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Integration of Crop Management and Genetics for Control of Preharvest Aflatoxin Contamination of Corn

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ABSTRACT

Aflatoxin contamination of corn in the field is influenced by several factors. In the southern U.S., insect populations are usually large every year. Drought caused by warmer and drier than normal weather is conducive to *A. flavus* infection and aflatoxin contamination of corn, *Zea mays* L. When loose-husked hybrids are used in the southern U.S., they accentuate insect damage and aflatoxin contamination. The development and breeding of ''southern-type'' hybrids is essential for control of preharvest aflatoxin contamination. Molecular biotechnology may make an impact on tackling the complexity of preharvest aflatoxin contamination of corn. Integration of crop management tactics and genetic

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strategies, conventional or molecular, may constrain the problem and help southern corn growers produce a quality, profitable crop.

Key Words: *A. flavus*; *A. parasiticus*; Mycotoxins; Maize; *Zea mays* L.

INTRODUCTION

The fungal metabolites called aflatoxins are among the most potent naturally occurring carcinogens, and are produced primarily by *Aspergillus* spp. fungi (Squire, 1981). Their production on corn, *Zea mays* L., can be dramatically influenced by several environmental factors (Lillehoj, 1983). Some of these are beyond control of producers; rainfall, ambient temperatures, humidity, and soil type (Lillehoj, 1983). Although considered uncontrollable, even these may be modified by appropriate management practices, e.g. irrigation, site selection, and crop rotation (Widstrom, 1996).

The importance of crop management to reduce contamination by aflatoxin became apparent when research revealed that most of the components that influence *Aspergillus* spp. infection and aflatoxin contamination are those which can be controlled by the producer (Lillehoj, 1983). A listing of these components would include; planting date selection (Widstrom, 1996), fertilization (Wilson et al., 1989), tillage (Jones, 1987), irrigation (Payne et al., 1986; Smith and Riley, 1992), choice of an appropriate hybrid (LaPrade and Manwiller, 1976; Lillehoj et al., 1982; Zuber, 1977), and control of insects (Lillehoj et al., 1980a; McMillian et al., 1980; Widstrom et al., 1975; Wilson et al., 1981), diseases (Campbell et al., 1993; Doupnik, 1972) and weeds (Glover and Krenzer, 1980). Each factor mentioned above, whether controlled or not, is inextricably related to plant stress in some manner. In general, a healthy nonstressed plant will be less likely to have high levels of infection or contamination than one subjected to stress, however, when conditions are favorable for aflatoxin contamination, no plant has been found to be immune.

Host plant resistance to biological and environmental factors, such as insects, diseases, weeds and drought that can indirectly cause plant stress, and sometimes contribute directly to the infection process have been the subject of much research effort. The early studies on host plant resistance were conducted prior to 1980 (LaPrade and Manwiller, 1976; Lillehoj et al., 1976; Widstrom et al., 1978), but others have followed since then (Darrah, 1987; Naidoo et al., 2002; Scott et al., 1991). These studies have clearly demonstrated that resistance in hybrids does in a real sense provide a protective barrier against the development of stress in the plant, even though that resistance is influenced by environmental and biological factors which

can contribute to ear infection and aflatoxin contamination (Fortnum, 1987; Lillehoj, 1983; Zuber and Lillehoj, 1979). Host plant resistance is, in fact, the most effective, efficient and dependable tool that we have in the long term to protect corn from the preharvest infection and aflatoxin contamination processes (Lillehoj et al., 1980a; Widstrom, 1992; Zuber, 1977). Our efforts will be best focused on identification of the most important and effective environmental factors and development through genetic improvement of chemical and physical traits in hybrids that reduce plant stress and contribute to host plant resistance. Our goal is to combine the major genetic traits using marker assisted selection (MAS), and to incorporate them into a management package that includes manipulation of critical environmental factors to minimize the risk of preharvest contamination by aflatoxin (Widstrom et al., 2000a). Our discussion in this paper will be centered around the development of progress to date and projecting a plan for continued research expected outcomes.

ENVIRONMENTAL FACTORS

Numerous environmental components were discussed in the previous section. In addition to their identification, each of the environmental and non-genetic components must be critically assessed to determine which are the most important in their influence on aflatoxin contamination. The field environment a logical place to search for clues that will assist in the identification of causes for poor plant health, reduced vigor, and other symptoms of abnormal development expressed by the growing plant. Identification of specific individual causes is often achieved with great difficulty due to the myriad of interactions that occur among the factors that impose their effects on plant environment. The major environmental influences will be discussed here individually, with the intent of assessing the relative importance of each, fully realizing that all are intricately interrelated in the imposition of stress on the plant, and in the development of contamination of the corn crop.

