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## Fumonisin in Maize: Can We Reduce Their Occurrence?

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# Fumonisin in Maize: Can We Reduce Their Occurrence?

## **Abstract**

In 1988, W. C. A. Gelderblom et al. reported that the fumonisins (Fig. 1), a new class of mycotoxins, had been identified from cultures of *Fusarium moniliforme* J. Sheld., and that these toxins had cancerpromoting activity (10). This report represented a major breakthrough in nearly a century of investigation into the animal and human diseases associated with consumption of maize contaminated with *F. moniliforme*. This also was the starting point for worldwide efforts to describe the structure, properties, and toxicology of this new group of toxins. Additionally, the report renewed interest in the phytopathology of the most notorious pathogen of maize.

## **Disciplines**

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## **Comments**

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# Fumonisin in Maize

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## *Can We Reduce Their Occurrence?*

In 1988, W. C. A. Gelderblom et al. reported that the fumonisins (Fig. 1), a new class of mycotoxins, had been identified from cultures of *Fusarium moniliforme* J. Sheld., and that these toxins had cancer-promoting activity (10). This report represented a major breakthrough in nearly a century of investigation into the animal and human diseases associated with consumption of maize contaminated with *F. moniliforme*. This also was the starting point for worldwide efforts to describe the structure, properties, and toxicology of this new group of toxins. Additionally, the report renewed interest in the phytopathology of the most notorious pathogen of maize.

*F. moniliforme* (teleomorph: *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura) was first described and associated with animal diseases in 1904 (50). This species and other anamorphs of *G. fujikuroi* (*F. proliferatum* and *F. subglutinans*) are the fungi most commonly associated with maize production in North America and many other temperate regions of the world. *Fusarium* species are capable of causing seedling diseases, root rots, stalk rots (Fig. 2), and ear rots of maize (Figs. 3 and 4), as well as damaging stored grain. Kernel damage by *G. fujikuroi* is typically minor compared with that caused by *G. zaeae*, with some notable exceptions (6) (Fig. 3B). Although yield usually is not much affected, kernel infection by *G. fujikuroi* is of concern because of the loss of grain and seed quality and the potential occurrence of fumonisins and other mycotoxins. This situation is further complicated by the common occurrence of fumonisins in symptomless infected kernels (33,34,36,39).

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*G. fujikuroi* consists of at least seven distinct mating populations, designated A through G (24); populations A, D, and E are the most common on maize. Most of the mating populations correspond to particular anamorph species in the Liseola section of *Fusarium*, but both the A and the

F mating populations are considered to be *F. moniliforme* on the basis of morphology. This is an important distinction because the A population contains many prolific fumonisin-producing strains, while members of the F population produce little or no fumonisin. Most strains found on maize are

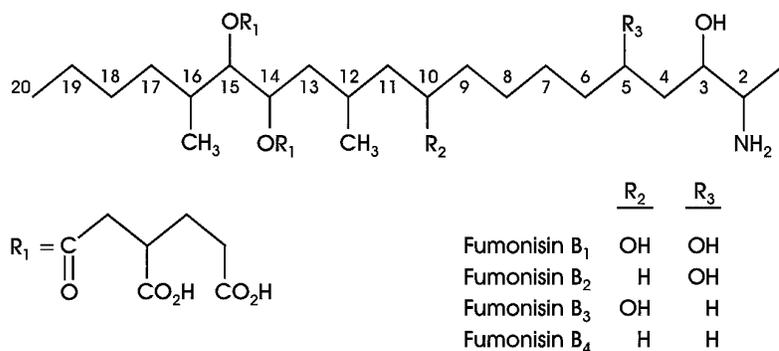


Fig. 1. Chemical structures of the fumonisins.



Fig. 2. Decay of maize stalk base caused by *Fusarium moniliforme*.

population A, and most found on sorghum are F (26). In this article, further reference to *F. moniliforme* will pertain only to population A. Population D (*F. proliferatum*) also contains many strains that produce copious amounts of fumonisins, but strains in populations B and E (*F. subglutinans*) produce little or no fumonisin (26). Several other species have recently been shown to be capable of producing fumonisins, including *F. anthophilum*, *F. napiforme*, and *F. nygamai*. Some complications occur because other *Fusarium* species have frequently been misidentified as *F. moniliforme*. In maize grown in temperate regions, *F. moniliforme* and *F. proliferatum* represent the greatest threat for fumonisin production.

A large body of literature on *F. moniliforme* and fumonisins has developed, including some excellent review articles (29,38,39). In spite of more than 90 years of research on the biology, pathology, toxicology, and genetics of this fungus, there are still few options for the effective management of the diseases it causes in maize. Recent international developments in mycotoxin regulation have increased pressure to find strategies to manage the contamination of maize by fumonisins. This problem is unique because of the close associa-

tion of *F. moniliforme* with maize plants and the implications that this plant disease has for animal and possibly human health. The focus of this article is to describe the scope of problems caused by *F. moniliforme* and other fumonisin-producing species and to explore existing and potential strategies for reducing the occurrence and/or impact of fumonisins in maize.

