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Review

Using biotechnology to enhance host resistance to aflatoxin contamination of corn

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Host resistance is the most widely explored strategy for eliminating aflatoxin contamination by *Aspergillus flavus*. Breeding strategies for developing resistant corn germplasm have been enhanced by the development of new screening tools for field inoculation and for laboratory screening. RFLP analysis of corn populations has highlighted the possibility that different resistance traits can be successfully pyramided into agronomically useful germplasm, while proteomics has impacted the identification of proteins associated with resistance (RAPs). The identification of RAPs has also been enhanced by the discovery of near-isogenic corn lines in progeny generated in a West African breeding program. The characterization of genes of the aflatoxin biosynthetic pathway has provided a foundation for a genomics investigation aimed at understanding the biochemical function and genetic regulation of aflatoxin biosynthesis. Successful inhibition of aflatoxin elaboration may require not only the action of antifungal compounds, but of compounds that block biosynthesis of toxins as well.

Key words: Aflatoxin contamination, corn, molecular biology, biotechnology, host resistance.

INTRODUCTION

Aflatoxins, the highly toxic and carcinogenic secondary metabolites of *Aspergillus flavus* and *A. parasiticus,* are the most widely investigated of all the mycotoxins. This is due to their role in establishing the significance of mycotoxins in animal diseases (Wyllie and Morehouse, 1978). Presently, more than 50 countries have established or proposed regulations for controlling aflatoxins in foods and feeds (Haumann, 1995); the U.S. Food and Drug Administration (FDA) has limits of 20 ppb, total aflatoxins, on interstate commerce of food and feed, and 0.5 ppb of aflatoxin B_1 on the sale of milk. However, many countries, especially in the developing world, experience contamination of domestic-grown commodities to alarmingly greater degrees than does the U.S. A recent study revealed a strong association between exposure to aflatoxin in West African children and both stunting (a reflection of chronic malnutrition) and being underweight (a reflection of acute malnutrition) (Gong et al., 2002).

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Abbreviations: EST: expressed sequence tag, GUS: b – glucuronidase, RAPs: resistance-associated proteins, KSA: kernel screening assay, RFLP: restriction fragment length polymorphism, RNAi: RNA interference.

The need to control contamination of food and feed grains by aflatoxins is greatly recognized, and preharvest prevention, especially through host resistance, is probably the best and most widely explored strategy. *A. flavus* infects all affected crops prior to harvest (Lillehoj, 1987). Reliance upon good cultural and management practices, when possible, can, at times, reduce preharvest aflatoxin contamination, but not eliminate it. In addition, even the best management practices are sometimes negated by biotic and abiotic factors that are hard to control and by extremes in environmental conditions.

The preeminence of the host resistance strategy is also due to several recent research advances, such as the identification of resistant genotypes in corn through plant breeding. There is, however, an urgent need to optimize the host resistance strategy for controlling aflatoxin contamination by employing state-of-the-art technologies currently available. The present review highlights recently published and high-impact research involving biotechnological approaches that have been accomplished and that enhances a host plant resistance strategy for controlling aflatoxin contamination.

PREVENTION OF CONTAMINATION IN CORN THROUGH ENHANCEMENT OF HOST RESISTANCE

By far, most studies aimed at incorporation of antifungal resistance against mycotoxigenic fungi have been applied toward improvement of resistance against preharvest aflatoxin contamination in corn (reviewed in Cleveland et al., 2003). With corn, the strategy of enhancing host resistance to aflatoxin contamination through breeding has gained prominence because of: 1) the successful identification of germplasm resistant to aflatoxin contamination, and 2) the significant advances in the identification of natural resistance mechanisms and traits (Brown et al., 1999; Cleveland et al., 2003). However, these investigations indicated that resistance to aflatoxin contamination involves multiple chromosome regions and several genes (Davis and Williams, 1999). Therefore, attempts to select for resistance traits in the development of commercial corn varieties, while maintaining desirable agronomic characteristics, have been slowed due to a failure to identify expressed genes and proteins involved in resistance. This is especially needed since resistance, thus far identified, is in poor genetic backgrounds. Therefore, research is needed to elucidate the biochemical mechanisms that confer resistance in corn kernels that are vulnerable to aflatoxin contamination. These resistance mechanisms could then be used to enhance germplasm through marker-assisted breeding and/or genetic engineering (reviewed in Brown et al., 1999). Gaining an understanding of the natural resistance mechanisms in corn could serve as “nature’s lesson” about the specific requirements for seed-based resistance against fungal attack.