Temperature

Drought is defined in terms of very dry conditions, and in reference to crop production is usually associated with higher than normal temperatures. Such weather is usually accompanied by insect injury and fungus infection of the corn ear (Taubenhaus, 1920). *Aspergillus* spp. are uniquely thermotolerant, and fit ideally into the environmental niche that produces drought and heat stress in the corn field. The optimal temperature for production of

aflatoxin is approximately 30°C (Boller and Schroeder, 1974; Sorenson et al., 1967) while that for growth of corn is about 27°C (Aldrich et al., 1975; Shaw, 1977), and lower still when the plant is subjected to drought conditions (Zuber and Lillehoj, 1987). Average daily temperatures during grain fill reach or exceed this value in several southern states (Zuber and Lillehoj, 1987). Therefore, during years with even short periods of time as temperatures rise above 27°C, the fungus will be increasing its aflatoxin production activity while the plant is reducing its capacity for growth and grain filling, and thus less able to defend against fungal infection.

The establishment of temperature as an important component of infection by *A. flavus* and subsequent aflatoxin contamination has been clearly demonstrated under controlled greenhouse conditions (Payne et al., 1988; Thompson et al., 1980). The concept was corroborated by several field studies in which temperatures were monitored (Jones et al., 1980; Zuber et al., 1983). Some efforts to illustrate a relationship between temperature and aflatoxin contamination were unsuccessful (Stoloff and Lillehoj, 1981). The reason for this phenomenon can be traced to the finding that a detectable relationship exists only during years when amounts of contamination are high. McMillian et al. (1985b) conducted a six year study in which the three years with the highest contamination also had the highest average daily temperatures during the growing season. Similarly, in a five year study, a significant positive correlation between aflatoxin contamination and temperatures was obtained only during the two years with exceptionally high amounts of aflatoxin contamination (Widstrom et al., 1990). We conclude, therefore, that high temperatures do significantly contribute to the contamination process and the ultimate amount of aflatoxin produced.

Rainfall

The interrelationship of climatic factors such as temperature and precipitation cannot be ignored, but each has its own unique contribution to the aflatoxin contamination problem. It has been suggested that differences in precipitation amounts from region to region as contributing to contamination (Lillehoj et al., 1978b). If so, this has especially important implications for the producer who grows dryland corn while the effect can, to a large extent, be ignored if irrigation is available. A clear indictment of rainfall as a major component contributing to aflatoxin contamination has not been possible, probably due to the sporadic and variable amounts that occur during the growing season. Seasons with very low rainfall produce stress conditions for dryland corn and the high temperatures which usually

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accompany low rainfall are related to the severity of aflatoxin contamination. When late-season rainfall prevents timely harvest of the corn crop, the grain obtained from the delayed harvest can be expected to have increased aflatoxin contamination (Jones and Duncan, 1981).

Relative Humidity—Net Evaporation

Relative humidity and net evaporation are intricately interrelated since the two traits themselves are a result of the interaction between water and temperature. Lillehoj (1983) discussed these inter-relationships in terms of water activity and pointed out that water activities of 0.9 and above are ideal for *A. flavus* growth and aflatoxin synthesis while those less than 0.85 severely reduce aflatoxin production. Significant amounts of aflatoxin are not generated in inoculated samples of corn when incubated for seven days at relative humidities less than 91% (Guo et al., 1996). The determination and application of environmental limits for fungus growth and elaboration of aflatoxin in laboratory studies can be misleading if the information is extrapolated directly for use in the field, but is vital to initiating experiments to study those factors under highly variable field conditions (Lillehoj, 1983). Sisson (1987) monitored field conditions in several corn growing states, and determined that high humidity and high temperatures are both conducive to high concentrations of aflatoxin contamination. The common occurrence of heavy dews in the southern U.S., simulated by ear wetting at least three times each week during grain fill, can also significantly increase aflatoxin concentrations in mature ears (McMillian et al., 1985a). Field measurements of mean temperature and net evaporation are significantly correlated with aflatoxin concentrations of grain samples taken at harvest. These measurements are both judged to be more important than relative humidity or total precipitation in determining contamination (McMillian et al., 1985b).