### The Maize-*F. moniliforme* Relationship

*F. moniliforme* is associated with disease at all stages of maize plant development, infecting the roots, stalk, and kernels. This fungus is not only the most common pathogen of maize; it also is among the most common fungi found colonizing symptomless maize plants. *F. moniliforme* is an almost constant companion of maize plants and seed. In many cases, its presence is ignored because it is not causing visible damage. Symptomless infection can exist throughout the plant, and seed-transmitted strains of the fungus can develop systemically to infect the kernels (20,35). By most definitions, this relationship can be referred to as endophytic (52).

If *F. moniliforme* is an endophyte of maize, we may have much to learn from research on other endophytic microorgan-

isms. While the relationship between *F. moniliforme* and maize is not as intimate as that between tall fescue and its endophytes (*Neotyphodium* spp.), there are some striking similarities. In both systems, the plant-microbe symbiosis can result in toxicoses in livestock. In both systems, the toxic effects of the microorganism have been addressed by attempts to eliminate it from the host. These efforts have met great challenges in the case of tall fescue, because the endophytic fungi are beneficial to their host plants. These benefits include enhanced root development, drought tolerance, and protection against pathogens (52). Therefore, it is very difficult to produce endophyte-free tall fescue populations that retain desirable agronomic characteristics. In addition, forage grass endophytes protect the plants from herbivores by producing toxic compounds: a benefit to the plant, but a detriment to livestock producers.

Is *F. moniliforme* beneficial to maize plants? In some ways it clearly is not, but this possibility has not been thoroughly investigated. Exploring possible benefits of *F. moniliforme* infection is challenging because the fungus is ubiquitous in maize; therefore, it is difficult to establish noninfected control plants. There have been



Fig. 3. (A) Moderate *Fusarium moniliforme* ear rot symptoms common in the midwestern United States. (B) Severe *F. moniliforme* ear rot symptoms common in central California (photo courtesy B. Anderson, Pioneer Hi-Bred International).

some reports that *F. moniliforme* infection can stimulate growth and development of maize plants, possibly due to the production of plant-growth promoting hormones (65). Perhaps *F. moniliforme* infection protects maize plants against more destructive pathogens or insects. Some reports indicate reduced *Aspergillus flavus* infection and reduced aflatoxin development in maize ears co-inoculated with *F. moniliforme* (66). *F. moniliforme* also has been reported to protect maize seedlings from infection by *F. graminearum* (59). Effects on other pathogens have not been investigated, nor have other possible benefits of infection of maize plants by *F. moniliforme*.

While many infected plants are symptomless, damage to stalks and ears is sometimes dramatic (Figs. 2 to 4). When severe kernel damage is present, strategies to manage infection by this fungus are similar to those used for managing other ear rot diseases. Careful adjustment of the combine, followed by grain cleaning, removes the vast majority of rotted kernels. Proper drying and storage of grain prevents further fungal development. In this context, disease resistance can be detected by a visual rating of disease severity, and selections can be made for more resistant genotypes.

Symptomless infection is more difficult to manage. These kernels cannot be removed from grain by any standard cleaning method. Seed cleaning methods, such as gravity tables, may remove some infected seeds, but the prevalence of the fungus in commercial corn seed indicates that this procedure is not completely effective. Further work is needed on seed condition-

ing methods to remove symptomless infected kernels. Another complication with symptomless infection is that most resistance screening relies on visual ratings. With *F. moniliforme*, there is often a poor relationship between levels of symptomatic and symptomless infection.

The cycle of infection and disease in the *F. moniliforme*-maize system (Fig. 5) is complex, and the relative importance of its components is still under debate. The fungus survives in crop residue but is usually not among the common *Fusaria* found there (25). It does not produce chlamydospores, but it can produce thickened hyphae that apparently prolong its survival (21). *F. moniliforme* is seedborne and seed-transmitted (31). This phase of the disease cycle has been associated primarily with seedling disease; the role of seed transmission in stalk and kernel rot is not as clear, but strains from the seed can be found throughout the plant in some cases (20,35). Seed can be infected with no detrimental effects on the seedlings (21,31). *F. moniliforme* produces macroconidia and abundant microconidia, which are airborne in maize fields (21,38). Microconidia may also facilitate movement within the plant. The source of airborne conidia is believed to be crop residue, but sporulation of the fungus on the tassels may contribute to silk infection (27). Maize silks can be infected by airborne or water-splashed conidia. Exact conditions that favor silk infection are not known, but infection is enhanced by maintaining moisture on the silks, and some researchers have shown a positive correlation between infection and late-season rainfall (21). The physiological state of the silks also affects susceptibility (17).

Some factors may predispose maize ears to *F. moniliforme* infection, such as damage caused by other ear rot fungi (47).

Insects play an important role in infection of maize plants by *F. moniliforme*. Injuries caused by insects are common sites of infection of maize ears and stalks (Fig. 6). Infection of the wounded tissue often occurs due to airborne or rain-splashed inoculum that arrives subsequent to the insect injury, but some insects can act as vectors. The most commonly cited insect associate is the European corn borer, *Ostrinia nubilalis*. Various other insects have been investigated as vectors or wounding agents involved in *F. moniliforme* infection. These include the corn earworm, corn rootworm (larval and adult stages), Western flower thrips, and sap beetles (family Nitidulidae, particularly *Glischrochilus quadrisignatus*). The fungus can be isolated externally from several of these insect species, but strong evidence for a vector relationship has been presented only for the European corn borer (53) and the Western flower thrips (6). Movement of *F. moniliforme* by European corn borer larvae is probably limited to spread from plant surfaces to kernels (53). It is likely that other, more mobile insects such as *G. quadrisignatus* (21) and the corn rootworm beetle also can act as vectors, spreading the fungus over longer distances.

