Preharvest origins of toxigenic fungi in stored grain

D. T. Wicklow*

Abstract

The discovery of *Aspergillus flavus* and aflatoxin in preharvest maize in 1975 has invited considerable interdisciplinary research on the ecology and pathology of a fungus that seed pathologists had classified as a 'storage mould' and evidence of poor grain storage practices. *A. flavus* colonises maize as follows: (1) airborne or insect-transmitted inoculum contaminates the silks and grows into the developing ear; (2) portions of the ear having kernels damaged by insects or birds become infested with *A. flavus* and contaminated with aflatoxin; (3) nitidulid beetles (Nitidulidae:Coleoptera) also carry *A. flavus* inoculum directly from moulded crop residues to damaged maize kernels; (4) climatic/-cultural factors that stress the plant, particularly high evening temperatures (> 24°C) during kernel filling, increase susceptibility of maize ears to fungal infection. The seed coat (testa) is the most important barrier preventing *A. flavus* from infecting the grain proper (e.g. embryo and endosperm), where aflatoxin contamination occurs. The outcome of these interactions determines the degree to which *A. flavus* colonises grain and contaminates it with aflatoxin.

Introduction

This paper examines the preharvest origins of *Aspergillus flavus* Link:Fr. and aflatoxin in maize (*Zea mays* L). I shall attempt to characterise the ecology and pathology of *A. flavus* in maize production and storage ecosystems as a model for examining the dynamics of other fungi commonly isolated from grain. The presence of *A. flavus* in preharvest maize had been reported by Taubenhaus (1920) and Tuite (1961). However, it was the discovery of preharvest aflatoxin contamination (Anderson et al. 1975), particularly in the midwestern corn belt of the USA (Rambo et al. 1974; Hesseltine et al. 1976; Lillehoj et al. 1976; Hurburgh 1991) that has invited considerable interdisciplinary research on the ecology and pathology of a fungus that seed pathologists had earlier classified as a 'storage mold' (Christensen and Kaufmann 1965). Until these discoveries, individuals responsible for maintenance of grain quality in storage were advised that the growth of *A. flavus* from surface-sterilised maize grain was evidence of poor grain storage practices (Christensen and Kaufmann 1974). Maize kernels invaded by *A. flavus* or other storage molds before storage deteriorated more rapidly under conditions favorable to the fungi than kernels free of storage fungi (Qasem and Christensen 1960). With the exception of *A. flavus* (Diener et al. 1987; Wicklow 1991) we have little understanding of the life history and ecology of 'storage fungi' in agricultural fields and almost no information about their ecological role in native plant communities (Raper and Fennell 1965; Domsch et al. 1980; Christensen and Tuthill 1985).

* National Center for Agricultural Utilization Research, U.S. Department of Agriculture, 1815 N. University Street, Peoria, Illinois 61604, U.S.A.

Sources of *Aspergillus flavus* Inoculum

Aspergillus flavus produces yellow-green conidia that function in dispersal and as infective inoculum, in addition to sclerotia, long-lived survival structures. Both types of propagules are associated with damaged maize kernels and are dispersed onto the ground during combine harvesting (Wicklow and Horn 1984; Wicklow et al. 1984). Wicklow and Wilson (1990) observed that sclerotium germination (sporogenic) occurred in maize fields just before silking. Shade provided by the maize canopy may help to retain moisture at the soil surface and thus promote germination, since sclerotia exposed for the same interval on fallow, bare ground adjacent to each field did not germinate. Stack and Pettit (1984) reported sporogenic germination of buried sclerotia at soil moisture tensions between 0-10 bars. This was an important revelation since we now realise that simple burial of *A. flavus* sclerotia will not necessarily prevent them from germinating and colonising dead roots and other plant residues (Wicklow et al. 1993). Papa (1986) theorised that *A. flavus* sclerotia such as those reported to form on maize kernels (Wicklow et al. 1982, 1984) could provide the means by which genetically diverse populations, different heterokaryon-compatability (h-c) groups, would accumulate in a single field.

Aspergillus flavus is common in tropical and subtropical habitats (Raper and Fennell 1965; Domsch et al. 1980). Miller et al. (1957) reported that *A. flavus* occurred in 10-27% of the isolation plates made in a survey of forest and cultivated soils from Georgia, USA. *Aspergillus flavus* has also been isolated, although infrequently, from soil samples collected from cultivated fields in northern islands of Japan (Manabe and Tsuruta, 1978). At the same time, *Aspergillus flavus* and *A. parasiticus* are not reported from soils collected from native prairies in the midwest (Orpurt and Curtis 1957; Wicklow 1973; Clarke and Christensen 1981; Angle et al. 1982; Shearer et al. 1992). The fungal community of native prairie soils is significantly altered by ploughing and cropping practices (England and Rice 1957; Shearer 1988) and this apparently enables *A. flavus* to 'invade' such soils. For example, soils from several different maize cultural systems at Columbia, Missouri were all contaminated with *A. flavus* and *A. parasiticus* (25–279 colony forming units, CFUs/g dry soil) including plots that have received manure applications without change since 1888 at Columbia, Missouri (Angle et al. 1982). *A. flavus* was recovered from 70% of crop residues sampled in 1989 from 40 Iowa maize fields, within one year of the 1988 aflatoxin outbreak in Iowa, and the fungus persisted at low levels when the same 40 corn fields were sampled in 1990 (Shearer et al. 1992) and in 1991, 1992, and 1993 (D.C. McGee, pers. comm.). Visible sporulation of *A. flavus* was detected on waste maize spillage near cribs and storage bins on several Iowa farms, especially where there was extensive weed growth (Hoyos et al. 1992). Before these discoveries, reports of *A.*

flavus being isolated from agricultural systems in northern latitudes were limited to examples of grain moulded in storage (Christensen and Kaufmann 1974), hay sampled from barn lofts in Wisconsin (Smalley et al. 1972), or diseased insects (Burnside 1930; Wicklow and Cole 1982).

In cultivated fields, *A. flavus* may actively colonise the residues of maize and other crops (Zummo and Scott 1990; Shearer et al. 1992). Zummo and Scott (1990) report finding *A. flavus* sclerotia in the pith tissues of maize cobs that were dispersed onto the ground at harvest and had overseasoned (1-year) in Mississippi, USA. There is some evidence suggesting that the build-up of *A. flavus* populations in peanut field soils may be associated with crop rotation sequences (Griffin and Garren 1974; Pettit and Taber 1968). Griffin and Garren (1976) showed that low densities of *A. flavus* in field soil increased several–fold around buried rye grass. Maize following maize, and maize following peanut rotations were associated with increased *A. flavus* density in field soil (Griffin et al. 1981). Any effort to determine the source of storage fungi contaminating grain in maize production fields might begin by examining the fungal populations colonising crop residues just before maize flowering and during the interval of ear maturation. The fate of these residues is important, particularly since they may also be colonised by nitidulid beetles that vector moulds to ripening maize ears (Lussenhop and Wicklow 1990).