Soil Type

Crop history affecting the amount and kinds of plant refuse in the soil profile and on the soil surface does influence the myco biota available and the micro-environment for fungal development (Martyniuk and Wagner, 1978). Soil types may also exert great influence on aflatoxin contamination of crops grown on them. Preharvest samples obtained from corn grown on sandy Coastal Plain soils had higher amounts of aflatoxin contamination than those sampled from the crop grown on heavier clay soils (Jones et al., 1981). They attributed the difference to additional plant stresses incurred by reduced water availability provided by the lighter soils. It has been

demonstrated that both soil type and cultivation practices influence spore load and crop contamination by aflatoxin (Angle, 1987) . The sandy soils of the southeastern U.S. have less than one-half the water holding capacity of most soils in the Cornbelt, increasing the probability of drought stress during the growing season (Widstrom, 1992). Conservation tillage reduces the loss of water from soils, but conventional wisdom tells us that such practices increase the *A. flavus* spore load available for infection of the crop following the rotation (Angle, 1987).

CROP MANAGEMENT FACTORS

Crop management has not been considered to be an efficient approach to control preharvest aflatoxin contamination, although the influence of crop environment has been recognized since the time when aflatoxin was first recognized as a preharvest problem (Anderson et al., 1975). Crop management practices can often alter the environmental effects and their influence on preharvest aflatoxin contamination which have been known and studied extensively (Fortnum, 1987; Jones, 1987; Lillehoj, 1983). The use of these components to modify or manipulate toxin formation, however, has not been primary consideration for the control of preharvest aflatoxin contamination. The use of crop management as a key to preventing or limiting aflatoxin contamination of the preharvest crop has been recommended (Widstrom et al., 1984a; Wilson et al., 1989). The relative impact and ease of manipulation of each management component through management decisions to influence aflatoxin contamination will require additional assessment before an effective plan for control can be initiated. Additionally, the economics of the application of control measures will greatly influence their integration into an overall management system (Widstrom et al., 2000b).

Planting Date

The choice of a planting date to avoid plant stress during the critical grain filling period was first suggested by Zuber and Lillehoj (1979). Studies that supported this concept were reported shortly thereafter (Jones and Duncan, 1981; Jones et al., 1981; Lillehoj et al., 1980b). The data were conflicting, however; the 1980 paper reported that an early planting in Tennessee had the highest amount of aflatoxin while the 1981 papers suggested that early plantings had reduced amounts of aflatoxin in North Carolina. The most comprehensive study conducted on planting dates was published in 1990 with data accumulated over a five-year period including five or more planting dates sampled during each year, depending on the

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earliest available planting time (Widstrom et al., 1990). The study demonstrated a reduction in aflatoxin contamination for plantings delayed as late as June or July on the coastal plain soils at Tifton, Georgia. Unfortunately late plantings are also associated with increasing reductions in yield. The study did confirm the importance of temperature and net evaporation during the critical grain fill period (McMillian et al., 1985b). Significant correlations between aflatoxin contamination and both temperature and net evaporation (Widstrom et al., 1990) were quite similar to those reported earlier (McMillian et al., 1985b) and discussed in the previous section on environmental factors.

Irrigation

Many environmental components are responsible for imposing stress on the corn plant during development and maturation. The most common, and possibly the most significant of these, is drought (Zuber and Lillehoj, 1979). The obvious remedy is irrigation, especially in areas where rainfall is limited and/or soils are sandy, providing little water holding capacity (Lillehoj, 1983). Irrigation, however, does not always have a large impact on aflatoxin contamination of the corn crop (Fortnum and Manwiller, 1985). The amount of contamination is normally reduced to some extent if irrigation is applied to alleviate drought conditions (Jones et al., 1981). Irrigation effects have been described as being similar to adequate rainfall in reducing the incidence and amount of aflatoxin in the grain (Jones, 1987). Irrigation not only alleviates the moisture stress in the plant, but also changes the overall environment in the field, creating a generally cooler temperature in the plant canopy. The influence of irrigation in modifying temperature, a factor considered by many to be the most important factor in reducing preharvest aflatoxin contamination (Fortnum, 1987), cannot be ignored. Finally, a net beneficial effect of irrigation has been demonstrated by most research on the subject, as indicated in several published research reports (McMillian et al., 1991; Payne et al., 1986; Smith and Riley, 1992). A major concern in many areas, where corn is grown and aflatoxin is a problem, is that irrigation is not available. More than one-half of the corn acreage in the southeastern U.S. is corn grown under dryland conditions (McMillian et al., 1991).

