AFLATOXIN LEVELS IN COTTONSEED AT WEEKLY INTERVALS IN ARIZONA, MISSISSIPPI AND TEXAS MODULES

W. E. Batson, Jr. and J.Caceres
Dept. of Entomology and Plant Pathology
Mississippi State University
Mississippi State, MS
P. J. Cotty
Southern Regional Research Center, A.R.S., U.S.D.A.
New Orleans, LA
Tom Isakeit
Texas Agricultural Research and Extension Center
Weslaco, TX

Abstract

Two cotton modules were constructed near Odem, Texas, and two near Yuma, Arizona from producer fields in areas with histories of aflatoxin problems. Also, two modules were constructed in Mississippi from a field artificially infested with *Aspergillus flavus*. Aflatoxin levels increased significantly with time in one module from Texas and one from Mississippi. The relationship between aflatoxin level and time was positive but not significant for all other sites except TX-2. The significant decrease of aflatoxin level over time in TX-2 was attributed to termperatures unfavorable for aflatoxin production.

Introduction

Aflatoxins are potent carcinogenic mycotoxins produced by *Aspergillus flavus* Link and *A. parasiticus* Speare. These fungi may colonize and/or contaminate corn (Diener1983), cottonseed (Ashworth and McMeans 1966; Ashworth *et. al*. 1969), peanuts (Diener 1982), tree nuts (Cole 1983), and other crops(Stoloff 1976) and are distributed worldwide in air and soil. Concern over aflatoxin contamination of food and feed and of milk caused by feeding whole cottonseed has created an atmosphere wherein the aflatoxin content of cottonseed and cottonseed products is under increased scrutiny. Consequently, even relatively low levels of aflatoxin in cottonseed can be a significant marketing liability. Chronic aflatoxin contamination in cottonseed is generally confined to areas of the cottonbelt that have high temperatures but are usually free from significant amounts of mid-summer precipitation. Aflatoxin contamination of seed has been a major problem for the cottonseed industry in arid Western Arizona and Southern California. Recently, the cottonseed industry has recognized that *A. flavus* seed infection and aflatoxin contamination occurs sporadically over a large portion of the cottonbelt. *A. flavus* group fungi were found on the 1991 cottonseed crop produced in Alabama, Arkansas, Georgia, Louisiana, Mississippi, Oklahoma, Tennessee and Texas (Cotty, personal communication). Specifically, in an area of the mid-south populations of *A. flavus* group fungi exceeding 350 propagules/g were obtained from over 10% of samples analyzed. In surveys of the Mississippi Delta (Batson and Newman 1993; Batson and Caceres 1994) *A. flavus* was detected in washings from lint samples from 23 and 47% of fields sampled. Populations of *A. flavus* were rarely above 225 propagules/g of seed cotton. *A. flavus* infected seed were detected in samples from 24 of 118 modules (20%) at the Hope Gin in Thornton, MS in 1994(Batson and Caceres 1995).

Oil mill managers in the Mississippi Delta have suggested that increased aflatoxin contamination of cottonseed in the mid-south might be associated with storage of cotton in modules prior to ginning. Increases of aflatoxin in harvested cottonseed have been largely discounted although mature cottonseed is susceptible to infection by *A. flavus* and increases in aflatoxin content of the crop after boll opening but before harvest have been documented(Cotty1991). Studies of the effects of modular storage of seed cotton on levels of aflatoxin are scarce. In a study in Arizona, aflatoxin levels of seed highly contaminated with *A. flavus* increased with modular storage (Russell 1985). However, aflatoxin content of seed which had low *A. flavus* contamination did not increase with modular storage. To the contrary, regression analysis of mean aflatoxin concentration (mean of 16 internal sampling sites) versus storage time was positive and significant in one of two modules constructed in Mississippi in 1994 from a field in which selected areas and bolls were artificially inoculated with *A. flavus* (Batson and Caceres 1995). The relationship in the other module was also positive but was not significant. There was also a significant increase in aflatoxin concentration over time of storage for seven of the 16 internal sampling sites of one of the modules and for one site of the other module (Batson and Caceres 1995). Aflatoxin concentrations were low but in no case was there a significant reduction with time.

Low levels of natural field contamination of cotton by *A. flavus* to date, in the mid-south study area, have not permitted us to fully elucidate the impact of module storage of seed cotton on subsequent aflatoxin contamination. Consequently, in 1996 we studied modules constructed in areas of the cottonbelt where chronic aflatoxin contamination occurs.

