Prospects for Reducing Fumonisin Contamination of Maize through Genetic Modification

Jon Duvick

Disease Resistance Group, Trait and Technology Division, Pioneer Hi-Bred, International, Inc., Johnston, Iowa, USA

Fumonisins (FB) are mycotoxins found in *Fusarium verticillioides*-infected maize grain worldwide. Attention has focused on FBs because of their widespread occurrence, acute toxicity to certain livestock, and their potential carcinogenicity. FBs are present at low levels in most field-grown maize but may spike to high levels depending on both the environment and genetics of the host plant. Among the strategies for reducing risk of FB contamination in maize supplied to the market, development and deployment of *Fusarium* ear mold-resistant maize germplasm is a high priority. Breeding for increased ear mold tolerance and reduced mycotoxin levels is being practiced today in both commercial and public programs, but the amount of resistance achievable may be limited due to complicated genetics and/or linkage to undesirable agronomic traits. Molecular markers can be employed to speed up the incorporation of chromosomal regions that have a quantitative effect on resistance (quantitative trait loci). Transgenic approaches to ear mold/mycotoxin resistance are now feasible as well. These potentially include genetically enhanced resistance to insect feeding, increased fungal resistance, and detoxification/prevention of mycotoxins in the grain. An example of the first of these approaches is already on the market, namely transgenic maize expressing *Bacillus thuringiensis* (*Bt*) toxin, targeted to the European corn borer. Some *Bt* maize hybrids have the potential to reduce FB levels in field-harvested grain, presumably through reduced feeding of *Bt*-susceptible insects in ear tissues. However, improved ear mold resistance per se is still an important goal, as the plant will still be vulnerable to noninsect routes of entry to *Fusarium.* A second approach, transgene-mediated control of the ability of *Fusarium* to infect and colonize the ear, could potentially be achieved through overexpression of specific antifungal proteins and metabolites, or enhancement of the plant's own defense systems in kernel tissues. This has not yet been accomplished in maize, although promising results have been obtained recently in other monocots versus other fungal and bacterial pathogens. Achieving reproducible and stable enhanced ear mold resistance under field conditions will be immensely challenging for biotechnologists. A third approach, transgene strategies aimed at preventing mycotoxin biosynthesis, or detoxifying mycotoxins *in planta,* could provide further protection for the grower in environments where FBs present a risk to the crop even when the maize is relatively resistant to *Fusarium* mold. In one example of such a strategy, enzymes that degrade FBs have been identified in a filamentous saprophytic fungus isolated from maize, and corresponding genes have been cloned and are currently being tested in transgenic maize. *Key words:* aflatoxin, *Bacillus thuringiensis* toxin, chitinase, corn earworm, Cry1Ab, Cry1Ac, European corn borer, *Exophiala spinifera,* fumonisin, fumonisin deaminase, fumonisin esterase, gene silencing, quantitative trait loci, *Rhinocladiella atrovirens,* trypsin inhibitor. — *Environ Health Perspect* 109(suppl 2):337–342 (2001). *http://ehpnet1.niehs.nih.gov/docs/2001/suppl-2/337-342duvick/abstract.html*

Fumonisins (FBs) are a family of mycotoxins produced by *Fusarium verticillioides* (formerly *F. moniliforme;* teleomorph *Gibberella fujikuroi*) and related species that infect maize and cause ear mold and stalk rot in field-grown maize worldwide, especially in warmer climates where maize is grown (*1*). *F. verticillioides* is a major causal agent of symptomless infection of maize, in which FBs can also be present although usually at low levels (*2*). FBs disrupt sphingolipid biosynthetic pathways in both animal and plant cells, with potentially profound consequences on cellular metabolism (*3*). FBs are acutely toxic to certain livestock, especially horses and swine, and have carcinogenic properties in rats (see other chapters in this volume). FBs can be detected in a variety of processed and unprocessed maize grain products (*4*). Thus, they are a concern for both animal and human consumers of maize grain, especially in developing countries (*5*) and in population segments where maize is a major part of the diet.

