complexity. How simple or complex is the intervention to implement? Is it so complex that individuals must have advanced education and training, or can it be made sufficiently simple for farmers or even the general public to adopt effectively?

Population/individual. Is the intervention tailored, or can it be tailored, to a population-level approach (e.g. so that a government can broadly implement the intervention), or does the intervention rely on individuals for implementation?

Useful in emergencies. Is this intervention suitable for use in a time of emergency, e.g. when mycotoxin levels are known to be high in available foods, or in the event of acute poisoning?

Locality of resources. Can the intervention be manufactured using local resources and drawing on local expertise, or does it require importation of resources and/or trained operators?

Accessibility. Is the intervention applicable in low-income countries (LICs) and among subsistence farmers with respect to access, cost, and feasibility?

References. For further reading on the topic.

Before individual interventions for each mycotoxin are discussed, three broad categories of interventions are described: good agricultural practice, good manufacturing practice, and the hazard analysis and critical control point system. These categories of interventions apply for general control strategies across multiple mycotoxins.

1.1 Good agricultural practice

Good agricultural practice (GAP) involves good farm management. Many definitions exist, depending on local conditions, but in general GAP means maintaining healthy crops and sustainable agriculture by, among other activities: (i) planting with optimal row and seed spacing for local conditions, especially water availability, to reduce plant stress; (ii) maintaining adequate water supplies, by irrigation where practicable; (iii) reducing erosion by contouring, ditching, or hedging; (iv) controlling weeds, and mulching crops to reduce moisture stress; (v) controlling insects that damage developing grains or nuts and permit entry of the fungi that produce mycotoxins; (vi) rotating crops to reduce insect infestation and fungal infection, which are exacerbated by monoculture; (vii) applying fertilizers at appropriate times and concentrations, to benefit the crop but limit run-off of nutrients such as nitrogen and phosphorus; (viii) harvesting crops at or before full maturity, because overmature crops are liable to increased risk from insect damage and water stress and hence mycotoxin production; (ix) drying crops rapidly and completely, as soon as possible after harvest; and (x) maintaining good storage conditions on the farm (storage facilities should be soundly constructed to prevent water ingress, with raised floors to prevent moisture migration from soil; properly dried crops should be stored in closely woven sacks that permit air exchange; and rodents and insects should be controlled).

Drying of crops is a critical process in reducing development of mycotoxins. Grains and nuts are often dried in the field, with consequent poor control over conditions. In subtropical and temperate regions, the weather is usually drier at harvest time and field drying is effective. In tropical countries, groundnuts are frequently harvested and left to dry in stacks in the field, or are separated from plants at harvest and dried on the ground, on some form of matting, or on plastic sheets. Maize is sometimes shelled wet and then dried mechanically by middlemen. Storage practices in developed countries normally prevent development of mycotoxins after drying. However, less than ideal storage conditions in LICs may permit increases in moisture content, leading to increases in production of aflatoxins or ochratoxin A. Storage conditions can be improved by using dry, well-ventilated rooms with protection from sunlight (to prevent moisture migration) and control of insects and rodents. This is true not just for maize, cereals, and groundnuts (the major sources of mycotoxin exposure for humans) but also for tree nuts such as pistachios, for which there have been dramatic improvements in aflatoxin reduction in Iran over the past decade due to improved drying and storage conditions (Wu, 2008).

For further information about GAP, see FAO (2002).

1.2 Good manufacturing practice

Good manufacturing practice (GMP) involves a wide range of practices that maintain the quality of foods, in developed countries often through legislation. In the context of mycotoxins, GMP includes practices that prevent fungal growth and hence reduce mycotoxin formation and that reduce or remove mycotoxin contamination in crops after harvesting and drying. On the farm, this most commonly involves removal of defects, including immature nuts or grains and also weed seeds, sticks, stones, earth, husks, and so on,

by hand sorting, winnowing, and gravity separation or other methods. Sorting out obviously mouldy nuts or kernels by hand and eye has proven a particularly effective method of removing a large proportion of the mycotoxin contamination in the food (Turner *et al*., 2005; Van der Westhuizen *et al*., 2011). Practices downstream, involving middlemen, cooperatives, and factories, depend on the crop and the mycotoxin. These include practices such as extrusion (Bullerman and Bianchini, 2007) and nixtamalization (described in more detail in Section 2.2.2), both of which have been shown to reduce levels of multiple mycotoxins in food.