### *F. proliferatum*

Many *F. proliferatum* strains are capable of producing large quantities of fumonisins, but very little is known about the ecology and epidemiology of this species. In literature published prior to 1976, *F. proliferatum* was universally misidentified as *F. moniliforme*, and even subsequent to the published description of *F. proliferatum*, it continues to be misidentified frequently as *F. moniliforme*. *F. proliferatum* is nearly as common in temperate-region maize as *F. moniliforme*, and it can be isolated from symptomatic and symptomless tissues, including seed. *F. proliferatum* also is a common pathogen of other crops, particularly asparagus. This species shares many morphological characteristics with *F. moniliforme*. It seems likely that the two species also share a similar disease cycle, but this remains to be demonstrated.

### Toxicity of Fumonisins: Naturally Occurring Toxicoses

Although the fumonisins were discovered only recently, the toxicity of corn contaminated by *F. moniliforme* has been well-documented for more than a hundred years. A disease of farm animals known as "moldy corn poisoning" or "blind staggers" was first described in the United States in 1850 (13). The causative agent remained unknown until Sheldon (50) and others identified *F. moniliforme* and associated it with an outbreak of moldy corn disease of horses, cattle, mules, hogs, and



Fig. 4. *Fusarium moniliforme* mycelium and white streaking ("starburst") symptom on maize kernels.

chickens in Nebraska. The most dramatic manifestation of moldy corn disease is equine leucoencephalomalacia (ELEM), a fatal brain disease of horses, donkeys, mules, and rabbits (29,39,62). In horses, this disease typically results in death within a few hours to 1 week. In 1971, ELEM was produced by feeding horses material contaminated with a pure culture of *F. moniliforme* (62), which firmly established this fungus as the causative agent.

The South African research group that was the first to identify and characterize the fumonisins was also the first to demonstrate that pure fumonisin B<sub>1</sub> (FB<sub>1</sub>) is able to produce ELEM in a horse (30). Horses appear to be unusually sensitive to fumonisins. Surveys of feed samples associated with outbreaks of ELEM in North America, South America, and Africa found FB<sub>1</sub> levels ranging from 0.2 to 126 µg/g feed (29). Experiments to determine the minimum toxic dose of fumonisins indicate that ponies consuming naturally contaminated feeds containing FB<sub>1</sub> at levels as low as 8 µg/g feed are at risk for developing ELEM (63).

ELEM may be the most dramatic, but it is certainly not the only animal disease associated with consumption of feed contaminated with *F. moniliforme*. A 1981 study of the oral toxicity of *F. moniliforme*

culture material to various animal species reported the deaths of two of three treated pigs (22). The principal lesions, however, were not in the brain, but in the lungs, where a fatal edema developed. Subsequently, in 1989, consumption of corn contaminated with *F. moniliforme* and fumonisins was associated with numerous outbreaks of porcine pulmonary edema (PPE) in the central United States and in Georgia (14). Researchers from the University of Georgia and elsewhere soon demonstrated that PPE could be produced by intravenous injection of pure FB<sub>1</sub> (4,15). Surveys of feed samples associated with outbreaks of PPE in North and South America found FB<sub>1</sub> levels ranging from 2 to 330 µg/g feed (29). Although pigs are unusually sensitive to trichothecene mycotoxins produced by *Fusarium* species, they appear to be considerably less sensitive than horses to fumonisins. Survey data and feeding experiments suggest that pigs consuming naturally contaminated feeds containing approximately 100 µg of fumonisins per g (FB<sub>1</sub> and FB<sub>2</sub>) are at risk for PPE (29). To our knowledge, however, this lung disease has not yet been produced by oral dosage with pure fumonisins.

Although the concentrations of fumonisins associated with adverse health effects are variable, the American Associa-

tion of Veterinary Laboratory Diagnosticians (AAVLD) has recommended maximum safe concentrations for livestock species (Table 1) based on currently available data.

## Human Health Hazards

Although the role of fumonisins in some moldy corn diseases of livestock has now been well-established, their role in human diseases and, most particularly, their carcinogenic potential in humans are much more difficult to determine. The International Agency for Research on Cancer (IARC), the South Africa Medical Research Council, the U.S. Food and Drug Administration, the U.S. Department of Agriculture, and many other agencies are in the process of evaluating the carcinogenic potential of fumonisins. These efforts include studies of carcinogenicity in experimental animals, studies of the mechanism of action of fumonisins in various animal models and in cell culture systems, and epidemiological studies of humans.