Maize Insects and *Aspergillus flavus*

Insects were immediately implicated as potential vectors of *A. flavus* to maize because the fungus was found sporulating in insect-damaged portions of the ear (Taubenhaus 1920; Anderson et al. 1975). Initial attempts to identify insects that might vector *A. flavus* to maize proved inconclusive (Diener et al. 1987). Although many kinds of insects collected from maize ears carry *A. flavus* (Fennell et al. 1977), collections have typically been made late in the season when secondary inoculum of *A. flavus* is so abundant that insects are more likely to become contaminated. Moths of the corn earworm, *Heliothis zea* (Boddie), and European corn borer, *Ostrinia nubilalis* (Hubner), have been implicated as vectors of *A. flavus* inoculum to maize ears because earworm larvae and the kernels damaged by larvae may become contaminated with *A. flavus* (Lillehoj et al. 1978) and because adult moths collected during an 8-year period at a light trap (Coastal Plains Experiment Station, Tifton, GA) were contaminated with *A. flavus* (McMillian 1987; McMillian et al. 1990). Presumably, the moths contaminate the silks with *A. flavus* when depositing their eggs. In this scenario, both the fungus and the developing caterpillar larvae would enter the ear through the silk channel. Where these moths become contaminated with *A. flavus* is not known. Neither insect has actually been found to transport *A. flavus* to maize ears in the field. The corn earworm has also been implicated as providing an entrance into the ear for many insects, including rice weevil, pink scavenger caterpillar, sap beetles and mites (Zuber 1977).

Payne (1987) suggested that insects are not a major factor in bringing *A. flavus* to the ear but contribute to contamination through kernel injury. Lussenhop and Wicklow (1990) demonstrated the role of nitidulid beetles (Nitidulidae: Coleoptera) in transmitting *A. flavus* infective inoculum to preharvest maize ears in commercial maize fields in the Georgia Coastal Plain, USA. The nitidulids gain entry to the ears through wounds caused by other insects and birds, and are capable of entering some ears on their own, particularly loose-husked varieties where kernels near the ear tip are exposed (Connell 1956).

Downed and overwintered maize ears that become infested with *A. flavus* represent an important source of infective inoculum (Lussenhop and Wicklow 1990). This is because populations of nitidulid beetles also colonise these ears. Nitidulids may preferentially feed on rotted and yeast-contaminated plant tissues, using the yeast as food (Miller and Mrak 1954). *Candida guillermondii* (Castellani) Langeronet Guerra was the prevalent yeast isolated from insect-damaged and moulded maize grain sampled at harvest from fields throughout the state of North Carolina, USA. (Wicklow et al. 1980; Horn 1985). Nitidulids are generally more tolerant of individual mycotoxins than non-fungus-feeding insects (Dowd 1992). For example, adults of *Carpophilus hemipterus* L. (Nitidulidae: Coleoptera) are unaffected by 25 ppm aflatoxin B_1 (Dowd 1992) and larvae are 10 times more efficient in metabolising the *Fusarium*-produced trichothecene 4-monoacetoxyscirpenol than non-fungus-feeding caterpillars, the fall armyworm, *Spodoptera frugiperda* and the corn earworm, *Heliothis zea* (Boddie) (Dowd and Van Middlesworth 1989). Stored product beetles such as *Tribolium confusum* are generally resistant to mycotoxins, yet are also selective in their feeding behaviours (Wright et al. 1982). The feeding behaviours of nitidulids in corn fields could determine which mycotoxigenic fungi are carried to ears ripening in the field. Aflavinines, secondary metabolites distributed exclusively in *A. flavus* sclerotia, deter feeding by *C. hemipterus* (Wicklow et al. 1988a). The adult beetles will consume the mycelium and conidia of *A. flavus*, but not the intact or ground sclerotia. Four natural products with the aflavinine ring system were isolated as major components of *A. flavus* sclerotia with antifeedant activity against *C. hemipterus* (Wicklow et al. 1988a; Gloer et al. 1988). These sclerotial metabolites were not detected in the mycelium, conidia, or medium from which the sclerotia were harvested.

Combine harvesting is not totally efficient and 1.5-4% of the maize ears may be left lying on the ground following harvest. The grain on these maize ears may be consumed by seed-eating rodents, birds, deer, cattle, or swine that forage in maize fields following harvest. The ability of a fungus to contaminate the ears with toxic metabolite(s) that causes vertebrates to refuse the grain would thus protect the fungus from being consumed with the grain. *Aspergillus flavus* produces aflatrem, cyclopiazonic acid, and B-nitropropionic acid, all of which have neurotoxic effects and thus would represent an immediate deterrent to vertebrates (Wilson and Wilson 1964; Bush et al. 1951; Dorner et al. 1983). These moulded ears then become a source of infective inoculum that nitidulids may transmit to the next year's maize crop. Mycotoxin-producing ability is therefore under strong selection in such maize cultivation systems (Wicklow 1989).

Fungal Interactions and Aflatoxin in Maize

Aspergillus flavus coexists or competes with these other fungal colonists of cereals and the outcome of such species interactions is important in determining the extent of *A. flavus* colonisation and aflatoxin contamination (Wicklow et al. 1980). Infection by the ear rot fungus *Helminthosporium maydis* predisposed the kernels to *A. flavus* infection and aflatoxin (Doupnik 1972). Patterns of fungal association in individual seeds can point to relevant species interactions (Wicklow 1988). Individual maize grains infected with *F. moniliforme* were less likely to be infected with *A. flavus* or other fungal pathogens of maize. Other saprotrophs are known to prevent aflatoxin formation or detoxify aflatoxins. For example, *A. niger*, *Rhizopus arrhizus* Fischer and *Rhizopus nigricans* Ehrenburg have been found to metabolise aflatoxin B1 to a relatively non-toxic compound, aflatoxin B2a (Ciegler

et al. 1966; Cole et al. 1972) or to limit aflatoxin formation on autoclaved or irradiated grain and other culture media (Weckbach and Marth 1977; Wicklow et al. 1980; Horn and Wicklow 1983; Magan and Lacey 1988).