1.3 The hazard analysis and critical control point system

The hazard analysis and critical control point (HACCP) system for food safety management involves controlling critical points in food handling (FAO, 2001) and is important in managing the problem of mycotoxins in the food supply (Bryden, 2009; Chulze, 2010). Adopting guidelines of the United Nations Codex Alimentarius Commission (Codex Alimentarius Commission, 1995), the Food and Agriculture Organization of the United Nations (FAO) has outlined the following seven principles of HACCP for food safety (FAO, 2001). First, identify potential hazards associated with food production at all stages, assess the likelihood of hazard occurrence, and identify preventive measures for control. Second, determine points, procedures, and operational steps that can be controlled to eliminate, or reduce the likelihood of, hazards. These are the critical control points (CCPs). Third, establish critical limits that must be met to ensure that CCPs are under control. Fourth, establish a system to monitor control of CCPs by scheduled testing or observations. Fifth, establish corrective actions when monitoring indicates that certain CCPs are not under control. Sixth, establish procedures for verification to confirm that the HACCP system is working effectively. Seventh, establish documentation concerning all procedures and records appropriate to these principles and their application.

HACCP control for mycotoxins is an integrated approach (Bryden, 2009), which includes GAP and GMP (described above) as complementary approaches (Aldred *et al*., 2004). In developed countries, several HACCP programmes have been developed for aflatoxin in a variety of commodities as well as for ochratoxin A in coffee; these programmes rely on rapid diagnostic tools to monitor fungal occurrences and application of methods to quantify mycotoxins.

In LICs, some HACCP processes may not yet be technically and economically feasible (Bryden, 2009), necessitating other strategies to reduce mycotoxins. However, it can be useful to adopt HACCP principles as a way of thinking, regardless of economic and technical constraints. A 1999 conference on mycotoxins emphasized that GAP and GMP overlap and are prerequisites for HACCP. HACCP will ensure and improve food quality in a controlled environment; hence, such systems must be kept simple, practical, and understandable for those who use them to reduce mycotoxin risk (FAO/WHO/UNEP, 1999).

Table 9.1. Risk management strategies for major mycotoxins in pre-harvest, post-harvest, and dietary settings

Setting	Mycotoxins			
	Aflatoxins	**Fumonisins**	**Ochratoxin A**	**Deoxynivalenol and zearalenone**
Pre-harvest	GAP Developing drought-resistant cultivars Biocontrol Forecasting aflatoxin formation Timely harvesting	GAP Ensuring that cultivars are adapted to local environments Breeding for insect resistance Transgenic Bt maize Forecasting fumonisin formation Timely harvesting	GAP Timely harvesting	GAP Breeding for host plant resistance Using cultivars that mature over a range of dates Transgenic Bt maize Using fungicides at anthesis or silking Forecasting toxin formation
Post-harvest	GMP, HACCP, sorting, drying, nixtamalization	GMP, HACCP, sorting, drying, washing, nixtamalization	GMP, HACCP	GMP, HACCP, sorting, drying
Dietary	Enterosorbents (e.g. organic clays) Chlorophyll and chlorophyllin			

GAP, good agricultural practice; GMP, good manufacturing practice; HACCP, hazard analysis and critical control point.

Table 9.2. Likelihood of mycotoxin contamination in major commodities and current stage of development of potential or actual interventions

	Commodity				
	Maize	**Groundnuts**	**Tree nuts**	**Small grains**	**Others**
Mycotoxin					
Aflatoxins	X	X	X		Figs, copra, spices, cottonseed
Fumonisins	X				Sorghum, millet, soybeans, asparagus
Ochratoxin A	X			X	Dried vine fruits, wine, coffee, cocoa, chocolate
Deoxynivalenol and zearalenone	X			X	
Relevant intervention					
GAP	Practice	Practice	Practice	Practice	Practice
GMP	Practice	Practice	Practice	Practice	Practice
HACCP	Practice	Practice	Practice	Practice	Practice
Biocontrol via non-toxigenic strains	Pilot	Practice	Pilot		Practice (cottonseed)
Fungicides			Practice	Practice	
Plant breeding (conventional and transgenic)	Practice	Pilot	Pilot	Practice	
Sorting	Practice	Practice	Practice	Practice	Practice
Nixtamalization	Practice				
Enterosorbents	Practice (animal feeds in the USA)	Practice (animal feeds in the USA)	Practice (animal feeds in the USA)	Practice (animal feeds in the USA)	
Chlorophyllin	Promising; needs more research	Promising; needs more research	Promising; needs more research	Promising; needs more research	
Dietary chemoprevention	Promising; needs more research	Promising; needs more research	Promising; needs more research	Promising; needs more research	

GAP, good agricultural practice; GMP, good manufacturing practice; HACCP, hazard analysis and critical control point; Pilot, studied or tested in experimental studies or on a pilot scale, but not in commercial use; Practice, currently being used by growers or producers to control a particular mycotoxin.

The tables organize several key characteristics of the interventions presented here. Table 9.1 summarizes the information on applicable interventions by mycotoxin and by type of intervention, i.e. pre-harvest, post-harvest, or dietary. Although hepatitis B virus vaccination, described in Chapter 7, is a clinical intervention that may reduce the potency of aflatoxin in causing liver cancer, it is not included in this chapter because it does not control mycotoxin levels directly. Likewise, food replacement – sourcing clean food from another region to a region suffering high foodborne mycotoxin contamination – is useful in emergency situations; however, it is also not included in the table because it does not directly reduce mycotoxin levels in the original food supply.