Tillage

The recent trends toward conservation tillage and organically grown crops, may have some impact on the risk for aflatoxin contamination of corn. Studies examining the influence of tillage on contamination of

peanuts are expected to be more revealing since their seed pods are in contact with the soil (Griffin et al., 1981). Deep tillage or subsoiling is regularly practiced on sandy soils of the Coastal Plain in the southeastern U. S. as a means of breaking up subsurface hardpan layers that develop. This practice probably has its greatest effect in reducing drought stress by allowing good root development and penetration (Jones, 1987). Burying crop residue has obvious advantages such as covering inoculum sources for disease in high-risk mono-culture rotations (Cole et al., 1982). Individual tillage effects on aflatoxin contamination of the crop have not been verifiable, possibly because of interaction with other more significant factors, such as moisture availability.

Fertilization

The first definitive research reporting preharvest aflatoxin contamination of corn also reported that stressed growing conditions, such as low nitrogen level, appeared to increase the incidence of aflatoxin contamination (Anderson et al., 1975). Conflicting results were reported for 1976 and 1977 when interpreting the influence of nitrogen fertilization on the amount of field contamination (Jones, 1987; Zuber and Lillehoj, 1979). Adequate fertilization has been suggested as a cultural practice that will alleviate plant nutrient stress and reduces aflatoxin contamination (Widstrom et al., 1984a; Zuber and Lillehoj, 1979). Similar recommendations advocating sufficient nitrogen fertilization to minimize aflatoxin contamination have been proposed by several other researchers (Glover and Krenzer, 1980; Jones, 1987; Lillehoj, 1983; McMillian et al., 1991; Widstrom et al., 1984a). Research specifically addressing the effects of nitrogen have been the basis for most of these recommendations (Jones and Duncan, 1981; Payne et al., 1989; Wilson et al., 1989). However, a recent study in Mexico failed to show any effect on aflatoxin contamination due to fertilization (Bucio-Villalobos et al., 2001). A word of caution is given by Wilson et al. (1989) warning against applying excess nitrogen which can increase plant stress and aflatoxin concentration. This precaution probably only applies to those attempting to maximize, rather than optimize their yields.

Weed Control

Weed infestation of corn fields was one of the aflatoxin predisposing factors studied by Anderson et al. (1975) in terms of the way weed populations contribute to plant stress. The suggested consideration of weeds as potential contributors to field contamination (Jones, 1987) is obvious

because their control often requires additional tillage practices, and always involves a crop management decision that will alleviate plant stress through a reduction in competition for water and nutrients (Zuber and Lillehoj, 1979). These competition effects have been directly linked to amounts of aflatoxin found in kernels (Cobb, 1979). The influence of herbicides on aflatoxin contamination, and the interaction of weed populations, has not been investigated extensively (Lillehoj, 1983). Research to measure aflatoxin concentration in corn grown under three cultivation rates for weed control was inconclusive (Bilgrami et al., 1992). Since most producers practice effective weed control by chemical or other means, it has not been demonstrated to be a critical consideration in an aflatoxin management program (Widstrom, 1996).

Fungal Competition

The concept of introducing fungal competitors into the field has been considered because studies have demonstrated a reduction in aflatoxin production by *Aspergillus* spp. when other fungi are present (Ehrlich et al., 1985). Calvert et al. (1978) reported this phenomenon using mixed inocula of *A. flavus* and *A. parasiticus*. Similar results were obtained when *A. niger* was used as a competitive species (Horn and Wicklow, 1983). *Fusarium moniliforme* was also identified as a fungus that could effectively suppress aflatoxin synthesis by *A. flavus* (Wicklow et al., 1988). The development of hybrids with the ability to support nontoxin-producing microbes that could effectively compete with toxin-producing *Aspergilli* was suggested by Lillehoj (1987). *A. flavus* has been shown to be better adapted to infection of the corn ear than *A. parasiticus* (Zummo and Scott, 1990b). It was discovered that *A. flavus* and *F. moniliforme* invade different parts of the ear, but that infection by one fungus does influence colonization of the ear by the other fungus (Zummo and Scott, 1990a). The production of aflatoxin by *A. flavus* in the field was reduced when placed in competition with *F. moniliforme* in a subsequent study (Zummo and Scott, 1992). Similar results were obtained by Widstrom et al. (1994) when *A. flavus* was observed as a competitor with *F. moniliforme* and by Choudhary (1992) when *A. flavus* was pitted against other toxigenic molds. The effective use of other toxigenic fungi to suppress aflatoxin production by *A. flavus* (Dorner et al., 1999), or even the use on non-toxigenic strains of the same fungus as suggested by Brown et al. (1991), still leaves the producer with a potentially serious ear rot problem. Most, if not all, of the fungi that effectively reduce aflatoxin production by *A. flavus* are ear-rotting organisms that reduce the quality and yield of the grain crop.