The search for causes of the high rate of esophageal cancer in the Transkei region of South Africa and in central China led to

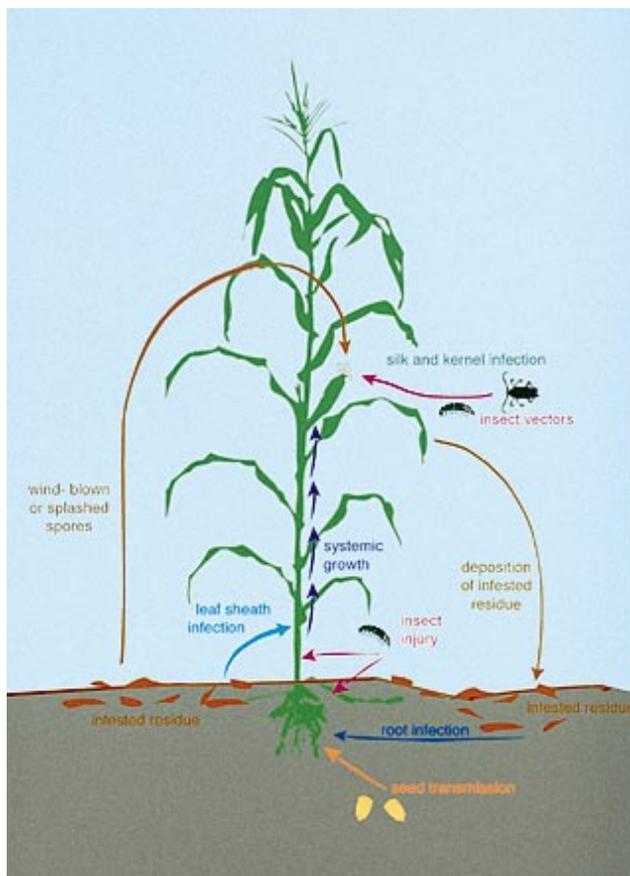


Fig. 5. Disease cycle of *Fusarium moniliforme* on maize. Various infection pathways are illustrated, but their relative importance is not indicated. The most common pathway to kernel infection is through silks or insect injuries.



Fig. 6. *Fusarium moniliforme* infection of insect-damaged maize ear.

Table 1. Recommended maximum fumonisin B<sub>1</sub> concentrations for livestock feed (32)

Species	Recommended max. conc.
Horses and other equine species	5 µg/g
Porcine species	10 µg/g
Beef cattle	50 µg/g
Dairy cattle	Not specified
Poultry	50 µg/g

the discovery of unusually high levels of fumonisins in corn that was being used for human consumption in these regions. The fumonisin (FB<sub>1</sub>) levels in some of these corn samples are certainly very high, up to 118 µg/g in the Transkei and up to 155 µg/g in central China (45,51). Nonetheless, the number of corn samples analyzed in these studies is quite small for an epidemiological analysis, comprising approximately 150 samples in the Transkei and only 31 samples in central China. The IARC has thus determined that there is not yet sufficient evidence to classify fumonisin itself as a human carcinogen, although it has classified "toxins from *Fusarium moniliforme*" as Class 2B, possibly carcinogenic to humans (3).

### Toxicity and Carcinogenicity in Experimental Animal Systems

The structural similarity of fumonisins to the long-chain base backbones of sphingolipids led Wang et al. (60) to propose and subsequently demonstrate that fumonisins affect sphingolipid metabolism. In cultured cells from rat liver and pig kidney, fumonisin B<sub>1</sub> inhibited the activity of sphingosine *N*-acetyl transferase (ceramide synthase). In vitro, this inhibition led to the rapid accumulation of high levels of the sphingoid base sphinganine, an increase in the level of the sphingoid base sphingosine, and the depletion of complex

sphingolipids. In fact, high levels of free sphinganine and/or sphingosine in serum and urine may be useful molecular biological markers for dietary exposure to fumonisins in humans and animals.

With pure fumonisins now more widely available, the carcinogenic potential of fumonisins is under study in a variety of experimental animals. Gelderblom and coworkers in South Africa (11) demonstrated that pure FB<sub>1</sub> can cause an increased incidence of hepatocellular carcinoma when fed to rats for 2 years at 50 µg/g feed. Subsequent studies demonstrated hepatocarcinogenesis of FB<sub>1</sub>, FB<sub>2</sub>, and FB<sub>3</sub> in short-term (3 week) initiation-promotion bioassays in the rat (9). Fumonisins are also acutely hepatotoxic, and it has been proposed that liver cell necrosis and regeneration play a role in carcinogenicity of fumonisins (9).

Although current models link the biological activities of fumonisins to sphingolipid biosynthesis, we still have much to learn about the mechanism(s) by which fumonisins are toxic and carcinogenic. A review of sphingolipids is beyond the scope of this paper; we refer the reader to the excellent recent review by Riley et al. (46). Sphingolipids are structural components of eukaryotic cell membranes; however, there is increasing evidence that sphingolipids also affect cellular proliferation and differentiation, and that they

regulate apoptosis, programmed cell death. Recent studies also demonstrate that treatment with FB<sub>1</sub> induces apoptosis and blocks cell proliferation in several types of cultured human and animal cells (56,61). The role of sphingolipids and the sphingolipid-analog mycotoxins in programmed cell death is a fast-developing field of research that should provide insights into the diseases caused by consumption of fumonisins.