Table 9.2 provides information about which food commodities are likely to be contaminated by which mycotoxins, and whether particular interventions are common agricultural practice or have only been tested in pilot experiments. Table 9.3 describes usage characteristics of each intervention: the local availability of resources needed to develop it, the technical simplicity of implementing the intervention,

Table 9.3. Usage characteristics of interventions: local availability of intervention materials, ease of implementation, usefulness in emergencies, and whether the intervention is applicable at the population or individual level

Intervention	Usage characteristic			
	Intervention materials available locally	Implementation technically simple	Useful in emergencies	Population or individual level
GAP	Yes	Yes	No	I, P
GMP	Yes	Yes	No	P
HACCP	Yes	Yes	No	P
Biocontrol via non-toxigenic strains	Yes (fungal strains and substrate)	Yes (after development)	No	P
Fungicides	Yes	Yes	No	P
Plant breeding	No	No	No	P
Sorting	Yes	Yes	Yes	I, P
Nixtamalization	Yes	Yes	Yes	I
Enterosorbents	No	No	Promising; needs more research	I, P
Chlorophyllin	No	No	Promising; needs more research	I
Dietary chemoprevention	No	No	Needs more research	I

GAP, good agricultural practice; GMP, good manufacturing practice; HACCP, hazard analysis and critical control point; I, individual; P, population.

its usefulness in emergencies, and whether the intervention is applicable at the population or individual level.

2. Aflatoxins

2.1 Pre-harvest interventions

2.1.1 Conventional breeding for host plant resistance

Description. Breeding methods have been explored to improve resistance to drought, insect herbivory, or other environmental stressors that would predispose groundnuts and maize to pre-harvest formation of aflatoxin. This has included work on identifying resistant crop lines and identifying biochemical and genetic resistance markers in crops. Sequencing of the *Aspergillus flavus* genome has been completed. Genes that potentially encode for enzymes involved in aflatoxin production have been identified, so that genomics as a tool for understanding aflatoxin biosynthesis has gained much ground (Yu *et al.*, 2008). It is hoped that these findings may have practical application in the future; for now, they have improved our understanding of the regulation and biosynthesis of aflatoxins. Also, recent proteomic studies involving the generation of > 20 000 expressed sequence tags from developing groundnut plants under drought stress have yielded several proteins potentially associated with resistance to aflatoxin production (Wang *et al.*, 2010).

Stage of development. Pilot. Much research has been conducted on resistance, but potentially resistant varieties lack other characteristics necessary for commercial application. For groundnuts, characteristics that confer drought tolerance may reduce pre-harvest aflatoxin accumulation. In addition, breeding for characteristics to reduce environmental stressors on maize has shown some efficacy in reducing aflatoxin.

Efficacy. Suggestive evidence of efficacy.

Geographical regions. Tested in the USA for maize. Several centres of the Consultative Group on International Agricultural Research (CGIAR) have conducted research.

Simplicity/complexity. High degree of complexity. Other strategies, such as giving germ plasm of improved open-pollinating lines of maize to indigenous farmers, have been proposed and possibly deserve reconsideration.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. Local crop lines should preferably be identified for suitability for each geographical region.

Accessibility. Likely to be accessible if appropriate hybrids are devel-

oped for different regions of the world and prices are established that are affordable for small-scale farmers.

References. Gorman and Kang (1991), Brown *et al*. (2001), Cleveland *et al*. (2003), Maupin *et al*. (2003), Menkir *et al*. (2006), Chen *et al*. (2007), Yu *et al*. (2008), Arunyanark *et al*. (2010), Girdthai *et al*. (2010), Wang *et al*. (2010), Warburton *et al*. (2011).

2.1.2 Biocontrol

Description. Biocontrol of aflatoxins relies on competitive exclusion. High numbers of spores of a non-toxigenic strain of *A. flavus* (or, less commonly, *A. parasiticus*) are introduced into the soil where crops are being grown, where they compete with existing toxin-producing spores for sites on the developing crop, thus reducing aflatoxin production. Usually the selected strain is introduced to the field on a carrier substrate that permits growth of the fungus with consequent production of high numbers of spores.

Stage of development. Commercial in the USA, and pilot.

Efficacy. Depending on climatic factors that affect biocontrol agent growth, and concentration of toxigenic spores in a given field, aflatoxin reductions may range widely, from no reduction at all to high levels (0–80%).

Geographical regions. Used commercially in cottonseed and groundnut fields in the USA. Tested in pilot studies on maize in the USA, Nigeria, Kenya, and Thailand. Tested in pilot studies on tree nuts in the USA.

Simplicity/complexity. Selecting and maintaining non-toxigenic strains is a specialist undertaking; producing substrates for field inoculation requires a feed mill or similar factory with suitable protection for workers because *A. flavus* is a known human pathogen. Application of the product onto fields can be carried out by trained farmers.