Aspergillus flavus appears to require at least 16–17.5% moisture to allow for colonisation of stored maize (Lopez and Christensen 1967; Sauer and Burroughs 1980). Drying maize to a moisture content below 13% effectively halts aflatoxin build-up (Shotwell et al. 1981). When preharvest maize kernels are damaged by insects there is a rapid dry-down to moisture levels at which the osmotolerant fungus *A. flavus* is at a competitive advantage over potential fungal competitors (Northholt et al. 1977; Diener et al. 1987; Jones et al. 1980). It is in these damaged kernels and among kernels 1-2 rows removed from the wound site where the bulk of the aflatoxin is produced (Lee et al. 1980; Wicklow et al. 1988b). Some of the kernels will fluoresce a bright greenish-yellow (BGYF) under ultraviolet light (365 nm). The fluorescence is produced by the oxidative action of peroxidase enzymes in living seed on kojic acid, which is formed by *A. flavus*. Samples of maize with lower BGYF counts had less aflatoxin (Shotwell et al. 1975; Shotwell et al. 1981; Shotwell and Hesseltine 1981; Dickens and Whittaker 1981). In yellow maize lots from North Carolina the average aflatoxin concentration in the non-BGYF kernel fraction was 49 µg/kg while an average of 8665 µg/kg was recorded for the BGYF kernel fraction (Dickens and Whittaker 1981). Dowd (1988a) has shown that kojic acid synergised the toxicity of aflatoxin B_1 to corn earworm and fall armyworm larvae. In the presence of kojic acid (25 ppm), only one-tenth the amount of aflatoxin B_1 (0.25 ppm) was needed to produce levels of toxicity/mortality seen for aflatoxin B_1 alone at 2.5 ppm. Dowd (1988b) suggests that kojic acid inhibits oxidative enzymes likely to be involved in aflatoxin B_1 detoxification. Does kojic acid also inhibit enzymes that function in protecting seeds from fungal attack?

Route of Kernel Infection

Fungal penetration of hyphae into the seed proper is resisted by the testa which becomes thicker as the grains mature, except over the embryo (Semeniuk 1954). Zuber (1977) and others have observed that *A. flavus* is a weak pathogen of maize, infection of the grain usually requiring a break in the pericarp (Koehler 1957; Guo et al. 1993). Smart et al. (1990b) showed that *Aspergillus flavus* gains entry to the seed proper through random microscopic breaks in the testa when the surrounding pericarp was intact. Aflatoxin is produced only after the fungus has invaded the embryo or endosperm. This explains the random pattern of aflatoxin occurrence among individual, undamaged kernels removed from the same ear (Jones et al. 1980; Lee et al. 1980; Smart et al. 1990a).

Temperature Stress and Aflatoxin

High temperatures during grain development have been associated with high levels of aflatoxin in the field (Anderson et al. 1975; Lillehoj et al. 1978; 1980; Widstrom et al. 1990) and greater numbers of infected kernels from silk-inoculated maize during the period of grain filling (Jones et al. 1980; Payne et al. 1988). In the midwestern corn belt, *A. flavus* colonisation and aflatoxin contamination of preharvest maize are associated with high temperatures and drought (Lillehoj et al. 1976; Hurburgh 1991). Zuber et al. (1983) observed that high temperatures proved more important than lack of moisture in enhancing aflatoxin levels. Lillehoj et al. (1978, 1980) compared summer temperature with the number of aflatoxin positive samples for 12 genotypes grown at 9 locations in 1976 and 12 locations in 1978. In general the locations with the highest July–August temperatures had the highest number of aflatoxin-positive samples in preharvest maize. Aflatoxin levels were significantly greater in kernels from plants grown at the hottest daily temperature in plant growth chambers (Thompson et al. 1980). Jones et al. (1980) suggest that 'at high temperatures this fungus (*A. flavus*) has increased parasitic ability.' While this may be true, stress injury to maturing grains grown at high temperatures leads to cracks in the testa and allows internal colonisation of the grains (Smart et al. 1990b). Stress-related damage to kernels may explain the substantial aflatoxin contamination recorded for silk-inoculated maize grown at elevated (30°C) night temperatures (Jones et al. 1981; Smart et al. 1990b). Temperature stress results in poor kernel filling, vivipary and kernel abortion, all of which provide entry points for opportunistic kernel-rotting fungi such as *Aspergillus flavus*. High temperature stress is known to reduce the sink capacity of developing maize kernels (Jones et al. 1985; Reddy and Daynard 1983) and to increase the frequency of kernel abortion (Jones et al. 1981). Significant increases in the frequency of kernel abortion and damage to the kernel were found among kernels cultured at high temperatures 'in vitro' without any microorganisms present (Hanft and Jones 1986a,b).

Approximately 25 years ago maize growers in the southern United States stopped planting late-maturing 'old southern' lines and began planting early maturing maize hybrids that were initially developed for the corn belt (Zuber 1977). Zuber argued that corn belt hybrids are not adapted for southern latitudes, noting that they have inadequate husk coverage which makes them vulnerable to maize insects that damage the kernels and vector *A. flavus*. At the same time, when early maturing corn belt hybrids are planted in southern latitudes this often exposes them to 'unseasonably' high temperatures during intervals of kernel filling. Consider that in the corn belt evening temperatures during kernel fill are 60-68°F (16–20°C) while evening temperatures exceeding 75–80°F (24–27°C) are common in regions such as the Georgia Coastal Plain and the Rio Grande Valley of Texas and Mexico, where aflatoxin contamination of preharvest maize is more common than in the corn belt. At some locations the problem may be further exacerbated by early planting of these corn belt hybrids. LaPrade and Manwiller (1977) reported that short-season hybrids had a much greater build-up of aflatoxin B_1 than the long-season hybrids. A recent study has shown that with progressively later planting dates in southern Georgia the maize had progressively less aflatoxin at harvest (Widstrom et al. 1990). This came as a surprise since Widstrom and his colleagues had predicted increased aflatoxin levels with later plantings because populations of *A. flavus* and numbers of kernel damaging insects both increase during the summer. Widstrom et al. (1990) showed an association between later planting dates and exposure to cooler temperatures during kernel filling. The later-maturing old southern genotypes would normally have ripened when temperatures are less extreme. To identify maize lines fully resistant to *A. flavus* and aflatoxin we may also have to find lines with physiological mechanisms of resistance to temperature stress.