### Toxicity to Plants

The high frequency of fumonisin production among strains of *G. fujikuroi* mating population A from maize, and the high frequency of fumonisin contamination in maize, raise the possibility that fumonisins play a role in virulence on maize. Further indirect evidence is provided by the structural similarity of fumonisins to AAL-toxin, which is a virulence factor of *Alternaria alternata* f. sp. *lycopersici* in tomato (12). In addition, purified fumonisins at low (10<sup>-6</sup> M) concentrations have been shown to cause necrosis and other symptoms in maize seedlings, tomato seedlings, and other plants (1,12,23).

Classical genetic analysis in *G. fujikuroi* mating population A also provides some evidence that fumonisins are virulence factors in maize seedling blight. Segregation ratios in progenies from crosses between field strains showed that a single

**Table 2.** Occurrence of fumonisins in maize samples collected in various countries

Country	Sample type	Samples (no.)	Fumonisin incidence <sup>a</sup>	Fumonisin B <sub>1</sub> (µg/g) <sup>b</sup>		Detection limit (µg/g)	Ref
				Maximum	Mean		
U.S.A.	Feed-PPE <sup>c</sup>	83	88	330	63	1	51
U.S.A.	Feed-ELEM <sup>d</sup>	98	87	126	29	1	51
U.S.A.	Screenings	160	100	239	21	0.1	51
China	Maize-EC <sup>e</sup>	31	100	155	54	1	51
S. Africa	High EC	12	92	118	28	0.05	45
S. Africa	High EC	24	100	47	13	0.05	45
Brazil	Feed	21	67	38	9	1	51
Kenya	Maize	33	18	47	15	0.1	... <sup>f</sup>
Brazil	Maize	48	100	18	2-11	0.1	18
Italy	Maize	33	58	5	2	0.1	51
Italy	Foods	29	62	6	3	0.1	51
U.S.A.	Maize 1988	22	73	15	2	0.1	51
U.S.A.	Maize 1989	49	67	28	3	0.1	51
U.S.A.	Maize 1990	59	88	19	3	0.1	51
U.S.A.	Maize 1991	50	96	16	3	0.1	51
U.S.A.	Maize 1993	230	22	6		0.5	36
U.S.A.	Maize 1994	245	11	10		0.5	33
U.S.A.	Maize 1995	639	46	24	1-2	0.5	34
U.S.A.	Feed	51	37	9	4	1	51
S. Africa	Low EC	23	52	19	3	0.05	45
S. Africa	Low EC	15	87	11	2	0.05	45
U.S.A.	Foods	20	50	7	2	0.1	42
U.S.A.	Foods	35	86	3	1	0.05	51
S. Africa	Foods	81	72	0.5	0.1	0.05	51
Switzerland	Foods	120	37	0.8	0.1	0.05	51

<sup>a</sup> Incidence = percent positive samples.

<sup>b</sup> FB<sub>1</sub> was determined by high performance liquid chromatography.

<sup>c</sup> PPE = porcine pulmonary edema.

<sup>d</sup> ELEM = equine leucoencephalomalacia.

<sup>e</sup> EC = human esophageal cancer.

<sup>f</sup> C. J. Kedera and R. D. Plattner, *personal communication*.

locus, designated *fum1*, controls production of fumonisins. When progeny of such a cross were analyzed for virulence in a maize seedling assay, high levels of virulence were strongly associated with production of fumonisins (7). This evidence, however, is not conclusive because the parents of this test cross were field strains that were isolated from different geographic areas and are likely to differ at many genetic loci important for virulence. Localization of the *fum1* locus by physical mapping is in progress (44,64) and should facilitate specific disruption of the *fum1* gene to conclusively determine the importance of fumonisins as virulence factors of *G. fujikuroi* mating population A on maize.

### Occurrence of Fumonisins

Surveys of the natural occurrence of fumonisins in maize are necessary to accurately assess human and animal exposure to these toxins. A range of methods is available for detecting fumonisins at the ng/g and µg/g levels at which they commonly occur in naturally contaminated maize. Liquid chromatography of fluorescent, derivatized fumonisins is the most widely used method for detecting fumonisins in maize samples. Fumonisin detection limits for liquid chromatography are generally in the range of 0.01 to 0.05 µg/g sample dry weight (43).

Because *F. moniliforme* infects maize worldwide, it is not surprising to find that fumonisins contaminate maize from every geographic region tested to date. Table 2 presents a comparison of the incidence of positive samples, maximum level, and mean level of positive samples for FB<sub>1</sub> in a selection of 25 surveys of maize and maize-based foods and feeds. Surveys were selected for this comparative assessment based on a sample size greater than 10, a chromatographic analytical method, and a clearly stated FB<sub>1</sub> detection limit for that method. To simplify comparisons, only FB<sub>1</sub> levels are included, although FB<sub>2</sub> was also quantitated in several of these surveys. The 25 surveys are ordered in Table 2 by their maximum and mean FB<sub>1</sub> levels, with the most contaminated maize samples at the top.