Population/individual. Individual in current commercial applications; possibility for population-level approach.

Useful in emergencies. No.

Locality of resources. Appropriate fungal strains must be sourced locally for each region where the process is used. Different substrates may be more economical in various regions; economic barriers for subsistence farmers.

Accessibility. Unlikely to be highly accessible, unless local soil samples are tested and facilities are built and maintained to develop biocontrol strains appropriate for the geographical region.

References. Dorner *et al*. (1999), Bandyopadhyay *et al*. (2005), Pitt and Hocking (2006), Cotty *et al*. (2007), Dorner and Horn (2007), Atehnkeng *et al*. (2008).

2.1.3 Forecasting

Description. AfloMan is a forecasting system for the formation of aflatoxin in groundnuts, in use in Queensland, Australia.

Stage of development. Commercial.

Efficacy. The model accounts for up to 95% of the variation in aflatoxin accumulation in groundnut crops at harvest. As with all models of this type, it is highly dependent on the reliability of weather and crop development data. Similar systems have been explored in the USA, but not beyond the conceptual stage.

Geographical regions. South Burnett region of Queensland, Australia.

Simplicity/complexity. Requires access to reliable climatic data and sophisticated mathematical modelling, but AfloMan is used daily by South Burnett groundnut growers via the Internet.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. Not applicable.

Accessibility. High in geographical regions where predictive models have been developed and access to computers is readily available to growers. Low in other regions.

References. Henderson *et al*. (2000), Chauhan *et al*. (2010), DEEDI (2010).

2.2 Post-harvest interventions

2.2.1 Sorting, drying, and storage

Description. Post-harvest control methods are based on GMP. Some specific methods that apply to aflatoxins follow: community-based approaches and industrial sorting methods.

At the level of communities, basic visual hand sorting can remove a large proportion of nuts or kernels that are significantly contaminated with aflatoxins. Additional community-based methods to keep aflatoxin levels low in post-harvest settings include proper drying, storage in bags that allow for air circulation, and use of well-ventilated storage facilities that control for pests.

Industrial sorting methods vary with the crop.

Maize. Primary sorting of maize kernels is by examination using an ultraviolet (UV) light (365 nm) after cracking; grains containing appreciable aflatoxin fluoresce, enabling sorting of lots. Fluorescence is due to plant peroxidase enzyme reacting with kojic acid produced by *A. flavus*. Some *A. flavus* strains do not produce kojic acid and therefore do not cause fluorescence. Further, tropical temperatures induce isomerization of kojic acid, preventing the fluorescence reaction, so sorting under UV light is ineffective in the tropics.

Groundnuts. When fungi invade groundnuts, enzymatic changes cause nut discolouration; thus, sorting out discoloured nuts also removes those that contain aflatoxin. Colour sorting should be followed

by aflatoxin assays. UV sorting of groundnuts is possible using a somewhat higher wavelength. In severe circumstances, groundnuts are blanched and roasted, which increases discolouration, enabling more effective colour sorting, which again should be followed by aflatoxin assays. Blanched and roasted nuts are susceptible to oxidative rancidity and thus should be packed under nitrogen in gas-tight packaging.

Pistachios. Pistachios have been very difficult to sort by UV or discolouration and have traditionally not been sorted. However, recent studies have shown that sorting by fluorescence and discolouration may be potentially useful. Aflatoxin in pistachios results from shells of nuts opening before the nut is dry, permitting ingress of *A. flavus* spores. Because shell opening is a desirable characteristic, control has involved the development of cultivars with later shell opening.

Almonds. Aflatoxin in almonds is usually caused by insect damage, so control relates to insect control. UV light can be used for sorting almonds containing aflatoxins.

Brazil nuts. Brazil nuts are infected by *A. flavus* when allowed to remain for extended periods on the forest floor before harvest (by picking them up). Infection by *A. nomius*, which also produces aflatoxins, apparently occurs before harvest. No control measures, other than aflatoxin assays, exist for Brazil nuts at this time. Some studies have suggested that sorting by size or physical appearance may be useful, but more research is needed.

Figs. Aflatoxin in figs results from *A. flavus* infection carried by insects during pollination. Control is by examination of individual dried or fresh fruit under UV light to detect the presence of aflatoxin.

Stage of development. Nut-producing regions of developed countries use the control measures described above.

Efficacy. A pilot test in Guinea of a post-harvest intervention package for groundnuts (including education on how to sort and dry nuts, natural fibre drying mats and storage bags, wooden pallets on which to store groundnut bags, and pesticide for storage floors) achieved a 70% reduction in aflatoxin levels in groundnuts after 5 months of storage compared with untreated groundnuts, and a 57% mean reduction in aflatoxin–albumin adduct levels in individuals who implemented the post-harvest intervention package compared with controls.

Geographical regions. Control measures outlined above are of universal applicability.

Simplicity/complexity. Control measures vary in complexity, but many can be applied in all regions.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. No.