GENETIC CONTROL STRATEGIES

Conventional

The establishment of the aflatoxin contamination problem in preharvest corn alerted plant breeders to the need for developing of germplasm with genetically controlled resistance (Anderson et al., 1975). A series of review papers on genetic control of field contamination suggests various approaches for development of hybrids with 1) resistance to insects, 2) resistance to plant stress (adapted hybrids), and 3) resistance based on a relationship to other plant traits (Widstrom, 1987; Widstrom and Zuber, 1983; Widstrom et al., 1984a; Zuber and Lillehoj, 1979, 1987). Even before these reports appeared in print, the breeders were already busy screening germplasm in a search for resistance among commercial hybrids (LaPrade and Manwiller, 1977; Widstrom, 1987; Widstrom et al., 1978), experimental hybrids (King and Scott, 1982; LaPrade and Manwiller, 1976; Widstrom et al., 1978; Zuber et al., 1978) and varieties (Priyadarshini and Tulpule, 1978; Tulpule et al., 1977). Results from early screening were inconclusive in that significant differences were not always found among germplasm entries. Zuber (1977) had proposed a genetic solution to the aflatoxin problem, and this led to the establishment of procedures for identification of resistant types. While initial screening was being conducted, as indicated above, several other studies were being conducted to determine the best methods for field inoculation and evaluation (Campbell and White, 1994; King and Scott, 1982; Tucker et al., 1986; Widstrom et al., 1981; Widstrom et al., 1986). The ensuing genetic experiments provided convincing evidence of potential for genetic control of resistance to aflatoxin contamination and a genetic solution to the contamination problem (Gorman et al., 1992; Naidoo et al., 2002; Scott and Zummo, 1988; Thompson et al., 1984; Widstrom et al., 1984c, 1987; Zuber et al., 1983). Consequently, several sources of resistance were identified and released for use by public and private breeders (McMillian et al., 1993; Scott and Zummo, 1990b, 1992; William and Windham, 2001).

Related Plant Resistance Factors

Insects

Ear-feeding insects have been implicated as a contributing factor in aflatoxin contamination from the first substantiated instance of preharvest contamination of corn (Anderson et al., 1975). Fennell et al. (1975, 1977,1978) had reported an association between insect damage with

aflatoxin contamination in stored samples. These investigations were followed by the confirmation of aflatoxin contamination as a preharvest problem associated with insect feeding damage (LaPrade and Manwiller, 1977; Lillehoj et al., 1978a; McMillian et al., 1978; Widstrom et al., 1975). McMillian et al. (1985a) demonstrated a consistent association between insect damage and field aflatoxin contamination. Several insects have been found to be associated with contamination of corn kernels. Among these are the corn earworm, *Helicoverpa zea* (McMillian et al., 1978, 1990), the maize weevil, *Sitophilus zeamais* (Barry et al., 1985; McMillian et al., 1980), and the European corn borer, *Ostrinia nubilalis* (Guthrie et al., 1981; McMillian et al., 1988). The European corn borer is not yet a serious problem in the Southeastern U. S. where aflatoxin contamination is chronic. The other two insects will be discussed in conjunction with husk coverage.

Silk Maysin

The report of a 'lethal silk' factor in corn by Walter (1957), and subsequent studies to investigate these claims (Chambliss and Wann, 1971; Wann and Hills, 1966; Widstrom et al., 1977), led to the isolation and identification of maysin, a flavone glycoside in corn silks (Waiss et al., 1979) that has biological activity against the corn earworm (Elliger et al., 1980). Snook et al. (1993) found numerous germplasm sources for this compound, some which have been selected for extremely high maysin concentrations and have been publicly released (Widstrom and Snook, 2001d; Widstrom et al., 2002). The inheritance of silk-maysin concentration is known well enough so that transfer to commercial germplasm will not be difficult (Widstrom and Snook, 1994, 1998). Molecular studies have located numerous quantitative trait loci (QTL) that influence maysin concentration in corn silks (Byrne et al., 1996–1998), several of which are associated with loci found by conventional methods (Widstrom and Snook, 2001a). Butron et al. (2000) outlined a program of marker-assisted selection to improve resistance to the corn earworm. However, it must be remembered that insect resistance is only one necessary link in solving the problem of aflatoxin contamination in corn.