Two surveys of maize-based feed samples associated with animal disease problems ELEM and PPE present the highest FB<sub>1</sub> contamination, with 87 to 88% of the samples above detection limits of 1 µg/g, maxima of 126 to 330 µg/g, and means of 29 to 63 µg/g. Corn screenings from the central United States comprise the third most contaminated sample type, with a maximum of 239 µg/g and a mean of 21 µg/g. The next most contaminated group comprises maize collected from high esophageal cancer areas of China and South Africa, which shows a high incidence of FB<sub>1</sub>-contaminated samples, with maxima from 47 to 155 µg/g and means from 13 to 54 µg/g.

Fumonisins are commonly detected in symptomless maize kernels. However, fumonisin levels in randomly selected good quality maize are generally much lower than in maize samples associated with human and animal health problems. Surveys of 1,300 maize samples collected in the central United States from 1988 through 1995 indicate that although FB<sub>1</sub> is present in most samples, the levels are generally low, with maxima of 5 to 38 µg/g and means of 1 to 3 µg/g. Surveys of good quality maize and maize-based foods from other countries have generally been more limited but indicate that a majority of samples contain fumonisins, although the levels are generally less than 1 µg/g. These limited surveys do, however, indicate that human food samples from a number of countries, including Brazil, Italy, Kenya, and the United States, may occasionally be contaminated with FB<sub>1</sub> at levels of 5 to 10 µg/g or more, which would generally be considered a level of concern. It should also be kept in mind that many surveys report data for levels of FB<sub>1</sub> only, whereas samples that contain FB<sub>1</sub> usually contain lower levels of several other, closely related fumonisins.

### Risk Assessment of Fumonisins in Maize

Estimating human and animal risk due to the presence of fumonisins in maize-based foods and feeds involves many factors. First, toxicity tests indicate that fumonisins cause adverse effects in a wide range of animal species, with disease symptoms usually observed at FB<sub>1</sub> levels of 5 to 10 µg/g feed, although physiological changes may occur at lower concentrations. There are species differences in fumonisin sensitivity and target organ specificity that make it difficult to extrapolate from animal data to humans. Human consumption of maize-based foods varies widely among populations around the world and even within the United States. It is especially difficult to estimate the risks of long-term chronic exposure to low levels of fumonisins in humans.

Secondly, it seems reasonable to conclude from surveys conducted to date that maize usually contains fumonisins. Furthermore, because of the close relationship of *F. moniliforme* with maize, fumonisins cannot be completely eliminated without banning maize as a food and feed ingredient, an economically and politically unrealistic option. Maize is one of the most important agricultural commodities in the United States, largely because it is the major ingredient of animal feeds. Maize meal is also a human dietary staple in many regions of Africa, Asia, and Central and South America. In North America and Europe, maize products are important components of many processed foods, including breakfast cereals, snacks, soft drinks, and beer. Fumonisins are not de-

stroyed by many of the methods used for food processing. Thus, technology is not yet available to ensure that all maize-based foods and feeds are completely free of fumonisins.

Although fumonisins are natural contaminants, they are not inherent components of maize, and are thus considered to be "added substances" by the criteria of the U.S. Food and Drug Administration (FDA). Because fumonisins cannot be completely eliminated from the food supply, the goal of the FDA is to determine an Acceptable Daily Intake, "a level that would result in negligible risk or a reasonable certainty of no harm" (57). The goal of the FDA, and of similar agencies in other countries, is to protect public health while minimizing the costs of risk assessment and risk management. The FDA has a range of risk management options that have been used to regulate a number of mycotoxins and could be used to regulate fumonisins. Current FDA guidelines include "action levels" for aflatoxins in a number of crops and "advisories" for deoxynivalenol in wheat. Action levels do not have the force and effect of laws but serve to guide FDA enforcement actions, such as preventing import or distribution of contaminated products (57).

More than 70 countries are known to regulate mycotoxins in human foods and animal feeds (58). Regulated mycotoxins include aflatoxins in maize, peanuts, cottonseed, and milk; deoxynivalenol and other trichothecenes in maize and wheat; ochratoxins in grains; patulin in apple juice; and FB<sub>1</sub> in maize. Tolerance levels often vary widely among countries, which can impact both import and export of agricultural products. More specifically, recent decisions to regulate fumonisins in maize imported into some European countries may impact maize exports from the United States to Europe.

### Current Methods for Reducing Fumonisins

Maize producers in the United States currently direct very little effort specifically toward the reduction of fumonisins in grain. Because of the general lack of regulatory guidelines in the United States and the sporadic nature of PPE and ELEM outbreaks, maize producers have not considered fumonisin reduction to be a high priority. However, several current management practices can impact fumonisin concentrations. These fall into two general categories: genetic resistance to *F. moniliforme*, and grain handling and processing to remove infected kernels and prevent continued fungal development after harvest.