Locality of resources. Most elements of post-harvest control methods can be obtained or manufactured locally.

Accessibility. Community-based approaches are generally accessible among subsistence farmers, but education is necessary. Industrial sorting approaches are accessible in developed countries on the scale of large commercial farming.

References. Pearson and Slaughter (1996), Hadavi (2005), Turner *et al.* (2005), Kabak *et al.* (2006), De Mello and Scussel (2007), Magan and Aldred (2007), Wagacha and Muthomi (2008), Pacheco and Scussel (2009), Khlangwiset and Wu (2010), Pacheco *et al.* (2010).

2.2.2 Nixtamalization

Description. Alkaline cooking of maize in a solution of ash, lime, or other materials containing inorganic calcium. This process is useful for reducing concentrations of both aflatoxins and fumonisins (which are described in Section 3).

Stage of development. This process has been used for centuries to produce masa (a dough made from ground maize), by indigenous populations in regions throughout Latin America, especially Mexico and Central America, where maize is produced.

Efficacy. Under laboratory and commercial conditions, nixtamalization by the traditional process can reduce aflatoxin levels by up to 90%.

Geographical regions. Mexico, Central America.

Simplicity/complexity. Simple process; adequate clean water is required.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. Yes.

Locality of resources. The activated lime used in nixtamalization is widely available. However, this process appears to be culturally acceptable only in the Americas. Water can sometimes be difficult to obtain, which can cause problems if washing is inadequate after alkaline steeping. In addition, use of polluted water for steeping can present a different set of risks.

Accessibility. Likely to be accessible in geographical regions where this practice is accepted and where clean water is readily accessible.

References. Torres *et al.* (2001), Elias-Orozco *et al.* (2002), De La Campa *et al.* (2004), Méndez-Albores *et al.* (2004), Bullerman and Bianchini (2007).

2.3 Dietary interventions

2.3.1 NovaSil clay

Description. NovaSil is a dioctahedral smectite clay that can bind aflatoxin in the gastrointestinal tract and aid in its elimination. NovaSil can be included in food or feed or taken separately during mealtimes.

Stage of development. Commercial in animal feed; pilot in humans.

Efficacy. In a pilot study in humans in Ghana, after 3 months of treatment, NovaSil clay added to diets achieved a 59% reduction in aflatoxin M_1 levels, and a 25% reduction in aflatoxin–albumin adduct levels, in treated individuals compared with controls.

Geographical regions. USA (as an anticaking agent in animal feed), Ghana (humans).

Simplicity/complexity. Currently, NovaSil clay comes only from one mine in the USA, so although the material is cheap, importation costs must be considered. Inclusion in the diet, in bread or in maize or groundnut meal, should be reasonably simple. Studies indicate some limitations, including the risk of vitamin and mineral binding in a nutritionally compromised population. Further, distribution under government supervision will be essential because imitation clay materials, similar in appearance to NovaSil but without the potential benefit, are readily available.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. Promising; needs more research.

Locality of resources. So far, NovaSil clay has been mined only in the USA; would have to be exported to countries in need.

Accessibility. Will depend on cultural acceptance and cost.

References. Pimpukdee *et al.* (2004), Afriyie-Gyawu *et al.* (2008), Phillips *et al.* (2008), Wang *et al.* (2008).

2.3.2 Chlorophyll and chlorophyllin

Description. Chlorophyll and its derivative chlorophyllin, which are natural constituents of green vegetables, can sequester aflatoxin in the gastrointestinal tract and impede its absorption. In addition, these compounds may have enzyme-inducing properties that contribute to mechanisms of detoxification.

Stage of development. Pilot; clinical trials.

Efficacy. A clinical trial in humans in Qidong, China, achieved a 55% reduction in aflatoxin-N7-guanine levels in treated individuals compared with controls.

Geographical regions. Clinical trials have been carried out in the USA and China.

Simplicity/complexity. The intervention was administered as a chemopreventive pill, which requires regular and continued administration.

Population/individual. Individual.

Useful in emergencies. Promising; needs more research for effects in situations of very high exposure to aflatoxin in vivo.

Locality of resources. Depends on availability of the chemopreventive pills.

Accessibility. Will depend on the availability of the medication and cost.

References. Dashwood *et al.* (1998), Egner *et al.* (2001), Simonich *et al.* (2007, 2008), Groopman *et al.* (2008), Jubert *et al.* (2009).

2.3.3 Naturally occurring dietary constituents

Description. Green tea polyphenols, sulforaphane derived from cruciferous vegetables, and lactic acid bacteria.

Stage of development. Pilot.

Efficacy. In rat studies, green tea polyphenols have been shown to inhibit initiation of liver cancer induced by aflatoxin. In humans, inverse associations between green tea consumption and overall cancer risk have been observed. Sulforaphane, metabolized from glucoraphanin in cruciferous vegetables such as broccoli and cabbage, induces phase 2 enzymes such as the glutathione-*S*-transferases that prevent DNA damage induced by aflatoxin. In human studies, those individuals who converted more glucoraphanin to sulforaphane had lower aflatoxin-N7-guanine levels. Lactic acid bacteria from fermented vegetables, fruits, and dairy products have the ability to bind aflatoxin B_1 in laboratory tests; this has not yet been tested in animals or humans.