The incidence and severity of *Fusarium* ear mold and FB in maize varies widely with growing season, location, and genotype (*4*). *F. verticillioides* is often associated with symptomless infection of maize even where the extreme environments associated with ear mold are less prevalent (*6*). Typically, the Southeastern United States fosters conditions that allow heavy colonization by *Fusarium:* heat, high humidity, and often a delay in harvest. However, significant FB contamination can occur even in the Central United States maize belt in certain years (*7*). Other countries with significant maize acreage have also reported FB levels that are cause for concern (*8*).

As natural products of a nearly ubiquitous endophytic contaminant of maize, fumonisins will be difficult, if not impossible, to eliminate from the food/feed chain, but steps can be taken to minimize preharvest contamination of maize. Environmental factors play a major, although largely uncharacterized, role in incidence of FB contamination of maize (*9*), but host genetics undoubtedly plays a role as well. Genetic modification of maize (either through plant breeding or transgene mediated) represents one potential way to reduce exposure to this important mycotoxin in food and feed, through increased resistance to fungal infection and/or toxin production in maize tissues. This article describes current and proposed genetic and molecular approaches that can potentially lead to reduced exposure to FBs from maize.

Breeding for Increased *Fusarium* Ear Mold Resistance

Resistance to visible symptoms of *Fusarium* ear mold can be selected for in environments such as those mentioned above, where disease pressure is severe enough to provide consistent infection and visible mold (*10*). However, symptomless infections may not be taken into account with traditional screening methods, and toxin-based screening methods are also warranted. Fortunately, immunology-based screening kits for FBs are available from a number of manufacturers. Additional work is needed to better define environmental conditions that result in high levels of ear mold and/or high levels of FB accumulation (*9*). Genotype by environment interactions are likely to be very important (*11*), and an anecdotal observation has been that high-yielding northern lines, grown outside their area of adaptation in the South, will often fare worse than adapted lines under severe ear mold pressure (*12*). Risk management, based on well-characterized germplasm and better environmental predictors, could certainly help grain producers make the best choices for disease/mycotoxin management. Other tools, including quantitative screening assays and biomarkers for resistance, could potentially be exploited as well (*13*). Exotic germplasm can

This article is based on a presentation at the International Conference on the Toxicology of Fumonisin held 28–30 June 1999 in Arlington, Virginia, USA.

Address correspondence to J. Duvick, Disease Resistance Group, Trait and Technology Division, Pioneer Hi-Bred, International, Inc., 7250 NW 62nd Ave., Box 552, Johnston, IA 50131 USA. Telephone: (515) 270-3176. Fax: (515) 334-4729. E-mail: duvickj@phibred.com

Received 10 April 2000; accepted 17 January 2001.

be used as a source for increased ear mold resistance (*14*), but difficulty is often encountered in introgressing this resistance into elite material used for commercially viable hybrids. Overall, with these tools and information now available, prospects for improving the level of *Fusarium* and FB resistance in hybrid maize are good. However, the rate at which advances can be made and the ultimate resistance achieved may not match the requirements for FB-free maize. An additional problem is that complex traits like ear mold resistance may be difficult to dissociate from undesirable agronomic traits.

The seed industry has been revolutionized by the advent of genetic transformation, the process of inserting novel genes into the crop's genetic material, which results in enhancement of specific traits, often beyond the natural genetic variation within the crop. Transgene-derived insect resistance in maize and herbicide resistance in soybeans constituted a major part of the respective markets in the United States in 1999 (*15*). Most transgenic crops on the market today have traits whose benefits are seen most clearly by the grain producer, who enjoys a more stable yield with fewer chemical inputs. Although these benefits are not always readily apparent to the consumer, a newer generation of transgenic crops is being developed with improved nutritional properties (*16*) and other qualities that will more directly affect the consumer. Given the difficulty in conventional breeding, the reduction in mycotoxin contamination, specifically FB contamination in maize, represents an area where transgene technology could bring benefits directly to the consumer. What are the opportunities, and how are they likely to be used in reducing the risk of FB contamination in food and feed?