Inheritance of resistance to *F. moniliforme* has been studied using both visual ratings and symptomless infection as selection criteria. Scott and King (48) showed that susceptibility to symptomless

infection was conditioned by the maternally inherited genotype of the pericarp. Other studies identified sources of resistance in sweet corn and confirmed maternal inheritance of resistance, which was expressed in the pericarp and silks (16). While these advances have been made, *F. moniliforme* resistance traditionally has not been a high priority in dent corn hybrid development. Most seed corn companies discard genotypes that are very susceptible, but little effort has been made to intentionally screen for resistance or incorporate known sources of resistance. This approach is problematic because selections are made based on visual ratings, which do not always reflect symptomless infection or fumonisin levels. Further complications arise because methods for inducing *F. moniliforme* symptoms are not always successful; it is sometime impossible to separate genotypes based on visual symptoms. Unfortunately, it is not practical to screen large numbers of genotypes for symptomless infection because the cost of culturing vast numbers of kernels is prohibitive. Currently used selection procedures discourage the utilization of genotypes that are highly susceptible to visible ear rot. Nevertheless, *Fusarium* ear rot can be found at low levels in the majority of maize fields in the United States. There is very little published information on the relationship between fumonisin levels and *Fusarium* ear rot symptoms in commercial hybrids. Generally, higher levels of fumonisins are found in visibly moldy kernels (29,39,45), but some studies have shown a very poor correlation between *Fusarium* ear rot symptoms and fumonisin concentrations (36). One possible explanation is that symptoms caused by *F. subglutinans* (a nonproducer of fumonisins) can be indistinguishable from those caused by *F. moniliforme* and *F. proliferatum* (fumonisin producers). Therefore, it is difficult to assess whether selecting against ear rot susceptibility results in lower fumonisin concentrations. However, it has been observed that commercial hybrids differ in their tendency to accumulate fumonisins, and hybrids grown outside of their adapted range tend to accumulate higher concentrations (49).

A frequent observation has been that fumonisin levels are highest in maize screenings (39), which are the broken kernels and other fine material removed from grain passed over a wire screen. Screenings are often sold cheaply for use as a component of animal feed. This practice has led to the most severe documented outbreaks of ELEM and PPE (39). Damaged and broken kernels that may be high in fumonisins can be removed from grain both during the harvesting process and through subsequent cleaning. Disposal of this component of the grain can significantly reduce the mean fumonisin concentration of a grain load. There is a cost associated with

this practice, because no income is realized from the screenings unless a user can be located who wishes to feed less susceptible livestock species. Therefore, while this is a readily available practice, its implementation is not universal.

Standard grain storage procedures should prevent the development of fumonisins in stored grain. *F. moniliforme* has not been reported to grow in grain at a moisture content below 18 to 20% (21), well above the recommended level of 13 to 14% for long-term maize storage. Generally, fumonisin concentrations are not believed to increase during storage as long as proper conditions of grain moisture and temperature are maintained.

Fermentation of maize does not reduce fumonisin concentrations; in fact, beer made from contaminated maize is suspected as a primary source of human fumonisin consumption in areas of Africa with high levels of esophageal cancer. However, distillation of fumonisin-contaminated maize yields fumonisin-free ethanol (37). When fumonisin-contaminated maize is wet-milled, the majority of detectable fumonisin ends up in the gluten, fiber, germ, and steep water fractions, with very little or no fumonisins in the starch fraction (2). These results indicate that maize products derived from the starch fraction may be relatively low in fumonisins, while the products developed from the other fractions will contain a significant proportion of the original fumonisins.

### Developing Methods for Reducing Fumonisin

A number of strategies for reducing fumonisin concentrations in maize are currently under development. Some approaches are directed toward resistance to infection or reduction of fumonisins in the grain, while others are aimed at detoxification of contaminated maize.

Genetic engineering may provide innovative solutions to the problem of fumonisins in maize. Among the possibilities are genetically engineered resistance to *Fusarium* infection, or genetic engineering approaches to detoxification of fumonisins in planta.

Engineering plants to produce antifungal proteins is a possible approach to enhancing resistance to fungi. Various proteins identified from microorganisms or plants have been shown to have antifungal activity and have been proposed as resistance factors. Some work is under way to use this strategy for engineering resistance to *Fusarium* species, but it is only in the early stages. Another possible approach would be to identify naturally occurring components in maize that inhibit fumonisin synthesis. Concentrations of these components could be enhanced through selection or genetic engineering. Unfortunately, no such components have been identified.

Another genetic engineering approach currently under development is in planta detoxification of fumonisins. There is little or no evidence that fumonisins are enzymatically metabolized or altered in maize under normal field conditions. Fumonisin detoxifying enzymes could, however, be introduced via genetic engineering to prevent the accumulation of fumonisins in *Fusarium*-infected grain. No such enzymes capable of using fumonisins as a substrate have been available until recently. Two species of saprophytic fungi isolated from moldy corn ears were shown to be capable of utilizing FB<sub>1</sub> as a sole carbon and energy source; these fungi were shown to possess enzymes capable of hydrolyzing and further metabolizing fumonisins (8). A cDNA encoding a tricarballylate ester hydrolase ("fumonisin esterase"), has been cloned from one of these fungi, the black yeast *Exophiala spinifera* (8). Research is currently under way to express this gene in transgenic maize in order to evaluate the effect on toxin accumulation and ear mold symptoms.

The exact cellular and subcellular sites of localization of fumonisin in *Fusarium*-infected grain are not known, leaving open the question of the toxin's bio-availability to a host-produced enzyme(s). Tejada-simon et al. (55) reported finding high levels of fumonisin in microconidia of *F. moniliforme* cultured in vitro. Since the toxin is water soluble, however, it could be reasonably expected to accumulate largely in the apoplasmic environment of an infection site where a host enzyme could also be sequestered. The transgene study mentioned above will address the feasibility of detoxifying fumonisins at their site of production in the plant, since the major product of fumonisin hydrolysis can be readily detected and quantitated, along with intact fumonisin, from organic extracts of *Fusarium*-infected seed expressing the esterase gene.