Development of aflatoxin-resistance screening tools

Several screening tools have been developed and used to facilitate corn breeding for developing germplasm resistant to fungal growth and/or aflatoxin contamination (King and Scott, 1982). Inoculation methods employed with corn include the pinbar inoculation technique (for inoculating kernels through husks with *A. flavus* conidia), the silk inoculation technique, and infesting corn ears with insect larvae infected with *A. flavus* conidia (King and Scott, 1982; Tucker et al., 1986). Two resistant inbreds (Mp420 and Mp313E) (Scott and Zummo, 1988; Windham and Williams, 1998) were discovered and tested in field trials at different locations, using the pin-bar technique, and released as sources of resistant germplasm.

A rapid laboratory kernel screening assay (KSA) was developed and used to study resistance to aflatoxin production in mature kernels and to preliminarily screen/rank corn lines for resistance to aflatoxin accumulation. (reviewed in Brown et al., 1999). The results of these studies indicate the presence of two levels of resistance: at the pericarp and at the subpericarp level. The subpericarp level of resistance was shown to require a viable embryo (Brown et al.,1999). The KSA has advantages over traditional field screening techniques, mainly because of the rapidity of the assay. However, field trials are irreplaceable for confirmation of resistance.

Recently, the KSA was improved by including a method to quantify fungal biomass using the β-glucuronidase (GUS) or green fluorescent protein (GFP) (Du et al., 1999; Windham and Williams, 1998; Windham et al., 1999) reporter gene-containing *A. flavus* tester strains. *A. flavus* tester strains were genetically engineered with a gene construct consisting of the GUS reporter gene linked to an *A. flavus* β-tubulin gene promoter for monitoring fungal growth (reviewed in Brown et al., 1999) or with the reporter gene linked to an aflatoxin biosynthetic pathway gene which could also provide a quick and economical way to indirectly measure aflatoxin levels (Payne, 1997; Brown-Jenco et al., 1998; Brown et al., 2003). Thus, it is now possible to accurately assess fungal infection levels and to predict the corresponding aflatoxin levels in the same kernels, as a result of fungal infection. Using this approach, it is also possible to determine whether kernel resistance mechanisms are affecting fungal growth or aflatoxin biosynthesis.

Plant breeding strategies for enhancing host resistance to aflatoxigenic fungi

The KSA confirmed sources of resistance among 31 inbreds tested in Illinois field trials (Brown et al., 1999; Campbell and White, 1995). Several resistant inbreds among the 31 tested in Illinois and highlighted through the KSA, have been incorporated into an aflatoxin-

resistance breeding program whose major objective is to improve elite Midwestern corn lines such as B73 and Mo17. In this program, the inheritance of resistance of inbreds in crosses with B73 and/or Mo17 was determined (White et al., 1995, 1998; Hamblin and White, 2000; Walker and White, 2001), and in the case of several highly resistant inbreds, genetic dominance was indicated.

Chromosome regions associated with resistance to *A. flavus* and inhibition of aflatoxin production in corn have been identified through Restriction Fragment Length Polymorphism (RFLP) analysis in three Illinois "resistant" lines (R001, LB31, and Tex6), after mapping populations were developed using B73 and/or Mo17 elite inbreds as the "susceptible" parents (White et al., 1995, 1998) . In some cases, chromosomal regions were associated with resistance to Aspergillus ear rot and not aflatoxin inhibition, and vice versa, whereas other chromosomal regions were found to be associated with both traits. This suggests that these two traits may be at least partially under separate genetic control. Also, it was observed that variation can exist in the chromosomal regions associated with Aspergillus ear rot and aflatoxin inhibition in different mapping populations, suggesting the presence of different genes for resistance in the different identified resistant germplasm. RFLP technology may provide the basis for employing the strategy of pyramiding different types of resistances into commercially viable germplasm, while avoiding the introduction of undesirable traits. Other breeding programs using this technology are attempting to pyramid insect and fungal resistance genes into commercial germplasm (Guo et al., 2000; Widstrom et al., 2003).

The KSA was also used to screen corn inbreds that had been selected for ear rot resistance in West and Central Africa, for aflatoxin-resistance (Brown et al., 2001) . The large number of promising lines observed in these experiments provides the basis for a current collaborative effort between the International Institute of Tropical Agriculture (IITA) and USDA-ARS, New Orleans. The best African lines (as determined by the KSA) were crossed with U.S. aflatoxin-resistant lines at IITA, while in the U.S., resistance markers for progeny generated through these crosses are being identified and characterized (Brown et al., 2003b). The goal is to develop aflatoxin-resistant, agronomically-superior germplasm for use in both West Africa and the U.S.