Molecular Marker-Based Breeding

Quantitative trait loci (QTL) are defined as chromosomal regions that can be identified by their statistical association with a measurable trait in a segregating population. They represent the cumulative contribution of one or more genes whose individual contribution to resistance may be too small to detect. Traits such as ear mold resistance that rely on more than a few genes for optimal expression can be mapped as QTL using large segregating populations (*17*). Molecular markers linked to these QTL could potentially provide an aid to conventional breeding in introgressing complex traits during inbred development. Advances in marker technology have made this feasible (although still expensive) today in field crops (*18*). However, complex traits may depend upon the action of other genes that may vary by genetic background, so it is questionable whether marker-assisted selection will result in a universal set of genes with equal potency in all hybrids.

Several public researchers have made significant progress in mapping natural resistance to *Aspergillus* ear mold, and we may now be in a position to test the usefulness of marker-assisted selection for ear mold resistance in maize hybrid development. There are currently no similar publicly funded projects to map *Fusarium* ear mold loci, although resistant germplasm has been identified (*19*). Work is underway to identify and map loci associated with *Gibberella* ear mold resistance (*20*), although it is likely that resistance factors involved in resistance to *Gibberella* are different from those responsible for *Fusarium* ear mold resistance.

Genes for Ear Mold Resistance in Maize

What about the genes themselves? Map-based cloning of genes has become feasible in several plant species, including soybean (*21*). QTL mapping, together with the candidate gene approach to linking expressed cDNAs to resistance-related loci (*22*), could conceivably allow the identification of genes that contribute to quantitative resistance to *Fusarium* ear mold. The large size of the maize genome makes this exceedingly difficult at present, but technologic advances including large-scale genomic sequencing and physical mapping may allow breakthroughs.

Transgenic Resistance to Ear-Feeding Insects

Genes that reduce insect feeding would be predicted to result in lower mycotoxin levels in maize under conditions where insects are a major factor in dispersal of the fungus and subsequent infection through insect-wounded kernels. Genes coding for insecticidal proteins from *Bacillus thuringiensis* (*Bt*) (*23*) have been expressed in transgenic maize hybrids to counteract the effects European corn borer feeding on leaf and stalk tissues. Maize hybrids containing genes coding for the δ-endotoxins Cry1Ab and Cry1Ac have been widely planted in the United States since 1997 (*15*). In several commercialized versions, *Bt* protein is expressed in kernels as well as in green tissues, and thus could confer protection against corn borer-mediated ear damage. Recent data from multiyear experiments involving a range of *Bt* hybrids grown in the Midwest (*24*) indicate that presence of the *Bt* gene can result in reduced FB levels relative to the same hybrid without the *Bt* gene. The effect was seen under conditions of both natural and artificial infestation with European corn borer, but was absent or less pronounced in some cases. Visible symptoms were also typically reduced. These data suggest that *Bt* maize can be part of a strategy for reducing ear molds and mycotoxins. If additional *Bacillus* genes are eventually deployed to control a broader range of insects that feed in ears, the degree of effectiveness may be improved even further. However, there is a caveat, in that insects may not be a limiting factor in ear mold severity in all environments. In addition, the use of *Bt* genes expressed in kernel tissues has been restricted in certain areas of the Southern United States where corn earworms can also feed on cotton (*25*), and this area includes regions where *Fusarium* ear mold is often a problem. In summary, *Fusarium* has multiple routes of entry to the ear (26), and resistance to ear mold per se will continue to be the most important part of a strategy to reduce mycotoxins in maize grain.

Transgenic Strategies to Reduce *Fusarium* Ear Mold

Transgene-enhanced disease resistance has not yet achieved the same level of success as insect resistance in crop plants. Despite the early success of genes for transgenic virus resistance (*27*), achieving a convincing level of resistance to fungi has proven more difficult, although success has been reported in model system species (*28*) and in some crop species (*29*). Even with moderate success in greenhouse or growth chamber, the ability of transgene efficacy to hold up under diverse environments and genotypes in the field is even more challenging. Perhaps as a reflection of this difficulty, no transgenic crops with enhanced resistance to fungi are currently on the market in 2001.

