Proceedings of the 2006 Annual Multi-Crop Aflatoxin/Fumonisin Elimination & Fungal Genomics Workshops

October 16-18, 2006
Ft. Worth, Texas

Please join us in thanking the following industry organizations for their generous support which made this workshop possible:

National Corn Growers Association

Corn Refiners Association

Pioneer

National Grain and Feed Association

Monsanto

North American Millers' Associaton

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Aflatoxin and Fumonisin Elimination and Fungal Genomics Workshop – 2006
Ft. Worth, Texas

Aflatoxin research continues to provide solid advances in fungal genome sequences and functional genomics, identification of resistant corn traits and strains. Successful competitive exclusion is being expanded to additional crops and growing areas beyond cotton and peanuts. Agronomic practices, including insect control, are being evaluated to provide immediate available controls for the growers. High points of these advances, along with discussion points are presented below. I want to thank Jeff Wilkinson, Peggy Ozias-Akins, Wenwei Xu, Russell Nuti, Peter Cotty, and Jeff Palumbo for giving me the material for each of their respective sessions for the following summarization.

Fungal Genomics, Fumonisins and Regulation of Aflatoxin Biosynthesis

Opening Talk - John Headrick gave an excellent overview of Monsanto's work on improving resistance to insect predation and aflatoxin accumulation in maize. Maize is a staple crop that a vast majority of the world depends on for nutrition; and in spite of increased yearly outputs, maize stores have been steadily declining due to increased demand. Thus to minimize losses in maize, Monsanto has focused their efforts on increasing resistance to insects and on improving drought tolerance. Yield Guard Bt Pro, a second generation Bt plant Monsanto is preparing for release, has proprietary genes that improve resistance above the first generation Bt plants, and is expected to further reduce insect damage caused by the corn borer.

As shown by Williams and Windham reductions in fungal infections and mycotoxin accumulations associated with these insect vectors is reduced by elimination or reduction of the corn borer. However, with dual fumonsin and aflatoxin contamination a complex solution is required. To that end Monsanto is also testing lines for drought tolerance, examining such traits as leaf rolling and leaf temperature in their selection process aimed at stacking these and other genes to increase drought tolerance. As with all of Monsanto's work, regular tests of aflatoxin levels assist screening efforts and compliment the many tests for other traits used during screens of hybrid lines.

Many advances and promising discoveries of the genetic basis and functional genomics of fumonisin and aflatoxin production were made over the past year. To reduce the time and cost of identification of *Fusarium verticillioides* during maize infection, a simple, reproducible and highly sensitive real-time PCR method was presented (Kendra). This real-time PCR method was sufficiently sensitive to detect and quantify *F. verticillioides* infection from the seedling stage to ear development. Detection of micro spores at node plates showed that tracking of seed infection can be a route to colonization of stalk and ear tissue of more mature plants (Kendra).

Studies of the mechanisms of polyketide synthases in fumonisin production demonstrated that they are not the key enzymes for generating fumonisins, and that by substituting ketosynthases, new non-aromatic polyketides can be generated (Du).

To identify what genes may control the regulation of the FUM cluster genes, twenty ESTs with homology to DNA binding proteins, transcription factors, regulatory genes, etc., were selected from a comparison of two Expressed Sequence Tag (EST) libraries. Disruption of six of the 20 ESTs that resulted in the loss of GUS expression also resulted in the loss of FUM gene expression and toxin production (Butchko). FUM21, a gene known to be a specific differential regulator of the FUM genes, was examined to determine the effects that alternative splice forms have on fumonisin biosynthesis. RT-PCR showed that the OE plasmid made transcripts earlier than wild-type, but not all make fumonsins. This may be an indication that alternative splice forms are controlling regulation, and the next generation of arrays will contain sequences to allow studies of alternative splice forms (Brown).