Identification of resistance-associated proteins (RAPs) in corn inhibitory to *A. flavus* growth/aflatoxin contamination

Developing resistance to fungal infection in wounded as well as intact kernels would go a long way toward solving the aflatoxin problem (Payne, 1992). Studies demonstrating subpericarp (wounded-kernel) resistance in corn kernels have led to research for identification of subpericarp resistance mechanisms. Examinations of kernel proteins of several genotypes revealed differences between genotypes resistant and susceptible to aflatoxin contamination (Guo et al., 1998). Imbibed susceptible kernels, for example, showed decreased aflatoxin levels and contained germination-induced ribosome inactivating protein (RIP) and zeamatin, both inhibiting *A. flavus* growth *in vitro* (Guo et al., 1997). In another study, two kernel proteins, one 28 kDa and inhibitory to *A. flavus* growth, the other over 100 kDa in size and primarily inhibitory to toxin formation, were identified from a resistant corn inbred (Tex6) (Huang et al., 1997). When a commercial corn hybrid was inoculated with aflatoxin and nonaflatoxin-producing strains of *A. flavus* at milk stage, one induced chitinase and one β- 1,3-glucanase isoform was detected in maturing infected kernels, while another isoform was detected in maturing uninfected kernels (Ji et al., 2000).

An investigation of kernel protein profiles of 13 corn genotypes revealed a constitutively-expressed 14 kDa trypsin inhibitor protein (TI), present at relatively high concentrations in seven resistant corn lines, but at low concentrations or absent in six susceptible lines (Chen et al., 1998). The mode of action of TI against fungal growth may be partially due to its inhibition of fungal-amylase, limiting *A. flavus* access to simple sugars (Chen et al., 1999b) required not only for fungal growth, but also for toxin production (Woloshuk et al., 1997). TI also demonstrated antifungal activity against other mycotoxigenic species (Chen et al., 1999a). The identification of these proteins and their corresponding genes may provide markers for plant breeders, and facilitate the introduction of antifungal genes through genetic engineering into other aflatoxin-susceptible crops such as cotton (Rajasekaran et al., 2000).

Using proteomics to identify RAPs

To increase protein resolution and detection sensitivity by 10 to 20 fold and, thus, enhance ability to identify more RAPs, a proteomics approach was recently employed. Kernel proteins from several resistant and susceptible genotypes were compared using large format 2-D gel electrophoresis), and over a dozen such protein spots, either unique or 5-fold up-regulated in resistant lines, were identified, isolated from preparative 2-D gels and analyzed using ESI-MS/MS after in-gel digestion with trypsin (Chen et al., 2000, 2002). These proteins can be grouped into three categories based on their peptide sequence homology: (1) storage proteins, such as globulins (GLB1, GLB2), and late embryogenesis abundant proteins (LEA3, LEA14); (2) stress-responsive proteins, such as aldose reductase (ALD), glyoxalase I (GLX1) and heat shock proteins, and (3) antifungal proteins, including TI.

No investigation has been conducted, thus far, to determine the possible direct involvement of stress-related proteins in host fungal resistance. However, increased temperatures and drought, which often occur together, are major factors associated with aflatoxin contamination of corn kernels (Payne, 1998). Possession of unique or of higher levels of hydrophilic storage or stress-related proteins, such as the aforementioned, may put resistant lines in an advantageous position over susceptible genotypes in the ability to synthesize proteins and defend against pathogens under stress conditions. Further studies including physiological and biochemical characterization, genetic mapping, plant transformation using RAP genes, RNAi gene silencing experiments (Brown et al., 2003b) and marker-assisted breeding should clarify the roles of stress-related RAPs in kernel resistance.

The screening of progeny generated in the IITA and USDA-ARS program identified potential near-isogenic lines from the same backcross differing significantly in aflatoxin accumulation (Table 1), and proteome analysis of these lines is being conducted (Brown et al., 2003). Investigating corn lines sharing close genetic backgrounds should enhance the identification of RAPs clearly without the confounding effects experienced with lines of diverse genetic backgrounds.

Table 1. Screening for aflatoxin in West African breeding progeny and selection of potential near-isogenic lines[1].