Husk Coverage

Both the length and tightness of husk coverage around the ear appear to be important in reducing aflatoxin contamination. Research supports the concept that complete and tight husk coverage helps protect the ear against

invasion by ear-feeding insects and against *A. flavus* infection with or without the presence of insect damage (Lillehoj et al., 1978a). The importance of husk traits to prevent ear damage by insects has been emphasized in the literature (Lillehoj and Zuber, 1975; Widstrom et al., 1976), and when it was discovered that insecticide treatments reduced but did not eliminate aflatoxin contamination, it was concluded that differences in husk protection also reduced contamination. Wiseman et al. (1977) determined that husk coverage beyond the ear tip was not sufficient to provide resistance, but that husk tightness was a necessary condition to prevent ear damage by corn earworm. Similarly, husk coverage and tightness are necessary to protect the ear from invasion and damage by the maize weevil (Barry et al., 1985, 1986; McMillian et al., 1980). Two loose-husked hybrids were contaminated with more than twice as much aflatoxin as two tight-husked hybrids in an inoculation study (Widstrom et al., 1981). Five hybrids, each with a different level of husk tightness, had significantly reduced aflatoxin contamination concentrations as husk tightness increased (Barry et al., 1986). Widstrom et al. (1993a,b) concluded that many corn hybrids depend heavily on husk protection for their resistance against aflatoxin contamination, although none give complete or consistent protection. Finally, high silk-maysin concentrations will not protect the ear against corn earworm unless husk coverage is sufficient to force the insect to feed on silks when entering the ear (Rector et al., 2002; Widstrom and Snook, 2001c).

Plant Resistance to Aflatoxin Formation

Plant Stress

Plant stress was recognized as a factor that enhances aflatoxin contamination in the field even prior to the firm establishment of hybrid differences as being important (Zuber and Lillehoj, 1979). Stress is most often associated with periods of extreme drought which, in turn, have been associated with aflatoxin contamination (Lillehoj, 1983). Several of the factors already discussed are responsible for imposing stress on the plant (Widstrom and Zuber, 1983), complicating the interpretation of research data (Widstrom, 1987; Widstrom et al., 1984a). Plant stress is sometimes equated with adaptation, thus the recommendation for planting adapted hybrids with the ability to buffer against local stresses caused by several factors, especially those with greatest impact on yield (Zuber and Lillehoj, 1987). Efforts are now being made to identify drought stress resistant germplasm and to incorporate that trait into other germplasms along with resistance to insect and aflatoxin contamination (Li et al., 2000).

Kernel Resistance

The search for resistance to contamination was begun shortly after it was defined as a preharvest problem (Anderson et al., 1975). Differences among germplasm sources were often inconsistent (Widstrom et al., 1984a; Zuber, 1977) causing some to be skeptical as to whether genetic differences existed for aflatoxin accumulation (Davis et al., 1985). In 1980, two types of kernels, distinctly different in appearance, were selected from the same open-pollinated ear on a hybrid plant and used to generate two different breeding populations (Widstrom et al., 1987). These populations were tested extensively in the field and laboratory, and found to be distinctly different in their resistance to aflatoxin production phenotypic appearance. The differences persisted when tested in experimental crosses to several southern inbred lines (McMillian et al., 1991). The resistant population, called GT-MAS:gk, was released in 1992 and registered as a germplasm source of resistance in 1993 (McMillian et al., 1993). Four years of experimentation on kernels of commercial hybrids and varied endosperm types indicated that sweet or sugary endosperm types supported greater colonization by *A. flavus* and higher aflatoxin production than starchy endosperm types (Widstrom et al., 1984b). Inconsistent results had been obtained in previous testing of endosperm types. However, sugary endosperm types were not included in those tests (Lillehoj et al., 1975, 1983).