Resistance to *Aspergillus flavus* or Aflatoxin

Identifying and developing maize genotypes with resistance to *Aspergillus flavus* Link:Fr. is considered by many to be the most logical approach for reducing aflatoxin contamination of the grain (Zuber 1977; Scott et al. 1991). At the same time, results have been disappointing because of the variable amounts of aflatoxin at different geographical locations and from year to year (Davis et al. 1986; Payne 1992). Widstrom (1987) listed four major genetically controlled traits that could

influence aflatoxin contamination: (1) resistance to the infection process; (2) resistance to aflatoxin contamination once infection has occurred; (3) resistance to insect damage; and (4) resistance to environmental stress. These traits may be expressed separately or in various combinations, depending on the location and year. Consider the problems in interpreting the results of aflatoxin resistance trials over 2-3 years if the test hybrids are simply planted in field plots at different locations and subject to natural/local sources of *A. flavus* inoculum:

Levels of infective inoculum may vary from year to year and location to location. The set of environmetal conditions that produce an *A. flavus* population 'bloom' in fields with the midwest corn belt are not understood.

The percentage of wild isolates that produce aflatoxin in northern latitudes is much lower than in subtropical latitudes (Manabe and Tsuruta 1978) and in the midwest corn belt such atoxigenic strains could function naturally in dampening the amplitude of any aflatoxin outbreak.

There may be so little infective inoculum present that relatively few of the ears become infected, or inadequate amounts of toxin may be formed, thus making contrasts among corn varieties difficult or impossible (Widstrom et al. 1981).

While *A. flavus* and aflatoxin are associated with insect-damaged ears (Lillehoj et al. 1983), estimates of insect damage are often poorly correlated with levels of aflatoxin contamination (Tucker et al. 1986). One explanation is that all of the kernels damaged by insects do not necessarily become infected by *A. flavus*.

The ability of a hybrid to resist damage from ear-feeding insects represents an indirect form of resistance to *A. flavus* infection and aflatoxin. For example, Lillehoj et al. (1983) reported that the greatest levels of insect damage over all locations was observed on kernels of Mo17 × B73. In the southern United States, increased losses in maize from insect damage is attributed to planting early-maturing hybrids with loose, open husks and quick dry-down attributes (McMillian 1987). Barry et al. (1986) showed that kernels from tight husk maize hybrids contained significantly less aflatoxin than did kernels from hybrids with loose husks. Furthermore, the ears produced by maize lines express significant antifeedant resistance mechanisms to maize insects resulting in significantly less ear damage (Waiss et al. 1979; Wiseman et al. 1983; Classen et al. 1990; Dowd 1990). At the same time, however, environmental stress to the maize plant may result in poorly developed husks or grain with fewer defensive chemicals.

Some maize varieties have a greater tendency for splits to occur in the seed coat or testa, such as occurs with a phenomenon characterised as 'silk cutting,' thus allowing *A. flavus* to infect the germ and endosperm, contaminating the grain with aflatoxin.

Some maize varieties exhibit greater resistance in the germ or embryo to *A. flavus* infection and aflatoxin (Brown et al. 1993). While the chemical/biochemical mechanism(s) that account for this resistance have not been elucidated, a general effort is under way to identify antifungal peptides in corn kernels (Duvick et al. 1992) and to isolate compounds from the kernel tissues such as the pericarp or alureone layers that may stimulate or inhibit *A. flavus* and aflatoxin formation (Norton 1994).

Temperature stress during kernel filling also causes kernels to split or abort, enabling *A. flavus* to enter the seed proper (see above). Drought has been associated with increased aflatoxin in maize (Payne et al. 1986) but the causal factors are not understood.

From the above it should be apparent that in evaluating data from aflatoxin resistance trials one must first consider the method of inoculation and kernel sampling that was used before attempting to distinguish genetic sources of resistance from confounding environmental variables. Plant pathologists have had to inoculate maize ears with *A. flavus* to obtain adequate aflatoxin contamination for differentiating resistance of genotypes each year. However, few studies involving maize hybrid comparisons have utilised the same method of inoculation and this makes comparisons of the results particularly difficult (Tucker et al. 1986). Any method involving wound-inoculation of the ears with *A. flavus* produces substantial quantities of aflatoxin in the wounded kernels (Gardner et al. 1987; Wicklow et al. 1987; Wicklow et al. 1988b). If the wound-inoculated kernels are included in the sample (Zuber et al. 1978) the high levels of aflatoxin in these wounded kernels may mask our ability to distinguish varietal resistance of intact kernels to *A. flavus* infection or aflatoxin.

King and Scott (1982) and Wicklow et al. (1987) provide methods for evaluating varietal resistance to preharvest infection and aflatoxin contamination of maize by separately evaluating the undamaged kernels adjacent to sites of wound-inoculation. Scott et al. (1991) demonstrated that the pin-bar inoculation technique is a suitable inoculation technique for distinguishing maize genotypes for response to inoculation with *A. flavus*. Aflatoxin levels in the adjacent undamaged kernels was equivalent to aflatoxin levels in undamaged kernels on wound-inoculated ears grown in the Biotron, University of Wisconsin (Wicklow et al. 1987, 1988b). Total aflatoxin concentration and BGYF were significantly correlated when the row of kernels adjacent to the pin-bar inoculated row was assayed (Tucker et al. 1986). Using the pin-bar technique, hybrids previously classified as resistant to kernel infection by *A. flavus* had fewer kernels infected and lower aflatoxin concentration in the grain at harvest (Scott and Zummo 1988; Scott et al. 1991). There are numerous other reports in the literature claiming some measure of maize resistance to *A. flavus* and aflatoxin (Payne 1992).

Identifying or developing maize varieties resistant to *A. flavus* and aflatoxin is just one component of our research program 'Integrated control of *Aspergillus flavus* and aflatoxin in the midwest corn belt' at the National Center for Agricultural Utilization Research. We seek to control *A. flavus* infection of preharvest maize through an integrated approach to disease management. The research investigates the ecology and pathology of *A. flavus* in preharvest maize, conceives and evaluates strategies for controlling sap beetle vectors of kernel rotting, mycotoxin-producing fungi, identifies maize resistance factors, and determines the important biotic interactions and environmental conditions that contribute to an aflatoxin outbreak in the midwest corn belt.

Specialty Maize Hybrids

It is important for grain pathologists and entomologists to recognise that the maize seed industry in the United States is actively developing specialty hybrids for specific end-users: food corn and snack foods; swine and poultry feeds; alcohol fuels; specific oils, proteins, starches, etc. Following harvest, the grain from these maize hybrids must be identity preserved. If a specialty hybrid is particularly susceptible to one or more mycotoxin-producing kernel-rotting fungi, then in mycotoxin outbreak years, maize products from these hybrids would be unacceptable to many end-users. This is particularly true if traditional maize hybrids represent an unacceptable alternative. Specialty maize and identity preserved grain lots will introduce new problems for grain pathologists and those involved in stored product protection, with increased risk to industries requiring reliable supplies of a specialty maize product.

