2004 BELTWIDE COTTON CONFERENCES, SAN ANTONIO, TX – JANUARY 5-9

ASPERGILLUS FLAVUS COMMUNITIES ASSOCIATED WITH COMMERCIAL COTTONSEED IN SOUTH TEXAS

R. Jaime-Garcia
Agricultural Research Service
United States Department of Agriculture
P.J. Cotty
College of Agriculture
University of Arizona
Tucson, AZ

Regulatory limitations on the quantity of aflatoxins permitted in foods and feeds exist throughout most of the world. Where contamination is common, diverse communities of aflatoxin producing fungi reside. Aflatoxin producers are asexual fungi belonging to *Aspergillus* section *Flavi*. Communities of section *Flavi* differ by region in both species composition and aflatoxin producing potential. The most common aflatoxin-producing species, *Aspergillus flavus* can be divided into two strains based on morphological, genetic, and physiologic criteria. The S strain produces numerous small sclerotia and high levels of aflatoxins, while the L strain produces fewer, larger sclerotia and, on average, less aflatoxin. The *A. flavus* S strain has been implicated as an important causal agent of aflatoxin contamination in several areas worldwide including Arizona, Texas, Louisiana and Mississippi in North America, Thailand in Southeast Asia, Benin in West Africa, and Argentina in South America. Aflatoxin contamination of commercial cottonseed in South Texas presents both temporal and spatial variation. Temporal variation occurs both between and within seasons. *A. flavus* community structure also is known to present spatial autocorrelation. However, relationships of contamination to fungal community structure have not been described. Furthermore, communities of aflatoxin-producing fungi associated with cotton crops in South Texas have not been characterized. The current study sought to relate incidence and spatial distribution of *A. flavus* on cottonseed in South Texas to aflatoxin contamination with the objective of determining the relative importance of the S strain to aflatoxin contamination. Cottonseed samples from trucks transporting cottonseed from several gins in South Texas were taken upon receipt at the Valley CO-OP Oil Mill in Harlingen, Texas during 1999 to 2001. Most gins were sampled several times during the season. For data analysis, gins were grouped into three different regions, the Rio Grande Valley, the Coastal Bend and the Upper Coast. Six cores each consisting of 3 to 5 kg of seed were taken from each truck and immediately subdivided resulting in a single 1 to 1.5 kg sample that was shipped to the lab for analysis. Samples were stored (1 to 3 weeks) dry at room temperature until analyzed. Cottonseed was washed in 0.006% Triton X-100 and members of *Aspergillus* section *Flavi* were isolated from the washing by dilution plating onto a modified Rose Bengal agar (Cotty, 1989). Isolates were assigned to *A. flavus* S or L strains on the basis of colony characteristics and strain morphology after subculturing on 5/2 agar (5% V8 juice and 2% agar) for 5 to 7 days at 31 C. The quantity of *A. flavus* on the cottonseed samples were calculated as colony forming units (CFU) of *A. flavus* per gram of cottonseed. The percent of *A. flavus* isolates belonging to the S strain (Percent S) was obtained by dividing the number of S strain isolates by the total number of *A. flavus* obtained for each sample and multiplying by 100. Data on aflatoxin contamination for each of the sampled gins was obtained from the Valley CO-OP Oil Mill in Harlingen, Texas. The average aflatoxin content in ppb and the percent of cottonseed with aflatoxin content equal to or higher than 20 ppb for each gin was obtained as previously reported (Jaime-Garcia and Cotty, 2003). *A. flavus* communities described by CFU and Percent S resident on cottonseed from gins in South Texas, vary across regions and seasons. Both, the quantity (CFU) and strain composition (Percent S) of *A. flavus* communities influence aflatoxin contamination in cottonseed in South Texas. CFU differ significantly both among seasons and among regions. Average CFU across regions was significantly higher for the 1999 season than the 2000 and 2001 seasons. Percent S differed significantly across regions for the average of the three years, as well as for each of the individual years. The Rio Grande Valley region had consistently significantly lower Percent S compared to the Coastal Bend and Upper Coast regions for the average of the three years. Multiple linear regression analyses by the stepwise method of SAS included both CFU and Percent S in the regression model for aflatoxin contamination. In general, the transformation of both aflatoxin contamination and CFU to their natural logarithm improved the regression models. The best model was obtained with the natural logarithm of aflatoxin contamination analyzed as a function of the natural logarithm of CFU and Percent S. Each season had different models and the variables included in models varied. Geostatistical analyses indicated spatial continuity for both the Percent S and CFU, with seasonal variation in the range of influence. Maps of both Percent S and CFU for the 1999 to 2001 seasons obtained by ordinary block kriging show recurrent patterns for Percent S, but not for CFU. Results from the current study suggest that seasonal variation in aflatoxin contamination is driven by factors that influence *A. flavus* growth and reproduction as quantified by the density of *A. flavus* propagules on crop surfaces. On the other hand, the data also supports variation in population structure, as reflected in the percentage of *A. flavus* communities composed of the S strain, as a factor closely related to spatial variation in aflatoxin contamination (Jaime-Garcia and Cotty, 2003). The quantity of *A. flavus* propagules on cottonseed varies among seasons and regions. However, the current results indicate that *A. flavus* quantity influences aflatoxin contamination across seasons, but not between regions. The 1999 season, a season with high aflatoxin contamination (Jaime-Garcia and Cotty, 2003), had significantly higher CFU than the 2000 and 2001 seasons. Increases in aflatoxin during the second phase (Bock and Cotty, 1999) might mainly reflect increased growth of *A. flavus* resulting from exposure of mature seed to high humidity at temperatures favorable for this fungus. In the

current study, *A. flavus* growth is represented by colony forming units (CFU). The current results reveal a clear geographic structure in the distribution of the S strain on commercial cottonseed in South Texas. The S strain is markedly less common on the crop from the lower Rio Grande Valley than from the Coastal Bend Area. Temporal variation in the Percent S on cottonseed during the three years of study was not detected. Results of the current study are consistent with the S strain being an important causal agent of aflatoxin contamination in South Texas. The S strain of *A. flavus* is the most important contributor to the average aflatoxin producing potential of fungal communities in Arizona and elsewhere (Cotty, 1989). Results of the current study suggest that the S strain has similar importance in parts of South Texas and may be an important cause of aflatoxin contamination in this region.

References

Bock, C. H. and Cotty P. J. 1999. The relationship of gin date to aflatoxin contamination of cottonseed in Arizona. Plant Dis. 83:279-285

Cotty, P. J. 1989. Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. Phytopathology 79:808-814.

Jaime-Garcia, R., and P. J. Cotty. 2003. Aflatoxin contamination in commercial cottonseed in South Texas. Phytopathology 93:1190-1200.