Geographical regions. China (green tea polyphenols, sulforaphane).

Simplicity/complexity. These interventions would be simple in the parts of the world where the foods or drinks containing these dietary constituents are already common in diets. They could be complex where this is not the case already. Optimized consumption patterns to modify aflatoxin metabolism over extended periods would need to be developed.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. No.

Locality of resources. Common.

Accessibility. Readily accessible in developed countries where these dietary constituents are affordable; less accessible in LICs, where diets are much less varied.

References. Haskard *et al.* (2000), Fujiki *et al.* (2002), Kensler *et al.* (2005), Yates and Kensler (2007), Hernandez-Mendoza *et al.* (2009), Gao *et al.* (2010), Gross-Steinmeyer *et al.* (2010).

3. Fumonisins

3.1 Pre-harvest interventions

3.1.1 Breeding for host plant resistance

Description. Breeding and selection methods have been used for centuries to improve maize resistance to fungal and insect infection or other environmental stressors, stressors that have been discovered recently to predispose plants to fumonisin contamination. This has included work on developing resistant inbred crop lines and identifying biochemical and genetic resistance markers in crops.

Stage of development. It is reliably known that improved insect and

drought tolerance results in reduced risk for fumonisin accumulation. This approach is being applied to the extent that hybrids suitable for particular areas have become available.

Efficacy. In years of high insect pressure and drought, such resistant hybrids can increase the percentage of the crop suitable for human consumption.

Geographical regions. USA, Europe.

Simplicity/complexity. Although the commercial breeding process involves significant expertise and expense at first, seeds resistant to fungal development and fumonisin formation can be disseminated as readily as other types of seeds, within the area of adaptation.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. Local inbred maize lines can be identified for suitability to the geographical region; questions of access and affordability for LICs.

Accessibility. Likely to be widely accessible after local varieties with improved traits are developed.

References. Miller (2001), Clements *et al.* (2004), Afolabi *et al.* (2007), Henry *et al.* (2009), Loeffler *et al.* (2010), Parsons and Munkvold (2010).

3.1.2 Transgenic Bt maize

Description. Transgenic Bt maize contains a gene from the soil bacterium *Bacillus thuringiensis* that results in the accumulation of proteins toxic to key insect pests of maize. Insect damage predisposes maize to fumonisin contamination by facilitating fungal infection.

Stage of development. Commercial.

Efficacy. Depending on the severity of insect infestation in a given year, fumonisin reductions afforded by Bt maize can greatly increase the percentage of the crop acceptable for human consumption.

Geographical regions. USA, Canada, Argentina, Brazil, Uruguay, South Africa, Honduras, Philippines, Hungary.

Simplicity/complexity. Although the commercial breeding process involves significant expertise and expense at first, Bt seeds can be disseminated as readily as other types of seeds, within the area of adaptation.

Population/individual. Individual; possibility for population-level approach in countries with commercial agriculture.

Useful in emergencies. No.

Locality of resources. Highly variable; requires reliance on biotechnology companies to permit small-scale farmers access to seed at prices under fair conditions.

Accessibility. High in developed countries that have permitted Bt maize planting and commercialization; low elsewhere worldwide.

References. Munkvold *et al.* (1999), Bakan *et al.* (2002), Hammond *et al.* (2004), de la Campa *et al.* (2005), Papst *et al.* (2005), Wu (2007), Folcher *et al.* (2010).

3.2 Post-harvest interventions

3.2.1 Sorting and washing

Description. Hand sorting of obviously contaminated kernels of home-grown maize, and washing before consumption.

Stage of development. Traditional hand and eye sorting methods are well developed; optical sorting using two wavelengths is possible but requires expensive equipment.

Efficacy. Sorting and washing maize kernels can reduce fumonisin contamination by > 84% in maize grains and by > 60% in maize porridge.

Geographical regions. Traditional sorting methods are used in many maize-producing regions.

Simplicity/complexity. Sorting and washing techniques are simple to implement; education can improve confidence in the results.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. Yes.

Locality of resources. These post-harvest control methods can be carried out locally.

Accessibility. Generally accessible techniques worldwide.

References. Desjardins *et al.* (2000), Pearson *et al.* (2004; 2010), Fandohan *et al.* (2005), Afolabi *et al.* (2006), Kimanya *et al.* (2009a, 2009b), Van der Westhuizen *et al.* (2010, 2011).

3.2.2 Nixtamalization

Description. Alkaline cooking of maize in a solution of ash, lime, or other materials containing inorganic calcium.

Stage of development. This process has been used for centuries to produce masa, by indigenous populations in regions throughout Latin America, especially Mexico and Central America, where maize is produced.