Materials and Methods

Two cotton modules were constructed near Odem, Texas, and two near Yuma, Arizona from producer fields in areas with histories of aflatoxin problems. The Texas modules, TX-1 and TX-2, were constructed on August 13 from cotton plots comparing Ginstar/Cyclone and Harvade/Cyclone harvest-aid materials, respectively. The Arizona modules were constructed on September 25. Texas and Arizona modules were transported to the gin yard for storage. In

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:116-118 (1997)
National Cotton Council, Memphis TN

addition, two modules were constructed at the Plant Sciences Research Center at Starkville, MS from fields infested at boll-crack with 7 lbs./A of wheat kernels colonized with a highly toxigenic isolate of *A. flavus* (Cotty '94). These modules remained in the field throughout the study. Module sampling ports (MSP) were installed within all modules during construction to provide access to the module interior for repeated sampling. Samples were taken from each sampling port at module construction and weekly for three weeks for the Texas modules and four weeks for the Arizonia and Mississippi modules. Also, two samples were also taken weekly from the exterior of each module. Samples from Texas and Arizona were shipped over night to Mississippi for aflatoxin analysis.

All seed cotton samples were ginned, fuzzy seed were acid-delinted, neutralized, passed through a Straub Model 4E grinder and screened to remove as much seed coat material as possible. Aflatoxin levels were determined individually for each individual port and on triplicate samples of a composite sample of all ports of a module for a sampling period. Procedures recommended by Vicam Co., Watertown, MA were used to determine aflatoxin concentration. A 50-g sample of finely ground cottonseed meal and 5 g NaCl were blended with 100 ml of MeOH-H_2O (8:2) for 60 sec. The mixture was filtered through M 901 filter paper (Schleicher & Schuell Inc., Keene, NH), and 10 ml of filtrate mixed with 40 ml of H_2O. This solution was filtered through glass filter paper (ZE 903, Schleicher & Schuell Inc.), and 10 ml passed through an Aflatest P immunoaffinity column (Vicam Co., Watertown, MA) with light pressure supplied with a glass syringe and plunger. The affinity was rinsed twice with 10 ml of H_2O and the aflatoxin eluted with 1 ml of MeOH. The eluent was combined with 1 ml of bromine developer (0.002% Br_2), mixed and the level of fluorescence determined in a fluorometer (TorBex, model FX-100 series 3, Vicam Co., Watertown, MA). Aflatoxin was expressed in nanograms per gram of cottonseed meal.

Results and Discussion

Mean aflatoxin concentration of composite samples composed of cottonseed meal from all sampling ports of TX-1, ranged from 0 ppb at the time of moduling to 43 ppb after three weeks of stroage (Figure 1). Regression analysis of aflatoxin levels versus time was positive and significant indicating that aflatoxin increased over time in the TX-1 module. Levels of aflatoxin were lower in TX-2 and regression of aflatoxin level on time was negative indicating that levels decreased over time (Figure 2). Temperatures within TX-2 were higher than in TX-1 and increased over time to above 40 C. Temperatures recorded within TX-1 were always below 38 C and decreased with time. Maximal production of aflatoxin in cottonseed has been reported to occur between 25 - 30 C (Ashworth et. al., 1968). Mean aflatoxin levels for MS-1 ranged from 0 ppb at moduling to 23 ppb after four weeks of storage (Figure 3). Aflatoxin levels increased weekly throughout the study. Regession analysis of aflatoxin level versus time was sigificant for MS-1. Levels of aflatoxin in composite samples from AZ-1 and AZ-2 were generally low and remained below threshold levels throughout the study. Modules in the Arizona study area constructed at the same time as ours were seldom rejected because of aflatoxin problems. However, aflatoxin problems did develop in modules constructed toward the end of the season in Arizona. The relationship of aflatoxin level and storage time in AZ-1, AZ-2, and MS-2 was also positive but not significant.

Acknowledgements

Financial support for this research was provided through a grant funded by USDA/ARS to address preharvest control of aflatoxin. We would like to thank Alan Brashers, USDA/ARS, Lubbock, TX, John E. Bremer and Rich Jahn, Texas A&M University, Corpus Christi, TX and Don Howell and Alfonso Tellez, University of Arizona, Yuma, AZ for assisting in construction of modules and taking samples. Thanks also to the producers in Texas and Arizona for their generosity in allowing us to work on their farms. A special thank you to Wes Graham, Mike Tagert, Derrick Taylor and Shannon White, students in my laboratory, for sample preparation.

Literature Cited

Ashworth, L. J.,Jr. and J. L. McMeans, 1966. Association of *Aspergillus flavus* and aflatoxins with a greenish yellow fluorescence of cottonseed. Phytopathology 56:1104-1105.

Ashworth, L. J., Jr., J. L. McMeans and C. M. Brown. 1968. Infection of cotton by *Aspergillus flavus*: The influence of temperature and aeration. Phytopathology 59:669-673.