Large amounts of both public and private research dollars are being spent attempting to generate transgenic plants with increased resistance to diseases, including *Fusarium* ear mold in maize. Several companies have maize field trials in the category *Fusarium* resistant or ear mold resistant in the U.S. Department of Agriculture Animal and Plant Health Inspection Service Field Test Release Database for 2000 (*30*). However, the process of delivering transgenes to market in a crop such as maize is complex and potentially lengthy (*31*). Ear mold resistance in maize presents a formidable challenge at this early stage in trait-directed transgene technology, in part because of our inadequate knowledge of the basic biology of the interaction: where, when and how *Fusarium* infects the kernel, how ear mold symptoms arise, and when and where mycotoxins such as FB are produced in the ear. Still, opportunities exist to evaluate genes and strategies that have shown promise in other host–pathogen systems. The following sections provide additional details of these strategies as they apply to ear mold resistance in maize.

Antifungal Proteins

Mature maize kernels contain several classes of proteins with known antifungal activity (*32–37*), and these may contribute to the general or specific resistance to infection by pathogens. However, establishing a role for individual proteins in disease resistance is often difficult. This is probably because plants use multiple or even redundant mechanisms that together result in a resistance response but individually may not play an obvious role. Nevertheless, genetic engineering can potentially be used to augment a given level of resistance by introducing novel antifungal proteins or protein combinations, as has been reported for several leaf pathogens in dicots (*38*). Few successful examples of transgenic disease resistance have been published for monocots (*39*). Antifungal proteins are generally much less potent against their targets than is *Bt* on a weight basis, and extremely high-level expression may be needed to affect fungal growth in tissue. Gene silencing may be brought on by high level expression of homologous gene sequences (*40*), so genes not of maize origin may be preferable. An additional consideration is that high-level expression of a seed protein could change kernel morphology in ways that could result in a change in susceptibility to ear mold. In a nontransgenic example, opaque-2 endosperm was reported to result in more severe *Fusarium* ear mold in already susceptible backgrounds (*41*). An alternative strategy would be to deploy an antifungal protein in a nonseed tissue that is critical for infection (e.g., silk, husk), although there is little data to suggest that silks are a limiting factor for infection, unlike the situation with *F. graminearum* (*20*).

The foregoing discussion illuminates some of the technical challenges that need to be overcome to successfully engineer resistance via one or several transgenes with direct antifungal activity. Thus far no commercial level of resistance has been reported using antifungal proteins.

Engineered Secondary Metabolites

Plants produce chemical defenses, either constitutively or in response to infection (*42*). Several secondary metabolites in the maize kernel have been implicated in resistance to *Aspergillus* and *Gibberella* ear molds, including hydroxamic acids (*43*), phenolics (*44*), and volatiles (*45*). Although some enzymes involved in secondary metabolite synthesis in maize have been cloned (*46*) and could thus be potentially manipulated by overexpression of pathway-limiting enzymes, such pathways are relatively uncharacterized in maize. Opportunities also exist to introduce novel metabolites into a crop species if a common precursor is present. The ideal metabolite is one that is already found in a food crop and can be readily ascertained to be safe. As an example, antifungal stilbenes, products of a single polyketide synthase, can be synthesized in plants that do not normally make them (*47*). One potential pitfall in this approach is the possibility that diversion of metabolic pathways into antifungals may compromise another biosynthetic route that shares intermediates with the pathway of interest. Although challenging, the area of metabolic engineering has long-term promise for engineering novel traits including disease resistance.