Brown et al. (1993) attributed differences between wounded and unwounded kernels of certain resistant genotypes to the presence of something in the living maize embryo. Some of the resistance in the same genotypes was attributed to the wax and cutin layers on the kernel pericarp (Guo et al., 1995). The finding was confirmed by Russin et al. (1997). Guo et al. (1996) concluded that an aflatoxin inhibitor was induced during germination of the seed, and later determined that a zeamatin-like kernel protein and at least one ribosome inactivating protein (RIP), present in the kernel, were capable of inhibiting growth of *A. flavus* (Guo et al., 1997). Studies of the protein profiles of kernels revealed that several proteins were found in resistant types in greater concentrations than in susceptible, and that others were present only in susceptible types (Guo et al., 1998). Additional research determined that RIP is primarily in the aleurone layer of the endosperm while zeamatin occurs mainly in the embryo (Guo et al., 1999). Both proteins uniquely protect kernels from pathogens and may provide important aspects of resistance to *A. flavus* and aflatoxin contamination in corn.

Genetic Studies and Selection

Genetic experiments were conducted before adquate screening techniques for resistance to aflatoxin were developed and available (Zuber et al.,

1978). The first genetic parameter estimates were made among single-crosses, within which, some inbreds gave large estimates of general combining ability (GCA), and the crosses provided evidence for resistance being recessively inherited (Zuber and Lillehoj, 1979). Some of the earliest screening was conducted among adapted southern open-pollinated varieties that were grown widely prior to the transition to hybrid corn production (Zuber et al., 1983). The test failed to reveal exceptional resistance in any of the open-pollinated varieties when compared to popular hybrids being grown in the 1980's. General combining ability effects were determined to be responsible for primary control of aflatoxin contamination among southern dent and sweet corn inbreds when tested as single crosses (Widstrom et al., 1984c). Controlled environment experiments by Thompson et al. (1984) revealed the importance of replication in detecting differences among genotypes. Gardner et al. (1987), using of the same germplasm tested by Zuber et al. (1978), concluded that experiments with eight replications provided a good compromise between controlling the variance estimates in an experiment and the cost for aflatoxin analyses. The germplasm evaluated by Zuber et al. (1978) and Gardner et al. (1987) was again evaluated in a five-state experiment (Darrah, 1987). The tests effectively accentuated the difficulties in repeating results in different environments and under varied inoculation techniques. Evaluations among genetically diverse varieties also illustrated difficulties in identifying germplasm with the most resistance in any given test (Kang et al., 1990).

The most efficient time to sample from field tests was determined to be at physiological maturity, since susceptible genotypes tend to accumulate aflatoxin at a higher rate than resistant genotypes (Widstrom et al., 1986). Widstrom et al. (1987) also demonstrated that two separate populations, generated from kernels collected off the same open-pollinated ear, were different in their ability to inhibit elaboration of aflatoxin by *A. flavus*. Kernel infection percentages have been used to identify resistant germplasm (Scott and Zummo, 1988, 1990a). When kernel infection percentages are compared to identifications made by other traits, such as total aflatoxin concentrations, regardless of the inoculation technique, the same germplasm sources are usually identified as resistant (Scott et al., 1991). The same problems of interaction with environments and large sampling variances, however, continue to plague all genetic experiments and germplasm evaluation procedures.

Some of the most recent genetic research efforts on *A. flavus* infection and aflatoxin contamination have been focused on ear rot symptoms and aflatoxin production (Campbell and White, 1995; Hamblin and White, 2000; Naidoo et al., 2002). In general, these studies have produced conflicting results in that resistance is sometimes attributed to additive

effects, sometimes to dominace, and sometimes to both. An encouraging common thread through all of these studies seems to be that the same hybrids and inbreds always fall into resistant and susceptible categories. Recurrent selection studies have been in progress for two breeding populations during the last 10–15 years (Widstrom and Snook, 2001b). One breeding population was generated by random mating of plants in a cross made between two released resistance sources, GT-MAS:gk and Mp313E. Two cycles of selection based on S_1 progeny performance have now been completed in this population. Replicated experiments for evaluation of selection progress reveal an aflatoxin contamination of 175 ng g^{-1} for the original population compared with only 67 ng g^{-1} for the C2 population. A slight reduction in husk tightness occurred in the C2 causing a large standard error for aflatoxin and prevented the decrease in contamination from being statistically significant. A second breeding population was generated by random mating of multiple crossings among eight dent single crosses and seven sommercial hybrids, all of which had demonstrated some resistance to aflatoxin contamination. Four cycles of selection based on S_1 progeny performance have now been completed in that population, and a replicated experiment to evaluate progress indicated that contamintion of the original population has been decreased an average of 30 ng g^{-1} per cycle, as determined by the regression of cycle means on selection cycles. The original population had an average aflatoxin concentration of more than 220 ng g^{-1} while the fourth selection cycle (C4) population was contaminated with 114 ng g^{-1} aflatoxin. Plant and ear height were reduced as a result of the selection while the C4 population also matured four days later than the original breeding population.