Acknowledgments

I thank Dr Bruce Champ, ACIAR, Canberra and the ASEAN Food Handling Bureau for providing support for my travel to the conference.

References

Anderson, H.W., Nehring, E.W., and Wichser, W.R. 1975. Aflatoxin contamination of corn in the field. Journal of Agricultural and Food Chemistry, 23, 775–782.

Angle, J.S., Dunn, K.A., and Wagner, G.H. 1982. Effect of cultural practices on soil populations of *Aspergillus flavus* and *A. parasiticus*. Soil Science Society of America Journal, 46, 301–304.

Barry, D., Lillehoj, E.B., Zuber, M.S., Kwolek W.F., and Guthrie, W.D. 1986. Effect of husk tightness and insect infestation on aflatoxin contamination in preharvest maize. Environmental Entomology, 15, 1116–1118.

Brown, R.L., Cotty, P.J., Cleveland, T.E., and Widstrom, N.W. 1993. Living maize embryo influences accumulation of aflatoxin in maize kernels. Journal of Food Protection, 56, 967–971.

Burnside, C.E. 1930. Fungous diseases of the honeybee. USDA Technical Bulletin No. 149, 1–42.

Bush, D.A., Touster, O. and Brockman, J.E. 1951. The production of B-nitropropionic acid by a strain of *Aspergillus flavus*. Journal of Biological Chemistry, 188, 685–693.

Christensen, C.M. and Kaufmann, H.H. 1965. Deterioration of stored grains by fungi. Annual Review Phytopathology, 3, 69–84.

Christensen, C.M. and Kaufmann, H.H. 1974. Microflora. In: C.M. Christensen, ed., Storage of cereal grains and their products. St. Paul, American Association of Cereal Chemists, 158–192.

Christensen, M. and Tuthill, D.E. 1985. *Aspergillus:* an overview. In: R.A. Samson and J.I. Pitt, Eds., Advances in *Penicillium* and *Aspergillus* systematics. New York, Plenum, 195–209.

Ciegler, A., Lillehoj, E.B., Peterson, R.E., and Hall, H.H. 1966. Microbial detoxification of aflatoxin. Applied Microbiology, 14, 934–939.

Clarke, D.C. and Christensen, M. 1981. The soil microfungal community of a South Dakota grassland. Canadian Journal of Botany, 59, 1950–1960.

Classen, D., Arnason, J.T., Serratos, J.A., Lambert, J.D.H., Nozzolillo, C., and Philogene, B.J.R. 1990. Correlation of phenolic acid content of maize to resistance to *Sitophilus zeamais*, the maize weevil, in CIMMYT'S collections. Journal of Chemical Ecology, 16, 301–315.

Cole, R.J., Kirksey, J.W., and Blankenship, B.R. 1972. Conversion of aflatoxin B_1 to isomeric hydroxy compounds by *Rhizopus* spp. Journal of Agricultural and Food Chemistry, 20, 1100–1102.

Connell, W.A. 1956. Nitidulidae of Delaware. Deleware Agricultural Experiment Station Bulletin No. 318, 67 pp.

Davis, N.D., Currier, C.G. and Diener, U.L. 1986. Aflatoxin contamination of corn hybrids in Alabama. Cereal Chemistry, 63, 467–470.

Dickens, J.W. and Whitaker, T.B. 1981. Bright greenish-yellow fluorescence and aflatoxin in recently harvested yellow corn marketed in North Carolina. Journal of American Oil Chemists' Society, 58, 973A–975A.

Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.S., and Klich, M.A. 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. Annual Review Phytopathology, 25, 249–270.

Domsch, K.H., Gams, W. and Anderson, T.H. 1980. A Compendium of Selected Soil Fungi, Vols. I & II. New York, Academic Press. 859 p.

Dorner, J.W., Cole, R.J., Lomax, L.G., Gosser, H.S. and Diener, U.L. 1983. Cyclopiazonic acid production by *Aspergillus flavus* and its effects on broiler chickens. Applied and Environmental Microbiology, 46, 698–703.

Doupnik, B. 1972. Maize seed predisposed to fungal invasion and aflatoxin contamination by *Helminthosporium maydis* ear rot. Phytopathology, 62, 1367–1368.

Dowd, P.F. 1988a. Synergism of aflatoxin B1 toxicity with the co-occurring fungal metabolite kojic acid to two caterpillars. Entomologia Experimentalis et Applicata, 47, 69–71.

Dowd, P.F. 1988b. Toxicological and biochemical interactions of the fungal metabolites fusaric acid and kojic acid with xenobiotics in *Heliothis zea* (F.) and *Spodoptera frugiperda* (J.E. Smith). Pesticide Biochemistry and Physiology, 32, 123–134.

Dowd, P.F. 1990. Responses of *Carpophilus hemipterus* larvae and adults to selected secondary metabolites of maize. Entomologia Experimentalis et Applicata, 54, 29–36.

Dowd, P.F. 1992. Insect interactions with mycotoxin-producing fungi and their hosts. In: D. Bhatnagar, E.B. Lillehoj and D.K. Arora, eds., Handbook of applied mycology, Vol. 5, Mycotoxins in ecological systems, New York, Marcel Dekker, 137–155.

Dowd, P.F. and van Middlesworth, F.L. 1989. In vitro metabolism of the trichothecene 4-monoacetoxyscirpenol by fungus- and non-fungus feeding insects. Experimentia, 45, 393–395.

Duvick, J.P., Rood, T., Rao, A.G., and Marshak, D.R. 1992. Purification and characterization of a novel antimicrobial peptide from maize (*Zea mays* L.) kernels. Journal Biological Chemistry, 267, 18814–18820.

England, C.M. and Rice, E.L. 1957. A comparison of the soil fungi of a tall-grass prairie and of an abandoned field in central Oklahoma. Botanical Gazette, 118, 186–190.

Fennell, D.I., W.F. Kwolek, E.B. Lillehoj, G.A. Adams, R.J. Bothast, M.S. Zuber, Calvert, O.H., Guthrie, W.D., Bockholt, A.J., Manwiller, A. and Jellum, M.D. 1977. *Aspergillus flavus* presence in silks and insects developing and mature corn ears. Cereal Chemistry, 54, 770–778.

Gardner, C.A.C., Darrah, L.L., Zuber, M.S., and Wallin, J.R. 1987. Genetic control of aflatoxin production in maize. Plant Disease, 71, 426–429.