Efficacy. Nixtamalization can reduce fumonisin B_1 levels in fried tortilla chips by up to 80%.

Geographical regions. All maize-growing areas in Latin America.

Simplicity/complexity. Simple process; adequate clean water is required.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. Yes.

Locality of resources. The activated lime used in nixtamalization is very widely available. However, this process appears to be culturally acceptable only in the Americas. Water can sometimes be difficult to obtain, which can cause problems if washing is inadequate after alkaline steeping. In addition, use of polluted water for steeping can present a different set of risks.

Accessibility. Likely to be accessible in geographical regions where this practice is accepted and where clean water is readily accessible.

References. Dombrink-Kurtzman *et al*. (2000), Voss *et al*. (2001, 2009), Palencia *et al*. (2003), De La Campa *et al*. (2004), Bullerman and Bianchini (2007), Torres *et al*. (2007), Burns *et al*. (2008).

4. Ochratoxin A

4.1 Pre-harvest interventions

Little evidence exists that any of the important fungi producing ochratoxin A (OTA) invade crops before harvest, so plant breeding is of little value in the control of OTA formation. The one exception is that the invasion of grapes by *Aspergillus carbonarius* (and *A. niger*) takes place before harvest. The evidence is that these fungi cannot infect intact grapes, so entry is dependent on infection by fungal pathogens such as powdery mildews or *Rhizopus stolonifer*, mechanical damage, or skin splitting due to rain. Some cultivars are more susceptible to skin splitting, so plant breeding is of value in that area.

Pre-harvest control of OTA production in grapes, and hence dried vine fruits and wine, is based on limiting damage by the powdery mildews (by fungicidal spray programmes) and by *Rhizopus* (by a defoliant spray to increase exposure of grapes to sunlight, which limits growth of *Rhizopus*).

4.2 Post-harvest interventions

Fungi producing OTA invade crops after harvest. Types of control vary with the crop.

Small grains. OTA is produced after harvest, during drying in small grain cereal crops (wheat and barley) in cool temperate zones, by *Penicillium verrucosum*. The problem is widespread, and the only effective control measure consists of rapid drying, where this is possible. Because *P. verrucosum* does not grow in warmer climates, OTA production in small grains is not a problem in warm temperate or tropical crops. OTA occurs in maize but appears to be a minor problem compared with aflatoxins, fumonisins, or deoxynivalenol.

Dried vine fruits and wines. As noted above, infection in grapes by *A. carbonarius* and *A. niger* may occur before harvest. In winemaking, fungal growth and toxin production cease when fermentation commences. Wine-making reduces the level of OTA in wine by up to 80%. In grape drying, mechanical damage during harvest and drying pretreatments increases the possibility of infection of grapes by *A. carbonarius* and *A. niger*, so rapid drying will reduce OTA formation.

Coffee. In coffee, OTA infection occurs immediately after harvest, when green coffee cherries are handled, hulled, and dried. The process is often slow because in many regions coffee is grown under climatic conditions that favour mist and rain at harvest time. Poor storage is also a documented factor causing increases in OTA levels. Partial control can be achieved during the early stages of manufacture, where defective cherries, which frequently contain most OTA in a sample, are sorted out. Roasting of coffee reduces OTA levels by amounts that vary with the severity of the roasting process, e.g. from a 50% reduction for a light roast (12 minutes at 180 °C) to a > 90% reduction for a dark roast (8 minutes at 240 °C).

Cocoa and chocolate. OTA occurs in cocoa, and hence in chocolate, but levels are usually low. Rapid and adequate drying of cocoa beans is the important control step.

References. Taniwaki *et al*. (2003), Leong *et al*. (2006), Copetti *et al*. (2010), Ferraz *et al*. (2010).

5. Deoxynivalenol and zearalenone

5.1 Pre-harvest interventions

5.1.1 Breeding for host plant resistance in small grains

Description. Breeding methods have been explored to improve host plant resistance either to fungal infection or to other environmental stressors that would predispose the plants to accumulation of deoxynivalenol (DON) and, secondarily, zearalenone (ZEA). This has included work on identifying resistant cultivars and identifying biochemical and genetic resistance markers in cultivars. Some regions (notably Germany and Ontario, Canada) have stringent registration rules that place specific weight on eliminating the worst cultivars in trials each year.

Stage of development. Commercial.

Efficacy. Efficacy varies, depending on climatic conditions by year and on agronomic factors, including the topology of the field. Current efforts are focusing on developing high-yielding cultivars with good quality characteristics and moderate resistance to Fusarium head blight and DON production.

Geographical regions. Cultivars with reasonable efficacy are in use in the USA, Canada, and Europe.

Simplicity/complexity. Although the breeding process involves significant expertise and expense at first, resistant seeds can be disseminated as readily as other types of seeds, within the area of adaptation. Because small grains are open-pollinated, seeds can be saved from year to year.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. Local lines can be identified for suitability to the geographical region through the International Maize and Wheat

Improvement Center (CIMMYT) shuttle breeding program.