Molecular Breeding

Conventional breeding has been proposed as a genetic solution to the preharvest aflatoxin contamination (Gorman et al., 1992; Naidoo et al., 2002; Widstrom et al., 1987; Widstrom et al., accepted). Recombination was unclear to breeders who must make selections based on phenotypic variation. Breeders increase genetic variation by crossing complementary lines with different desirable traits attempting to detect individuals with the desirable traits as identified by phenotype. Because of recombination events that occur, progenies are carrying chromosomal segments from both parents. The method of using genetic markers to tag useful traits or characters could provide tremendous potential when used in marker-assisted selection (MAS) and overcome many difficulties of conventional breeding (Dudley, 1993; Ribaut and Hoisington, 1998).

Because of the complexity of *Aspergillus*-corn interactions and the factors affecting aflatoxin formation, molecular breeding and MAS methods should enhance or improve general genetic resistance to fungal aflatoxin formation, ear-feeding insects and drought stress. Molecular procedures have been proposed to develop southern adapted corn hybrids that control/prevent preharvest aflatoxin contamination in corn (Guo et al., 2000; Widstrom et al., accepted). Markers based on DNA restriction fragment length polymorphism (RFLP) are being used extensively to build detailed genetic maps for a wide variety of crop species. RFLP has been proposed as a powerful analytical tool which has many practical applications in plant breeding (Tanksley et al., 1989). A sufficient number of difficult quantitative traits have been mapped to suggest that RFLP technology will have substantial utility in this area (Butrón et al., 2001; Guo et al., 2001, 2002; Ribaut and Hoisington, 1998).

Mapping studies to reveal the complexity of QTL have been used frequently to identify important chromosomal regions linked to agronomically important trait(s) (Agrama and Moussa, 1996). Molecular marker profiles should help improve the heritability or improve the power of selection and help to more efficiently and effectively organize, combine, and select new genotypic combinations (Inukai et al., 1996; Visscher et al., 1996). Butrón et al. (2000) conducted genetic mapping of the loci associated with reduced aflatoxin contamination from a F_2 population derived from (GT-A1 $\times$ GT119]). A major QTL for maysin was identified on chromosome 1S (Butrón et al., 2001), and QTLs for husk tightness were located on chromosomes 4L and 7S. The recombination of progenies with chromosome region 1S from GT-A1 and 2L from GT119 gave the lowest aflatoxin concentrations. A two locus model accounted for 24.7% of the phenotypic variance for aflatoxin concentrations. Further fine-mapping will be needed in conducting marker-assisted selection in a corn population, with the purpose of pyramiding the resistance to *Aspergillus* spp. infection and ear-feeding insects in an elite inbred source.

CONCLUSIONS

Aflatoxin contamination in corn is chronic problem in the southern U.S., but sporadic in the corn belt. Aflatoxin contamination of corn in the field is influenced by numerous factors, abiotic or biotic. We have knowledge concerning the contamination process to establish a guideline and management practices that will minimize the probability of contamination under an certain environment condition, but long-term solutions are needed if the problem is to be adequately controlled or resolved. The genetic strategies, conventional or molecular, are vital in long-term solution to develop hybrids

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resistant to infection by *A. flavus* and subsequent aflatoxin contamination. In the southern U.S. or especially southeastern U.S., high temperature coupled with drought and heat are favorable to *A. flavus* infection and aflatoxin production. Loose-husked hybrids used in the south are free invitation to ear-feeding insects and fungal spores. When grown in the south, these ''corn-belt type'' hybrids accentuate insect damage and aflatoxin contamination. The development and breeding ''southern-type'' hybrids, with good husk coverage and flint kernel character, is an important factor for control of preharvest aflatoxin contamination. In fact, several research programs in the southern States are conducting genetic manipulation of ''southern'' corn and introgression of tropic and subtropic corn genetics into U.S. germplasm to develop ''southern type'' hybrids in order to constrain the problem of aflatoxin contamination.

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