Transgene-Enhanced Defense Pathways

Host plant–pathogen interactions are complex and likely involve multiple proteins and metabolites with both sides taking part in a microscopic war for biomass and nutrients. Although our level of knowledge about molecular interactions between host and pathogens is increasing rapidly (*48*), we still do not have enough information about the genes and pathways to intervene successfully in most diseases. One recent focus has been the signal response pathways that transmit information about a stress or pathogen and generate a host response (often involving novel gene expression) to combat the stress or infection (*49*). The signaling pathways, similar to mammalian signal cascades, control a variety of cellular responses, using multiple steps or cascades involving protein–protein interactions that allow a few genes to control the action of many. This host response may or may not culminate in hypersensitive cell death in surrounding tissue and may also result in activation of defense pathways in adjacent tissues or even systemically in the plant (*50*). If the master signals that control defense gene expression can be identified, they could potentially be engineered to provide a more rapid response, a constitutive response, or a chemically induced defense response (*51*). Such a series of master genes potentially controlling cold tolerance in *Arabidopsis* has been identified (*52*). Overexpression of one of these factors, CBF1, is reported to result in enhanced cold tolerance in recipient transgenics, presumably by upregulating the entire suite of genes necessary for cold tolerance (*53*). Through genetic and molecular techniques, a number of genes that control disease resistance pathways have been identified in the model plant species *Arabidopsis,* and overexpression of these genes can result in an enhanced disease resistance phenotype (*54,55*). It would be somewhat surprising if this type of constitutive up-regulation did not come at some cost in energy availability or flexibility to combat other pathogens or pests, but no one has yet addressed these questions with meaningful experiments. It is encouraging that several transgenic routes to constitutive resistance have already been identified, and with time it should be possible to sort out which (if any) work best in actual field conditions. Many research groups are using powerful molecular techniques for detecting changes in expression of large numbers of genes simultaneously, with the aim of identifying defense pathways and controlling genes in plants such as maize (*56*).

Most of the experiments cited above involved dicot leaf pathogens. What about maize, and particularly, what about kernel tissues? Induction of defense pathways has been observed in maize (*57*), although it remains to be seen whether these defense pathways will provide effective control against maize pathogens. In addition, there is very little data on the effectiveness of induced cellular resistance pathways in developing seed tissues in any species, although a few studies of germinating seeds indicate defense gene expression can be enhanced by elicitor application following germination (*58*).

Gene-for-Gene Resistance to *Fusarium*

In some instances, plant resistance to a pathogen can be characterized genetically in terms of a dominant resistance gene (*R* gene) in the plant and a corresponding avirulence gene (*avr* gene) that must be present in the pathogen in order for a resistance response to occur. Several *R* genes and, in some cases, corresponding *avr* genes have been cloned and characterized (*59*). *R* genes fall into several related classes of proteins, often containing both potential protein-binding domains (leucine-rich repeats) and response regulator domains, whereas *avr* genes vary widely in size and structure. Attempts are underway to understand how interaction of these host and pathogen gene products could directly or indirectly lead to a signal cascade (*60*). In maize, *Rp1-D* conferring resistance to the common rust *Puccinia graminis* (an obligate fungal parasite) has been cloned (*61*).

There is no evidence of a gene-for-gene relationship in *Fusarium* ear mold of maize. As discussed earlier, known sources of resistance are likely to be polygenic. Several other *Fusarium* species have gene-for-gene interactions with their plant hosts. For example, in tomato an *R* gene conferring resistance to a race of *F. oxysporum* has been mapped and a candidate gene cloned (*62*). It is possible that through protein engineering/directed evolution (*63*), novel *R* gene specificities could be developed that would allow maize to recognize *F. verticillioides* in a gene-specific manner via factors secreted by the fungus during infection of maize. However, a hallmark of gene-for-gene interactions is the often rapid evolution of novel races of the pathogen that overcome resistance by loss of *avr* gene function (*64*). The diversity of toxigenic *Fusarium* species capable of infecting maize would create a significant challenge for an engineered recognition system.

Transgenic Strategies Aimed at Reducing Fumonisins

Although reducing fungal infection through host resistance remains the most desirable method to eliminate mycotoxins, strategies directed against a mycotoxin per se (either suppressing its synthesis or degrading it to nontoxic metabolites) represent an alternative or complementary means to address mycotoxin contamination. This approach may be desirable in two situations: *a*) in crop molds in which the mycotoxin itself is a participant in disease or symptom development, so reducing toxin levels would also be expected to reduce disease; and *b*) in crop genotypes or environments where there is minimal overt mold damage to grain overall, but a fungal toxin or family of toxins is still present and poses a potential threat to consumers.