Gloer, J.B., TePaske, M.R., Sima, J.R., Wicklow, D.T. and Dowd, P.F. 1988. Antiinsectan aflavinine derivatives from the sclerotia of *Aspergillus flavus*. Journal of Organic Chemistry, 53, 5457–5460.

Griffin, G.J. and Garren, K.H. 1974. Population levels of *Aspergillus flavus* and the *A. niger* group in Virginia peanut field soils. Phytopathology, 64, 322–325.

Griffin, G.J. and Garren, K.H. 1976. Colonization of rye green manure and peanut fruit debris by *Aspergillus flavus* and *Aspergillus niger* group in field soils Applied and Environmental Microbiology, 32, 28–32.

Griffin, G.J., Garren, K.H., and Taylor, J.D. 1981. Influence of crop rotation and minimum tillage on the population of *Aspergillus flavus* group in peanut field soil. Plant Disease, 65, 898–900.

Guo, B.Z., Russin, J.S., Cleveland, T.E., Brown, R.L., and Widstrom, N.W. 1993. The role of the pericarp of corn kernels to reduce infection and aflatoxin production by *Aspergillus flavus*. Phytopathology, 83, 1417–1418.

Hanft, J.M. and Jones, R.J. 1986a. Kernel abortion in maize. I. Carbohydrate concentration patterns and acid invertase activity of maize kernels induced to abort 'in vitro.' Plant Physiology, 81, 503–510.

Hanft, J.M. and Jones, R.J. 1986b. Kernel abortion in maize. II. Distribution of ^{14}C among kernel carbohydrates. Plant Physiology, 81, 511–515.

Hesseltine, C.W., Shotwell, O.L., Kwolek, W.F., Lillehoj, E.B., Jackson, W.K., and Bothast, R.J. 1976. Aflatoxin occurrence in 1973 corn at harvest. II. Mycological studies. Mycologia, 68, 341–353.

Horn, B.W. 1985. Association of *Candida guilliermondii* with amylolytic filamentous fungi on preharvest corn. Canadian Journal of Microbiology, 31, 19–23.

Horn, B.W. and Wicklow, D.T. 1983. Factors influencing the inhibition of aflatoxin production in corn by *Aspergillus niger*. Canadian Journal of Microbiology, 29, 1087–1091.

Hoyos, G., McGee, D.C., and Tiffany, L.H. 1992. Waste corn as an inoculum source for *Aspergillus flavus*. Phytopathology, 81, 1134. Abstract.

Hurburgh, C.R. 1991. Aflatoxin in midwest corn. In: O.L. Shotwell and C.R. Hurburgh Jr., eds., Aflatoxin in corn. Iowa Agriculture and Home Economics Experiment Station Research Bulletin No. 599, 343–350.

Jones, R.J., Gegenbach, B.G., and Cardwell, V.B. 1981. Temperature effects on in vitro kernel development in maize. Crop Science, 21, 761–766.

Jones, R.J., Roessler, J., and Outtar, S. 1985. Thermal environment during endosperm cell division in maize: Effects on number of endosperm cells and starch granules. Crop Science, 25, 830–834.

Jones, R.K., Duncan, H.E., and Hamilton, P.B. 1981. Planting date, harvest date, and irrigation effects on infection and aflatoxin production by *Aspergillus flavus* in field corn. Phytopathology, 71, 810–816.

Jones, R.K., Duncan, H.E., Payne, G.A., and Leonard, K.J. 1980. Factors influencing infection by *Aspergillus flavus* in silk-inoculated corn. Plant Disease, 64, 859–863.

King, S.B. and Scott, G.E. 1982. Field inoculation techniques to evaluate maize for reaction to kernel infection by *Aspergillus flavus*. Phytopathology, 72, 782–785.

Koehler, B. 1957. Pericarp injuries in seed corn. University of Illinois Agricultural Experiment Station Bulletin No. 617, 72 pp.

LaPrade, J.C. and Manwiller, A. 1977. Relation of insect damage, vector, and hybrid reaction to aflatoxin B_1 recovery from field corn. Phytopathology, 67, 544–547.

Lee, L.S., Lillehoj, E.B., and Kwolek, W.F. 1980. Aflatoxin distribution in individual corn kernels from intact ears. Cereal Chemistry, 57, 340–343.

Lillehoj, E.B., Fennell, D.I., and Kwolek, W.F. 1976. *Aspergillus flavus* and aflatoxin in Iowa corn before harvest. Science, 193, 495–496.

Lillehoj, E.B., Fennell, D.I., Kwolek, W.F., Adams, G.L., Zuber, M.S., Horner, E.S., Widstrom, N., Warren, H., Guthrie, W.D., Sauer, D.B., Findley, W.R., Manwiller, A., Josephson, L.M. and Bockholt, A.J. 1978. Aflatoxin contamination of corn before harvest: *Aspergillus flavus* association with insects from developing ears. Crop Science, 18, 921–924.

Lillehoj, E.B., McMillian, W.W., Guthrie, W.D., and Barry, D. 1980. Aflatoxin-producing fungi in pre-harvest corn: inoculum source in insects and soils. Journal of Environmental Quality, 9, 691–699.

Lillehoj, E.B., Zuber, M.S., Darrah, L.L., Kwolek, W.F., Findley, W.R., Horner, E.S., Scott, G.E., Manwiller, A., Sauer, D.B., Thompson, D., Warren, H., West, D.R., and Widstrom, N.W. 1983. Aflatoxin occurrence and levels in preharvest corn kernels with varied endosperm characteristics grown at diverse locations. Crop Science, 23, 1181–1184.

Lopez, L.C. and Christensen, C.M. 1967. Effect of moisture content and temperature on invasion of stored corn by *Aspergillus flavus*. Phytopathology, 57, 588–590.

Lussenhop, J. and Wicklow, D.T. 1990. Nitidulid beetles (Nitidulidae: Coleoptera) as vectors of *Aspergillus flavus* in pre-harvest maize. Transactions of the Mycological Society of Japan, 31, 63–74.

Magan, N. and Lacey, J. 1988. Mycotoxin formation by *Aspergillus flavus* and *Fusarium graminearum* in irradiated maize grains in the presence of other fungi. Journal of Food Protection, 51, 452–456.

Manabe, M. and Tsuruta, O. 1978. Geographical distribution of aflatoxin-producing fungi inhabiting southeast Asia. Japanese Agricultural Research Quarterly, 12, 224–227.