Line[2]	Toxin ppb[3]	
S-C	10197	a
22	**1693**	**b**
19	1284	bc
28	1605	bcd
27	1025	bcd
21	1072	bcd
26	793	bcde
20	574	cde
24	399	cde
U.S.	338	de
25	**228**	**e**
23	197	e
R-C	76	e

[1]Progeny are result of original cross between a U.S. resistant and an African resistant corn line, backcrossed to the U.S. line. All lines, thus, have the same parental background.

[2]Corn lines representing the fourth generation of lines (S4) from the original cross, generated through selfing and selecting for ear rot resistance and agronomic characteristics. Lines in bold print, #22 and #25, which differ significantly in aflatoxin accumulation, were selected for proteome analysis. S-C = susceptible control; U.S. = U.S. parent from original cross; R-C = resistant control.

[3]Aflatoxin B_1 was measured in parts per billion (ng/g), after being subjected to a KSA protocol (reviewed in Brown et al., 1999). Values followed by the same letter are not significantly different by the least significant difference test (P=0.05).

Exploiting the aflatoxin biosynthetic pathway

The fully characterized aflatoxin biosynthetic pathway and gene cluster comprising genes that govern this pathway, including the key regulatory gene (aflR) (Bhatnagar et al., 2003), may provide a sound basis for studies of the efficacy and mode of action of putative pathway -blocking compounds (e.g. Tex6 100 kDa protein). Also, regulation of these genes during invasion of the plant host is being investigated using a genomics approach. This approach is based on the fact that certain plant-derived natural products apparently have regulatory effects on aflatoxin biosynthesis (as reviewed in Bhatnagar et al., 2001). Investigating *A. flavus* genomics is an innovative strategy for simultaneous analysis of the biochemical function and genetic regulation of aflatoxin biosynthesis (Yu et al., 2002). *A. flavus* Expressed Sequence Tag (EST) technology facilitates rapid identification of the majority, if not all, of the genes expressed in the fungal genome and aids in understanding the coordinated regulation of gene expression. At present, over 7000 unique gene sequences have been identified within 14,000 cDNA sequences obtained.

CONCLUSIONS

Control of aflatoxin contamination of corn will likely be dependent upon the development and introduction into the commercial market, of germplasm, resistant to the growth of aflatoxigenic species, and/or biosynthesis of toxins by these species. The identification of chromosomal regions as well as proteins and their corresponding genes associated with resistance, and the subsequent confirmation of their role in resistance using biotechnological tools available such as RFLP analysis or RNAi should provide an efficient means for the development of this germplasm. Limiting the growth of aflatoxigenic fungi might at times not be enough to maintain aflatoxins at “acceptable” levels in corn crops. Therefore, the identification of compounds that block aflatoxin biosynthesis may represent the “magic bullet” needed to insure resistance. That the aflatoxin biosynthetic pathway and the gene cluster comprising genes that govern this pathway have been characterized, should provide a sound basis for experimentation in testing putative pathway-blocking compounds. Thus, the identification of resistance traits in corn can, through marker-assisted breeding, facilitate a more rapid development of resistant, commercially- useful germplasm. Genetic engineering provides a tool especially useful in testing gene functions, either using conventional gene constructs or RNAi constructs. It also provides a means of enhancing the resistance of other aflatoxin-susceptible species.

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REFERENCES

Bhatnagar D, Cotty PJ, Cleveland TE (2001). Genetic and biological control of aflatoxigenic fungi. In Wilson CL and Droby S (eds) Microbial Food Contamination, CRC Press, New York, NY, pp. 207-240.

Bhatnagar D, Ehrlich KC, Cleveland TE (2003). Molecular genetic analysis of aflatoxin biosynthesis and its application to understanding the biological role of aflatoxin. Appl. Microbiol. Biotech. In press.

Brown RL, Brown-Jenco CS, Bhatnagar D, Payne GA (2003a). Construction and preliminary evaluation of an *Aspergillus flavus* reporter gene construct as a potential tool for screening aflatoxin resistance. J. Food Prot. 66:1927-1931.

Brown RL, Chen Z-Y, Cleveland TE, Gonzalez P, Jackson T, Menkir A, Damann KE, Rajasekaran K (2003b). Progress in the identification and characterization of maize resistance traits against aflatoxigenic fungi. In: Dorner J, Robens R (eds) Proceedings of USDA-ARS Aflatoxin/Fumonisin Elimination and Fungal Genomics Workshop held at Savannah, GA (In press).

Brown RL, Chen Z-Y, Cleveland TE, Russin JS (1999) . Advances in the development of host resistance in corn to aflatoxin contamination by *Aspergillus flavus* (A mini-review). Phytopathology 89:113-117.