Maize is utilized in many end products and is subjected to various processing methods. The effects of some of these methods on fumonisin concentrations are under investigation. Additionally, other processing methods specifically aimed at detoxification have been attempted. A general complication to many of these studies is the fact that reductions in detectable fumonisins do not always result in reduced toxicity. The fumonisin molecules may be altered by various treatments in such a way that they are not detectable but are still toxic. Therefore, it is crucial that toxicity assays of the end products of these treatments accompany the fumonisin detection data.

The effects of heat on fumonisin toxicity are not completely clear. Some reports indicated significant reductions in fumonisin concentrations as a result of heating aqueous solutions to 150°C or higher (19), or heating moist maize kernels (37).

However, heating apparently causes hydrolysis of the primary amine group of the fumonisins, leaving the backbone of the molecule intact. Toxicity of the hydrolyzed product has been demonstrated in some experimental systems. Other reports found that fumonisin concentrations were not reduced by heat treatment (54).

When maize is made into tortilla flour, it is subjected to a process known as nixtamalization. During this process, the maize is treated with  $\text{Ca}(\text{OH})_2$  and heated. Research has shown that  $\text{Ca}(\text{OH})_2$  treatment can reduce detectable  $\text{FB}_1$  concentrations (37,40,54), but there are conflicting reports about the toxicity of the hydrolyzed end products. Murphy et al. (37) found that the hydrolyzed fumonisin may be nearly as toxic as unaltered  $\text{FB}_1$ . Similar results were reported by Park et al. (40), but these workers found that a modified nixtamalization process resulted in some detoxification.

Attempts to detoxify fumonisins by chemical methods have met with limited success. Several commercially available enzymes have been tested for their ability to detoxify fumonisins (37). None of these products significantly reduced recovery of  $\text{FB}_1$ . Ammoniation, tested as a detoxification method for other mycotoxins (particularly aflatoxins), may successfully detoxify the fumonisins when combined with high temperature (41). Low temperature ammoniation has not been successful (54). A promising method for detoxification was recently reported. Nonenzymatic browning is a reaction that occurs in the presence of a primary amine, a reducing sugar, and water at  $\text{pH} > 7$ . This reaction results in the removal of the primary amine group from the fumonisin molecule. Lu et al. (28) reported that treatment of  $\text{FB}_1$  with fructose under these conditions resulted in a significant reduction in detectable  $\text{FB}_1$ . More importantly, when a diet containing the product of the  $\text{FB}_1$ -fructose reaction was fed to rats, it was not toxic and did not result in cancer initiation (28). Another reaction that has been investigated is the treatment of maize with a combination of  $\text{H}_2\text{O}_2$  and  $\text{NaHCO}_3$ . Park et al. (40) reported that this reaction reduced fumonisin concentrations by up to 100% in contaminated maize. Toxicity of the end products of this reaction was greatly reduced compared with untreated maize. However, a great deal of work is needed before this or any detoxification method can be used in a commercial setting.

### Future Outlook

Currently, there are no proven, practical methods for significantly reducing fumonisin concentrations in maize. There is considerable debate over whether efforts in this direction are warranted. The health risks posed to humans are still unclear. Because of the lack of fumonisin regula-

tions in the United States and in major importing nations, maize producers have little motivation to support efforts to reduce fumonisins. Eventually, it is likely that more nations will impose restrictions on fumonisins in imported maize. What action will be taken by the FDA is less clear. The major motivation for fumonisin reduction currently comes from the industries that utilize maize: the livestock and food processing industries. Major maize seed producing companies have recognized that in the future, there will be a growing demand for fumonisin-free maize, and this realization is reflected in ongoing genetic engineering efforts.

Biological control of *F. moniliforme* by competitive exclusion is untested under field conditions. There will be many obstacles to successful fumonisin reduction by biological control because of the numerous pathways for infection by the fungi. The most feasible approach may be to reduce infection by fumonisin-producing *Fusarium* strains through competition with nonpro-

ducing *Fusarium* strains. This approach has been used successfully with *Aspergillus flavus* to reduce aflatoxin concentrations in cottonseed in small field experiments (5). The ubiquitous nature of naturally occurring, fumonisin-producing *Fusarium* strains will make this approach extremely challenging, and EPA approval of such a practice may also pose an obstacle.

It is difficult to evaluate the success of traditional breeding in controlling fumonisin levels. It is possible that currently used selection methods have prevented even higher levels of fumonisins; nevertheless, substantial levels are occurring in the maize crops of many nations. Unless specific sources of resistance are identified and incorporated into modern hybrids, breeding will not contribute further to management of fumonisins. Genetic engineering approaches are the most attractive methods now under development, and the future of fumonisin reduction may very well lie in the hands of these researchers.



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## Dedication

This paper is dedicated to the memory of Paul E. Nelson, who inspired and taught a generation of *Fusarium* researchers. Paul was a generous cooperator and a good friend; we will miss him greatly.

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