McMillian, W.W. 1987. Relation of insects to aflatoxin contamination in maize grown in the Southeastern USA. In: M.S. Zuber, E.B. Lillehoj and B.L. Renfro, eds., Aflatoxin in maize: A proceedings of the workshop. Mexico, D.F., CIMMYT, 194–200.

McMillian, W.W., Widstrom, N.W., Wilson, D.M. and Evans, B.D. 1990. Annual contamination of *Heliothis zea* (Lepidoptera: Noctuidae) moths with *Aspergillus flavus* and incidence of aflatoxin contamination in preharvest corn in the Georgia Coastal Plain. Journal of Entomological Science, 25, 123–124.

Miller, J.H., Giddens, J.E., and Foster, A.A. 1957. A survey of the fungi of forest and cultivated soils of Georgia. Mycologia, 49, 779–808.

Miller, M.W. and Mrak, E.M. 1954. Yeasts associated with dried fruit beetles in figs. Applied Microbiology, 1, 174–178.

Northholt, M.D., Van Egmond, H.P., and Paulsch, W.E. 1977. Difference between *Aspergillus flavus* strains in growth and aflatoxin production in relation to water activity and temperature. Journal of Food Protection, 40, 778–781.

Norton, R.A. 1994. Isolation and identification of steryl cinnamic acid derivatives from corn bran. Cereal Chemistry, 71, 111–117.

Orpurt, P.A. and Curtis, J.T. 1957. Soil microfungi in relation to the native prairie continuum in Wisconsin. Ecology, 38, 628–637.

Papa, K.E. 1986. Heterokaryon incompatibility in *Aspergillus flavus*. Mycologia, 78, 98–101.

Payne, G.A. 1987. *Aspergillus flavus* infection of maize: silks and kernels. In: M.S. Zuber, E.B. Lillehoj, and B.L. Renfro, eds., Aflatoxin in maize: proceedings of the workshop, Mexico, D.F., CIMMYT, 119–129.

Payne, G.A. 1992. Aflatoxin in maize. Critical Reviews in Plant Science, 10, 423–440.

Payne, G.A., Cassel, D.K., and Adkins, C.R. 1986. Reduction of aflatoxin contamination in corn by irrigation and tillage. Phytopathology, 76, 697–684.

Payne, G.A., Thompson, D.L., Lillehoj, E.B., Zuber, M.S., and Adkins, C.R. 1988. Effect of temperature on the preharvest infection of maize kernels by *Aspergillus flavus*. Phytopathology, 78, 1376–1380.

Pettit, R.E. and Taber, R.A. 1968. Factors influencing aflatoxin accumulation in peanut kernels and the associated mycoflora. Applied Microbiology, 16, 1230–1234.

Quasem, S.A. and Christensen, C.M. 1960. Influence of moisture content, temperature, and time on the deterioration of stored corn by fungi. Phytopathology, 48, 544–549.

Rambo, G.W., Tuite, J., and Caldwell, R.W. 1974. *Aspergillus flavus* and aflatoxin in preharvest corn from Indiana in 1971 and 1972. Cereal Chemistry, 51, 848–853.

Raper, K.B. and Fennell, D.I. 1965. The Genus *Aspergillus*. Baltimore, Williams and Wilkins. 686 p.

Reddy, V.M. and Daynard, T.B. 1983. Endosperm characteristics associated with the rate of grain filling and kernel size in corn. Maydica, 22, 339–355.

Sauer, D.B. and Burroughs, R. 1980. Fungal growth, aflatoxin production and moisture equilibration in mixtures of wet and dry corn. Phytopathology, 70, 516–521.

Scott, G.E. and Zummo, N. 1988. Sources of resistance in maize to kernel infection by *Aspergillus flavus* in the field. Crop Science, 28, 504–507.

Scott, G.E., Zummo, N., Lillehoj, E.B., Widstrom, N.W., Kang, M.S., West, D.R., Payne, G.A., Cleveland, T.E., Calvert, O.H., and Fortnum, B.A. 1991. Aflatoxin in corn hybrids field inoculated with *Aspergillus flavus*. Agronomy Journal, 83, 595–598.

Semeniuk, G. 1954. Microflora. In: J.A. Anderson and A.W. Alcock, eds., Storage of cereal grains and their products. St. Paul, MN, American Association of Cereal Chemists, 77–151.

Shearer, J.F. 1988. Soil fungi of virgin and restored tallgrass prairies in central Iowa. Ph.D. Dissertation, Iowa State University, Ames, Iowa.

Shearer, J.F., Sweets, L.E., Baker, N.K., and Tiffany, L.H. 1992. A study of *Aspergillus flavus/parasiticus* in Iowa crop fields: 1988–90. Plant Disease, 76, 19–22.

Shotwell, O.L., Goulden, M.L., Jepson, A.M., Kwolek, W.F., and Hesseltine, C.W. 1975. Aflatoxin occurrence in some white corn under loan, 1971. III. Association with bright greenish-yellow fluorescense in corn. Cereal Chemistry, 52, 670–677.

Shotwell, O.L. and Hesseltine, C.W. 1981. Use of bright greenish yellow fluorescence as a presumptive test for aflatoxin in corn. Cereal Chemistry, 58, 124–127.

Shotwell, O.L., Kwolek, W.F., and Hesseltine, C.W. 1981. Aflatoxin in freshly harvested 1979 Georgia corn and formation after collection. Journal of American Oil Chemists' Society, 58, 980A–983A.

Smalley, E.B., Marasas, W.F.O., and Daugherty, S. 1972. Development of *Aspergillus flavus* and aflatoxins in harvested forage. College of Agricultural and Life Sciences, University of Wisconsin, Madison. Research Bulletin No. R2412, 24 p.

Smart, M.G., Shotwell, O.L., and Caldwell, R.W. 1990a. Pathogenesis in *Aspergillus* ear rot of maize: Aflatoxin B_1 levels in grains around wound-inoculation sites. Phytopathology, 80, 1283–1286.

Smart, M.G., Wicklow, D.T., and Caldwell, R.W. 1990b. Pathogenesis in *Aspergillus* ear rot of maize: light microscopy of fungal spread from wounds. Phytopathology, 80, 1287–1294.

Stack, J. and Pettit, R.E. 1984a. Germination of *Aspergillus flavus* sclerotia in soil. Phytopathology, 74, 799. Abstract.

Taubenhaus, J.J. 1920. A study of the black and the yellow molds of ear corn. Texas Agricultural Experiment Station Bulletin No. 270.

Thompson, D.L., Lillehoj, E.B., Leonard, K.J., Kwolek, W.F., and Zuber, M.S. 1980. Aflatoxin concentration in corn as influenced by kernel development stage and post-inoculation temperature in controlled environments. Crop Science, 20, 609–612.