Deoxynivalenol, a mycotoxin produced by *F. graminearum,* has been proposed to play a phytotoxic role in both wheat scab and maize ear mold diseases caused by this fungus (*65,66*), and it is possible to envision resistance strategies that involve reducing the impact of this toxin on host tissue. FBs, although they are somewhat phytotoxic to many plant species including maize (*67*), are apparently not strongly associated with pathogenicity or symptom severity in *F. verticillioides* diseases of maize (*68*). Whether FBs are not involved at all in the etiology of *Fusarium* diseases of maize, or whether they play a role along with other toxic factors is not known. In any case, there is currently no compelling evidence to suggest that FB detoxification *in planta* will alter the course of *Fusarium* infection.

However, strategies targeted at FB reduction may be worthwhile nonetheless. Although high FB levels in grain are usually correlated with symptomatic (i.e., damaged or visibly moldy) kernels, FBs can also be detected in asymptomatic kernels, coincident with the asymptomatic presence of *Fusarium* (*2*). FB contamination can occur in grain that would not be flagged as moldy, and strategies to reduce or eliminate FB in this grain should be given consideration.

Modifying Mycotoxin Catabolic Pathways

Most mycotoxins are produced under specific cultural conditions, and there is evidence that plant host-produced signals can play either a positive or a negative role in toxin accumulation (*69,70*). Developmental changes in kernel composition can also influence mycotoxin production, as was reported recently in a study of FB production on sterilized maize kernels at different stages of development (*71*). One approach, therefore, would be to engineer a plant to produce a soluble signal to turn off the mycotoxin pathway in the fungus or, alternatively, fail to produce an essential signal that would normally turn on toxin production. Natural products that have a suppressive effect on mycotoxin production have been reported (*43*). Manipulation of lipid hydroperoxides represents a possible route towards the reduction of aflatoxin in grain (*69*). A recent report suggests that FB biosynthesis by *F. verticillioides* is subject to nitrogen repression (*72*), although this pathway may not be easy to manipulate without other effects on grain.

If mycotoxin biosynthetic pathway genes can be identified, molecular methods involving promoter/marker gene fusions can be used to simplify screens for modulators of the pathway (*73*). To date, these tools have not been used in high throughput screens, so their usefulness has not been put to the test. The first FB biosynthetic pathway gene has recently been cloned (*74*), opening up the possibility of metabolite screens for *Fusarium* as well. It is difficult to predict how difficult this strategy will be to put into practice for FB until the key factors that turn production on or off are identified.

Detoxification of Mycotoxins *in Planta*

Another transgene-based method for reducing mycotoxin accumulation involves the deployment of catabolic enzymes to detoxify the mycotoxin *in situ* before it can accumulate in the plant. This topic has been the subject of a recent literature review (*75*). This strategy, although an untested concept insofar as mycotoxins are concerned, is one that plants may deploy naturally in their defense against certain fungi that produce toxins as disease agents. For example, most maize genotypes produce an NADH reductase enzyme that inactivates a fungal toxin produced by race 1 of *Cochliobolus carbonum* (*76*). In the absence of *Hm1*, the gene coding for this reductase (*77*), seedlings are highly susceptible to this fungus. There are several reports in the literature in which microbial genes coding for detoxification enzymes have been expressed successfully in plants, with a resulting reduction in disease. An early example is that of tabtoxin and wildfire resistance developed in tobacco using an acetyltransferase enzyme from the pathogen itself, *Pseudomonas syringi* pv. *tabaci* (*78*). Lu et al. (*79*) have presented data indicating that transgenic expression of wheat oxalate oxidase, which detoxifies oxalic acid produced by *Sclerotinia sclerotiorum,* can reduce white mold disease caused by *Sclerotinia* in sunflower; Zhang et al. (*80*) reported the successful engineering of resistance to a pathogenic bacterium in sugarcane using a detoxifying gene, *AlbD,* obtained from a biocontrol organism. These reports demonstrate that in some cases not only the toxin but the disease can be controlled by a degradative enzyme.