Brown RL, Chen Z- Y, Menkir A, Cleveland TE, Cardwell K, Kling J, White DG (2001). Resistance to aflatoxin accumulation in kernels of maize inbreds selected for ear rot resistance in West and Central Africa. J. Food Prot. 64:396-400.

Brown-Jenco CS, Obrian GR, Sloan S, Payne GA (1998). Identification of the DNA binding site for the *Aspergillus flavus* AFLR in the NOR-1 promoter. In: Robens J, Wicklow, D (eds) Proceedings of the USDA-ARS Aflatoxin Elimination Workshop held at St. Louis, MO, p. 102.

Campbell KW, White DG (1995). Evaluation of corn genotypes for resistance to Aspergillus ear rot, kernel infection, and aflatoxin production. Plant Dis. 79:1039-1045.

Chen Z-Y, Brown RL, Damann KE, Cleveland TE (2000). Proteomics analysis of kernel embryo and endosperm proteins of corn genotypes resistant or susceptible to *Aspergillus flavus* infection. In: Robens J, Cary, JW, Campbell, BC (eds) Proceedings of the USDA-ARS Aflatoxin Elimination Workshop held at Yosemite, CA, p. 88.

Chen Z-Y, Brown RL, Damann KE, Cleveland TE (2002). Identification of unique or elevated levels of kernel proteins in aflatoxin-resistant maize genotypes through proteome analysis. Phytopathology 92:1084-1094.

Chen Z-Y, Brown RL, Lax AR, Cleveland TE, Russin JS (1999a). Inhibition of plant pathogenic fungi by a corn trypsin inhibitor over-expressed in *Escherichia coli*. Appl. Environ. Microbiol. 65:1320-1324.

Chen Z-Y, Brown RL, Lax AR, Guo BZ, Cleveland TE, Russin JS (1998). Resistance to *A. flavus* in corn kernels is associated with a 14 kDa protein. Phytopathology 88:276-281.

Chen Z-Y, Brown RL, Russin JS, Lax AR, Cleveland TE (1999b). A corn trypsin inhibitor with antifungal activity inhibits *Aspergillus flavus* α-amylase. Phytopathology 89:902-907.

Cleveland TE, Dowd PF, Desjardins AE, Bhatnagar D, Cotty PJ (2003). United States Department of Agriculture – Agricultural Research Service research on pre -harvest prevention of mycotoxins and mycotoxigenic fungi in U.S. Crops. Pest Manag. Sci. 59:629-642.

Davis GL, Williams WP (1999). QTL for aflatoxin reduction in maize. Maize Genetics Conference 41:22.

Du W, Huang Z, Flaherty JE, Wells K, Payne GA (1999). Green fluorescent protein as a reporter to monitor gene expression and food colonization by *Aspergillus flavus*. Appl. Environ. Microbiol. 65:834-836.

Gong YY, Cardwell K, Hounsa A, Egal S, Turner PC, Hall AJ, Wild CP (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. Br. Med. J. 325:20-21.

Guo BZ, Brown RL, Lax AR, Cleveland TE, Russin JS, Widstrom NW (1998). Protein profiles and antifungal activities of kernel extracts from corn genotypes resistant and susceptible to *Aspergillus flavus*. J. Food Prot. 61:98-102.

Guo BZ, Chen Z-Y, Brown RL, Lax A R, Cleveland TE, Russin JS, Mehta AD, Selitrennikoff CP, Widstrom NW (1997). Germination induces accumulation of specific proteins and antifungal activities in corn kernels. Phytopathology 87:1174-1178.

Guo BZ, Widstrom NW, Cleveland TE, Lynch RE (2000). Control of preharvest aflatoxin contamination in corn: Fungus-plant-insect interactions and control strategies. Recent Research Developments in Agricultural and Food Chemistry 4:165-176.

Hamblin AM, White DG (2000). Inheritance of resistance to Aspergillus ear rot and aflatoxin production of corn from Tex6. Phytopathology 90:292-296.

Haumann F (1995). Eradicating mycotoxins in food and feeds. Inform 6:248-256.

Huang Z, White DG, Payne GA (1997). Corn seed proteins inhibitory to *Aspergillus flavus* and aflatoxin biosynthesis. Phytopathology. 87:622-627.