Tucker, D.H., Trevathan, L.E., King, S.B., and Scott, G.E. 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.

Waiss, A.C., Chan, B.G., Elliger, C.A., Wiseman, B.R., McMillian, W.W., Widstrom, N.W., Zuber, M.S., and Keaster, A.J. 1979. Maysin, a flavone glycoside from corn silks with antibiotic activity toward corn earworm. Journal of Economic Entomology, 72, 256–258.

Weckbach, L.S. and Marth, E.H. 1977. Aflatoxin production by *Aspergillus parasiticus* in a competitive environment. Mycopathologia, 62, 39–45.

Wicklow, D.T. 1973. Microfungal populations from surface soils of manipulated prairie stands. Ecology, 54, 1302–1310.

Wicklow, D.T. 1988. Patterns of fungal association within maize kernels harvested in North Carolina. Plant Disease, 72, 113–115.

Wicklow, D.T. 1989. Maize cultivation selects for mycotoxin-producing ability among kernel-rotting fungi. In: C.H. Chou and G.R. Waller (Eds.) Phytochemical Ecology: Allelochemicals, Mycotoxins and Insect Pheromones and Allomones, Taipei, Institute of Botany, Academia Sinica Monograph Series No. 9, 263–274.

Wicklow, D.T. 1991. Epidemiology of *Aspergillus flavus* in corn. In: O.L. Shotwell and C.R. Hurburgh Jr., eds., Aflatoxin in corn: new Perspectives, Iowa Agriculture and Home Economics Experiment Station Research Bulletin No. 599, 315–328.

Wicklow, D.T. and Cole, R.J. 1982. Tremorgenic indole metabolites and aflatoxins in sclerotia of *Aspergillus flavus*: An evolutionary perspective. Canadian Journal of Botany, 60, 525–528.

Wicklow, D.T., Dowd, P.F., Tepaske, M.R., and Gloer, J.B. 1988a. Sclerotial metabolites of *Aspergillus flavus* toxic to a detritivorous maize insect (*Carpophilus hemipterus*, Nitidulidae). Transactions of the British Mycological Society, 91, 433–438.

Wicklow, D.T., Hesseltine, C.W., Shotwell, O.L., and Adams, G.L. 1980. Interference competition and aflatoxin levels in corn. Phytopathology, 70, 761–764.

Wicklow, D.T. and Horn, B.W. 1984. *Aspergillus flavus* sclerotia form in wound-inoculated corn. Mycologia, 76, 503–505.

Wicklow, D.T., Horn, B.W., Burg, W.R., and Cole, R.J. 1984. Sclerotium dispersal of *Aspergillus flavus* and *Eupenicillium ochrosalmoneum* from corn during harvest. Transactions of the British Mycological Society, 83, 299–303.

Wicklow, D.T., Horn, B.W., and Cole, R.J. 1982. Sclerotium production by *Aspergillus flavus* on corn kernels. Mycologia, 74, 398–403.

Wicklow, D.T., Horn, B.W., and Shotwell, O.L. 1987. Aflatoxin formation in preharvest maize ears coinoculated with *Aspergillus flavus* and *Aspergillus niger*. Mycologia, 79, 679–682.

Wicklow, D.T., Horn, B.W., Shotwell, O.L., Hesseltine, C.W., and Caldwell, R.W. 1988b. Fungal interference with *Aspergillus flavus* infection and aflatoxin contamination of maize grown in a controlled environment. Phytopathology, 78, 68–74.

Wicklow, D.T. and Wilson, D.M. 1990. *Paecilomyces lilacinus* (Thom) Samson, a colonist of *Aspergillus flavus* sclerotia buried in Illinois and Georgia. Mycologia, 82, 393–395.

Wicklow, D.T., Wilson, D.M., and Nelson, T.E. 1993. Survival of *Aspergillus flavus* sclerotia and conidia buried in soil in Illinois and Georgia. Phytopathology, 83, 1141–1147.

Widstrom, N.W. 1987. Breeding strategies to control aflatoxin contamination of maize through host plant resistance, In: M.S. Zuber, E.B. Lillehoj and B.L. Renfro, eds., Aflatoxin in maize: a proceedings of the workshop, Mexico, D.F., CIMMYT, 212–220.

Widstrom, N.W., McMillian, W.W., Beaver, R.W., and Wilson, D.M. 1990. Weather-associated changes in aflatoxin contamination of preharvest maize. Journal of Production Agriculture, 3, 196–199.

Widstrom, N.W., Wilson, D.M. and McMillian, W.W. 1981. Aflatoxin contamination of preharvest corn as influenced by timing and method of inoculation. Applied and Environmental Microbiology, 42, 249–251.

Wilson, B.J. and Wilson, C.H. 1964. Toxin from *Aspergillus flavus*: production on food materials of a substance causing tremors in mice. Science, 144, 177–178.

Wiseman, B.R., Widstrom, N.W., and McMillian, W.W. 1983. Influence of resistant and susceptible corn silks on selected developmental parameters of corn earworm (Lepidoptera: Noctuidae) larvae. Journal of Economic Entomology, 76, 1288–1290.

Wright, V.F., Vesonder, R.F., and Ciegler, A. 1982. Mycotoxins and other fungal metabolites as insecticides. In: E. Kurstak, ed., Microbial and viral pesticides, New York, Marcel Dekker, 559–583.

Zuber, M.S. 1977. Influence of plant genetics on toxin production in corn. In: J.V. Rodricks, C.W. Hesseltine and M.A. Mehlman, eds., Mycotoxins in human and animal health, Park Forest South, Pathotox, 173–179.

Zuber, M.S., Calvert, O.H., Kwolek, W.F., Lillehoj, E.B., and Kang, M.S. 1978. Aflatoxin B1 production in eight-line diallel of *Zea mays* infected with *A. flavus*. Phytopathology, 68, 1346–1349.

Zuber, M.S., Darrah, L.L., Lillehoj, E.B., Josephson, L.M., Manwiller, A., Scott, G.E., Gudauskas, R.T., Horner, E.S., Widstrom, N.W., Thompson, D.L., Bockholt, A.J., and Brewbaker, J.L. 1983. Comparison of open-pollinated maize varieties and hybrids for preharvest aflatoxin contamination in the southern United States. Plant Disease, 67, 185–187.

Zummo, N. and Scott, G.E. 1990. Relative aggressiveness of *Aspergillus flavus* and *A. parasiticus* on maize in Mississippi. Plant Disease, 74, 978–981.