The success of a detoxification approach for mycotoxins will depend in part on the extent to which the plant-produced enzyme reaches its target substrate and the effectiveness or stability of the detoxification step. For FBs in maize, little is known about their cellular location, so it is difficult to predict how successful a detoxification approach could be. However, FBs are water soluble, and it seems likely that most of the toxin would be available to soluble enzymes produced in host tissues. In addition, key functional groups (tricarballylate esters and a primary amine) that affect toxicity (*81*) make FB an attractive molecule for detoxification. However, much data suggest that merely removing tricarballylates does not render the molecule nontoxic (*82*), so multistep modification may be necessary. Generally, FBs appear resistant to the activity of known esterases and amine-modifying enzymes (*83,84*), so novel enzymes and genes must be sought.

The identification of several microbial species (both fungal and bacterial) that metabolize the C-20 backbone portion of FBs to CO_2 in liquid culture has opened up the possibility of engineering maize that detoxifies FBs (*85–87*). Two fungal species (*Exophiala spinifera* and *Rhinocladiella atrovirens*), belonging to the dematiaceous hyphomycetes known as black yeasts (*88*), can grow on FB_1 as a sole carbon source and produce enzymes in culture that metabolize FBs (*89*). One of these, *E. spinifera,* has been used as a source for genes in a strategy aimed at detoxification of FBs in transgenic maize. The initial step in FB metabolism by *E. spinifera* consists of deesterification of FB's tricarballylic acid esters by a FB-specific, soluble esterase, ESP-1 (*85*). Ester hydrolysis is followed by oxidative deamination of the resulting amine alcohol backbone by an amine oxidase enzyme (*84*), resulting in a 2-oxo polyalcohol (2-OP_1) that undergoes internal cyclization to form a hemiketal (*90*) (Figure 1). Together, these two catabolic steps are likely to detoxify FB, although toxicologic data to verify this are not yet available. We hypothesize that the esterase, amine oxidase, and other downstream enzymes together constitute a novel catabolic pathway that provides a unique carbon-source niche for microbes that express them. We have cloned genes corresponding to the two FB-specific enzymes that carry out the catalytic processes described above (deesterification and oxidative deamination) (*86,87,91*). Experiments are under way to evaluate the effect of these genes, when expressed in transgenic maize, on FB levels in the grain.

Unlike transgene-mediated herbicide resistance in which the successful phenotype is self-selecting (i.e., plant survival and growth), engineering the breakdown of a xenobiotic toxin like FB will require extensive and complex analysis to determine its efficacy. It will be important to establish a number of

benchmarks for the success of this approach to FB reduction, all of which will determine the extent of mycotoxin breakdown possible in a transgene. These include: *a*) enzyme localization in the seed in relation to mycotoxin substrate accessibility; *b*) kinetic parameters of the enzyme(s) in the context of its localization in the plant (substrate K_m, pH optimum, substrate range, potential inhibitors); and *c*) stability and activity of the enzyme during pre- and postharvest conditions conducive to fungal growth. Equally important will be experiments that verify the identity of mycotoxin breakdown products that accumulate in the transgenic grain under ear mold conditions, the toxicity of these products in a variety of test systems, in comparison to FBs, and the nutritional properties of the transgenic grain under various mold conditions. In addition, regulatory processes set in place by U.S. agencies that oversee transgenic food safety concerns under the Food, Drug and Cosmetics Act (*92*) will need to be met.

If all of these benchmarks are achieved, genes for reducing FB levels in maize could be incorporated into existing germplasm that already has adequate levels of *Fusarium* ear mold resistance overall but still carries a risk of FB contamination depending on the growing environment. In principle, other detoxification enzymes could be stacked together to provide broader protection from risk of adverse levels of a range of mycotoxins.