Accessibility. Likely to be widely accessible after local varieties with improved traits are developed.

References. Mesterhazy *et al*. (1999), Boutigny *et al*. (2008), Foroud and Eudes (2009), Snijders (2004), Müller *et al*. (2010).

5.1.2 Breeding for host plant resistance in maize

Description. Breeding methods have been explored to improve host plant resistance either to fungal infection or to other environmental stressors that would predispose the plants to accumulation of DON and, secondarily, ZEA. This has included work on identifying resistant cultivars and identifying biochemical and genetic resistance markers in hybrids. Some regions (e.g. Ontario, Canada) have stringent registration rules that place specific weight on eliminating the worst hybrids in trials each year.

Stage of development. Commercial.

Efficacy. Moderate resistance has been achieved.

Geographical regions. Cultivars with moderate resistance are in use in the USA, Canada, and Europe.

Simplicity/complexity. Although the breeding process involves significant expertise and expense at first, resistant seeds can be disseminated as readily as other types of seeds, within the area of adaptation.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. Local inbred varieties can be identified for suitability to the geographical region through the CIMMYT shuttle breeding program.

Accessibility. Likely to be widely accessible after local varieties with improved traits are developed.

References. Boutigny *et al*. (2008), Loeffler *et al*. (2010) and references therein.

5.1.3 Transgenic Bt maize

Description. Transgenic Bt maize contains a gene from the soil bacterium *Bacillus thuringiensis* that encodes for proteins toxic to key insect pests of maize. Insect damage predisposes maize to DON and ZEA contamination by facilitating fungal infection.

Stage of development. Commercial.

Efficacy. Reductions of DON levels by up to 59% and significantly lower ZEA levels in animal feed made from Bt maize compared with maize lacking the Bt gene.

Geographical regions. USA, Canada, Argentina, Brazil, Uruguay, South Africa, Honduras, the Philippines, Hungary, China.

Simplicity/complexity. Although the transgenic breeding process involves substantial initial expertise and expense, Bt seeds, once developed, can be disseminated as easily as other types of seeds.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. No.

Locality of resources. Not local; requires reliance on biotechnology companies to permit small-scale farmers access to seed at prices under fair conditions.

Accessibility. High in developed countries that have permitted Bt maize planting and commercialization; low elsewhere worldwide.

References. Munkvold *et al*. (1999), Aulrich *et al*. (2001), Magg *et al*. (2002), Schaafsma (2002), Wu (2007), Burachik (2010), Folcher *et al*. (2010), Loeffler *et al*. (2010).

5.1.4 Fungicides

Description. Chemicals that control fungal species; azoles, commonly used to control Fusarium head blight, inhibit sterol biosynthesis in *Fusarium* species. However, an important issue for high efficacy of fungicides is the correct timing of application during anthesis, the optimal time for *F. graminearum* infection and DON accumulation.

Stage of development. Commercial.

Efficacy. Varies depending on fungal infection risk by year.

Geographical regions. Worldwide.

Simplicity/complexity. Simple to implement, although basic education is required.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. No.

Locality of resources. Widely available in developed countries, much less so in LICs; cost implications for subsistence farmers.

Accessibility. Accessible in developed countries, much less so in LICs.

References. Hollingsworth *et al*. (2008), Odenbach *et al*. (2008), Paul *et al*. (2008), Zhang *et al*. (2009).

5.1.5 Forecasting

Description. The most evolved models for forecasting the formation of DON in small grains were developed in Ontario, Canada. Only preliminary work has been reported on a model for DON in maize.

Stage of development. Commercial in Ontario, Canada, and in Europe.

Efficacy. Analyses of data from maize produced a model with initial validation, i.e. data for enough years of variation in two countries to explain 70% of variation in fumonisin accumulation. A different model, developed in Italy, has had only limited validation thus far.

Geographical regions. These models have been applied in Uruguay, in France, and (since 2009) throughout Europe.

Simplicity/complexity. These models are used to enable management decisions, e.g. harvest time, whether foliar fungicides would be useful in a given year, and crop segregation. These models must be tested and validated in many areas, not only to

make the models more widely useful but also to test their robustness to climatic variation. As with all predictive models, these require reliable climatic and agronomic data and some sophisticated mathematics.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. To date, Canada and Europe.

Accessibility. High in regions of the world for which forecasting models have been developed (Canada, Europe); low elsewhere worldwide.

References. de la Campa *et al*. (2005), Hooker and Schaafsma (2005), Schaafsma and Hooker (2007), Maiorano *et al*. (2009), Müller *et al*. (2010), Van der Fels-Klerx *et al*. (2010).

5.2 Post-harvest interventions

Because *Fusarium* species do not grow at water activities below about 0.90, DON and ZEA are not produced in grains that have been even partially dried. Post-harvest treatments are not applicable to these mycotoxins in wheat or maize.

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