Ji C, Norton RA, Wicklow DT, Dowd PF (2000). Isoform patterns of chitinase and β-1,3-glucanase in maturing corn kernels (*Zea mays* L.) associated with *Aspergillus flavus* milk stage infection. J. Agric. Food Chem. 48: 507-511.

King SB, Scott GE (1982). Field inoculation techniques to evaluate maize for reaction to kernel infection by *Aspergillus flavus*. Phytopathology 72:782-785.

Lillehoj EB (1987). The aflatoxin-in-maize problem: The historical perspective. In: Zuber MS, Lillehoj EB, Renfro BL (eds) Aflatoxin in maize: A proceedings of the workshop held at CIMMYT, El Batan, Mexico, DF, pp 13-32.

Payne GA (1992). Aflatoxin in maize. Critical Rev. Plant Sci. 10:423-440.

Payne GA (1997). Characterization of inhibitors from corn seeds and the use of a new reporter construct to select corn genotypes resistant to aflatoxin accumulation. In: Robens J, Dorner, J (eds) Proceedings of the USDA-ARS Aflatoxin Elimination Workshop held at Memphis, TN, pp 66-67.

Payne GA (1998). Process of contamination by aflatoxin- producing fungi and their impact on crops. In Sinha KK, Bhatnagar D (eds.), Mycotoxins in Agriculture and Food Safety. Marcel Dekker, New York, NY, pp. 279-306.

Rajasekaran K, Hudspeth RL, Cary JW, Anderson DM, Cleveland TE (2000). High-frequency stable transformation of cotton (*Gosspium hirsutum* L.) by particle bombardment of embryogenic cell suspension cultures. Plant Cell Rep. 19:539-545

Scott GE, Zummo N (1988). Sources of resistance in maize to kernel infection by *Aspergillus flavus* in the field. Crop Sci. 28:505-507.

Tucker DH Jr, Trevathan LE, King SB, Scott GE (1986). Effect of four inoculation techniques on infection and aflatoxin concentration of resistant and susceptible corn hybrids inoculated with *Aspergillus flavus*. Phytopathology 76:290-293.

Walker RD, White DG (2001). Inheritance of resistance to Aspergillus ear rot and aflatoxin production of corn from CI2. Plant Dis. 85: 322-327.

White DG, Rocheford TR, Kaufman B, Hamblin AM (1995). Chromosome regions associated with resistance to *Aspergillus flavus* and inhibition of aflatoxin production in maize. In: Robens J, (ed) Proceedings of the USDA-ARS Aflatoxin Elimination Workshop held at Atlanta, GA, p. 8.

White DG, Rocheford TR, Naidoo G, Paul C, Hamblin AM, Forbes AM (1998). Inheritance of molecular markers associated with, and breeding for resistance to Aspergillus Ear Rot and aflatoxin production in corn using Tex6. In: Robens J, Wicklow, D (eds) Proceedings of the USDA-ARS Aflatoxin Elimination Workshop held at St. Louis, MO, pp. 4-6.

Widstrom NW, Butrón A, Guo BZ, Wilson DM, Snook ME, Cleveland TE, Lynch RE (2003). Control of preharvest aflatoxin contamination in maize through pyramiding resistance genes to ear-feeding insects

and invasion by *Aspergillus* spp. Eur. J. Agron (In press).
Windham GL, Williams WP (1998). *Aspergillus flavus* infection and aflatoxin accumulation in resistant and susceptible maize hybrids. Plant Dis. 82:281-284.
Windham GL, Williams WP, Davis FM (1999). Effects of the southwestern corn borer on *Aspergillus flavus* kernel infection and aflatoxin accumulation in maize hybrids. Plant Dis. 83:535-540.
Woloshuk CP, Cavaletto JR, Cleveland TE (1997). Inducers of aflatoxin biosynthesis from colonized maize kernels are generated by an amylase activity from *Aspergillus flavus*. Phytopathology 87:164-169.
Wyllie TD, Morehouse LG (1978) . Introduction. In TD. Wyllie TD, Morehouse LG (eds) Mycotoxic Fungi, Mycotoxins, Mycotoxicoses, an encyclopedic Handbook. Vol. 3, Marcel Dekker, Inc., New York, pp. vii.
Yu J, Whitelaw CA, Bhatnagar D, Cleveland TE, Nierman WC (2002). Report on *Aspergillus flavus* EST project – A tool for eliminating aflatoxin contamination. In: Brown RL, Robens J (eds) Proceedings of the USDA-ARS Aflatoxin/Fumonisin Elimination and Fungal Genomics Workshop held at San Antonio, TX, pp. 27.