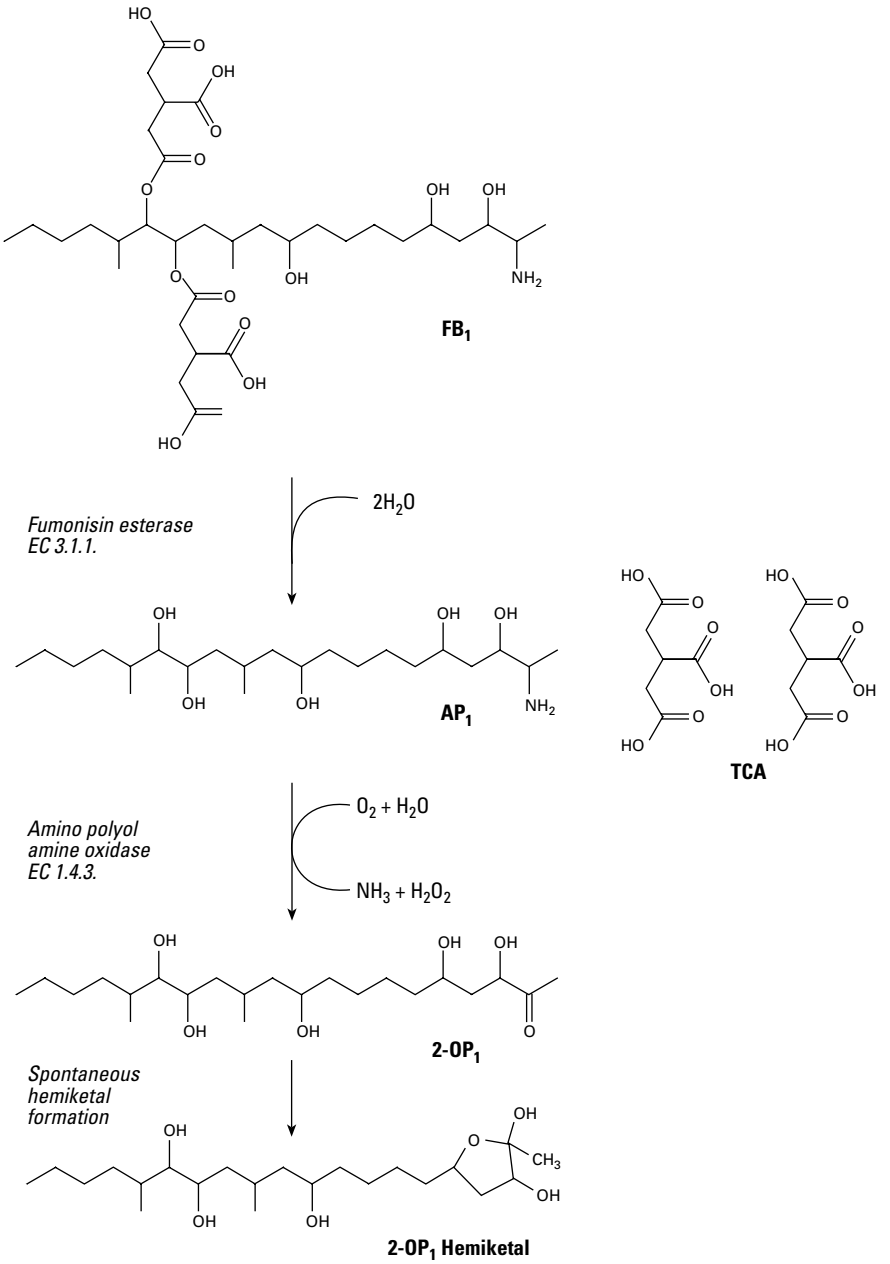

Figure 1. Initial enzymatic steps in breakdown of FB_1 by the black yeast *E. spinifera*. AP_1, aminopentol 1; TCA, tribal-lylic acid. See Duvick et al. (*85*) and Blackwell et al. (*90*). The conversion of keto alcohol ($2\text{-}OP_1$) to its corresponding hemiketal is presumed to occur spontaneously.

Summary and Conclusions

The potential for improvement of maize through molecular genetics-based enhancement of elite germplasm is now being realized in areas of insect resistance and herbicide tolerance. Insect resistance genes can have an impact on ear mold and mycotoxin levels, but will not eliminate the problem. First-generation genetic enhancement of maize will soon be augmented by additional improvements in both input and output traits, perhaps including increased resistance to fungal diseases such as *Fusarium*. Transgene-mediated fungal resistance has not yet reached its commercial potential in field crops, but advances in understanding of fundamental mechanisms of host resistance and fungal pathogenicity are setting the stage for this becoming reality. New strategies such as detoxification are also worth pursuing, as we need to test the limits of transgene technology to help solve these difficult food and feed safety problems.

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