Ashworth, L. J., Jr., J. L. McMeans and C. M. Brown. 1969. Infection of cotton by *Aspergillus flavus*: Epidemiology of the disease. J. Stored Prod. Res. 5:193-202.

Batson, W. E., Jr. and Debra Newman. 1993. Effect of module storage on incidence of *Aspergillus flavus* contamination of seed cotton in Mississippi in 1992: A preliminary report. Proceedings Beltwide Cotton Conferences. 209-210.

Batson, W. E., Jr. and J. Caceres. 1994. Investigations of the influence of modular storage on Aspergillus flavus infection of cottonseed and aflatoxin contamination. Proceedings Aflatoxin Elimination Workshop. p. 70.

Batson, W. E., Jr. and J. Caceres. 1995. Incidence of Aspergillus flavus seed infection and aflatoxin contamination in Mississippi cotton modules and the

influence of field storage. Proceedings Aflatoxin Elimination Workshop. p. 67.

Cole, R. J., T. H. Sanders, P. D. Blankenship and R. A. Hill. 1983. Mode of formation of aflatoxin in various nut fruits and gross and histological effects of aflatoxins in animals. *In* Xenobiotics in Foods and Feeds, ed. J. W. Findley and D. E. Schwass, pp. 223-239. ACS Symp. Ser. 234. Washington, DC.

Cotty, P. J. 1991. Effect of harvest date on aflatoxin contamination of cottonseed. Plant Dis. 75:312-314.

Cotty, P. J. 1994. Influence of field application of an atoxigenic strain of *Aspergillus flavus* on the population of *A. flavus* infecting cotton bolls and on the aflatoxin contentr of cottonseed. Phytopathology 84:1270-1277.

Diener, U. L., R. E. Pettit and R. J. Cole. 1982. Aflatoxins and other mycotoxins in peanuts. *In* Peanut Science and Technology, ed. H. E. Pattee and C. T. Young. pp. 486-519.

Diener, U. L., R. L. Asquith and J. W. Dickens, eds. 1983. Aflatoxin and *Aspergillus flavus* in Corn. So. Coop. Ser. Bull. 279. Auburn University, AL.

Russell, T. E. 1985. Effect of modular storage of Arizona seed cotton on levels of aflatoxin in seed. JAOCS 62:515-517.

Stoloff, L. 1976. Occurrence of mycotoxins in foods and feeds, *In* Mycotoxins and other Fungal Related Food Problems, ed. J. V. Rodricks, pp. 23-50.

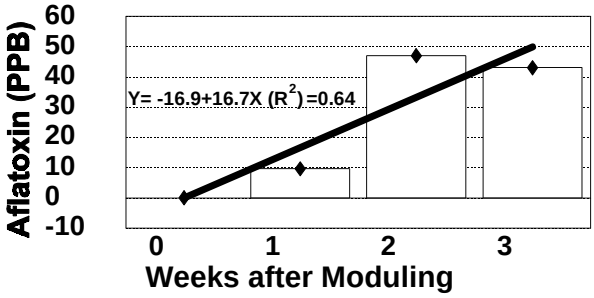

Figure 1. Mean aflatoxin levels of bulked cottonseedmeal from 18 ports over time for the TX-1 module.

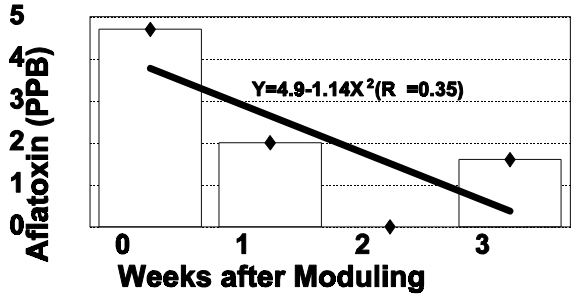

Figure 2. Mean aflatoxins levels of bulked cottonseed meal from 18 ports over time for the TX-2 module.

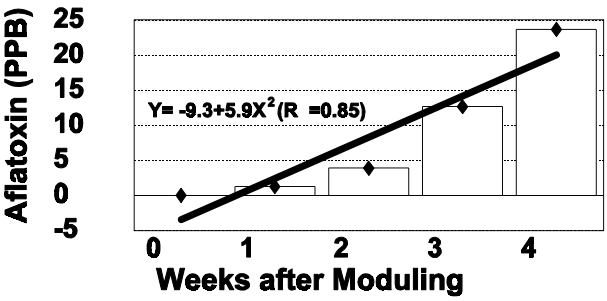

Figure 3. Mean aflatoxin levels of bulked cottonseed meal form 18 ports over time for the MS-1 module.