The whole genome sequence of *Aspergillus flavus* has been completed and is estimated to contain 13,800 genes, larger than either *A. nidulans* or *A. fumigatus*. The 33.1 Mb of the preliminary draft consists of 17 scaffolds on 2995 contigs. Manual annotation of this genome is coordinated through North Carolina State University and will be made available at www.Aspergillusflavus.org. This genome sequence will be the basis of two *A. flavus* whole genome array platforms (Payne). An affymetrix based array containing the 13,800 *A. flavus* genes probes and an additional 10,000 probes from maize yield a comprehensive *A. flavus*/maize kernel expression array (Payne); in addition a 70mer oligo array contains 11813 genes from *A. flavus* and 278 genes from *A. parasiticus* (Yu). Though these future arrays promise much, interesting studies are ongoing with the 5031-element *A. flavus* amplicon microarray (Yu). Several candidate genes that may function to regulate aflatoxin have been identified in investigations of *A. flavus* and *A. parasiticus* (Yu, Campbell, Wilkinson). Several research avenues seem to support oxidative stress as a major inducer of aflatoxin biosynthesis. Caffeic acid modulated the antioxidative stress-response system in a screen of compounds that have antioxidative properties and inhibit aflatoxin biosynthesis using gene-deletion mutants of *Saccharomyces cerevisiae,* (Campbell). Significant down-regulation of genes in the aflatoxin biosynthetic gene cluster occured using gene expression analysis of *A. flavus* treated with caffeic acid (Campbell). Likewise, suppression of the aflatoxin biosynthesis was demonstrated using microarray analysis comparisons of tryptophan supplemented *A. flavus*. *A. parasiticus* samples do not show the same reduction in transcripts seen in *A. flavus* (Wilkinson).

Crop Resistance – Genetic Enginneering

Genetic engineering to reduce aflatoxin contamination continues to be productively explored. When attempting to interfere with mycotoxin production by the fungus, there are two levels of control that need to be considered, chromatin structure and specific regulatory gene control (Nierman). Genes whose overexpression in the plant confer a level of resistance to *A. flavus* growth when tested using in situ seed assays include chloroperoxidase (Ozias-Akins, peanut), peptide D4E1 (Cary, cotton), and ribosome inactivating protein (Weissinger, peanut). Functional assays for endogenous genes using gene silencing or knockout techniques in maize also are beginning to reveal their specific roles in *A. flavus* resistance or aflatoxin production (Guo, lipoxygenase; Chen, PR-10 trypsin inhibitor). Economic analysis of the impact of aflatoxin contamination may help to justify their deployment (Wu).

In addition to chloroperoxidase-expressing peanut for Aspergillus resistance, an anti-apototic gene (Bel-x) that has conferred resistance to multiple fungal and even viral pathogens in tobacco is being tested. Resistance to oxidative stress induced by exposure of leaf disks to the herbicide paraquat has been shown in a protein expressing line (Ozias). It is speculated that a threshold level of protein expression is required in order to observe herbicide resistance. In field studies, transgenic D4E1 cotton lines have shown higher germination stands that non-transgenic controls, probably due to greater resistance to seedling pathogens (Fusarium wilt). It is not possible to detect the peptide D4E1 on a western due to technical reasons; therefore, expression in transgenic plants must be assayed solely by RT-PCR to detect transcription. In vitro evidence does support the mode of action for the peptide to be disruption of the fungal membrane rather than an indirect effect. It is hoped that a D4E1 dimer will be more antigenic yet, retain antifungal activity (Cary). Concerning additional peanut transgenics, *mod1* (ribosome inactivating protein) lines are being are being used as pollen parents rather than female parents to increase the probability of successful crosses even though there is no apparent reduction in seed set on the transgenic plants. Chloroplast engineering is preferable to nuclear engineering when high levels of stable gene expression are desired along with a reduced probability of transgenic transmission through pollen.

Chloroplast engineering has not become standard practice, however, because it is difficult to accomplish transformation in plants other than tobacco. Peanut chloroplast transformation has not yet been achieved. The expression pattern of M*od1* in peanut seeds is in the cotyledonary epidermis, but it has not yet been determined whether there is an expression difference between abaxial and adaxial epidermis (Weissinger). Nevertheless, multiple independent transformants have shown significant reduction in aflatoxin production *in vitro*. With regard to RNA silencing of PR-10 in maize, the level of silencing as detected by protein assay was positively correlated with the level of aflatoxin production, i.e., less PR-10, more aflatoxin (Chen). This provides direct evidence for a role of PR-10 in reducing aflatoxin contamination of maize. Also in maize, a lipoxygenase gene, *Zmlox*3, is induced by *A. flavus* (Guo). Maize mutants for this gene showed significantly higher aflatoxin levels than wild type in the field. Responding to a question about jasmonic acid (JA) levels in the maize *lox* mutants, Dr. Guo stated that the *Zmlox*3 mutant does produce higher levels of JA. These mutants also were more susceptible to root-knot nematode and insects.

Dr. Wu has spoken and published on the economics of aflatoxin contamination in crops; her most recent publication on Bt corn can be found in *Transgenic Research* 15:277 (2006) and others are in the *Journal of Toxicology and Toxin Review* and *Environmental Science and Technology*. Her publications include US and international data, but not health-related costs since aflatoxin levels are below the threshold for concern in the diets of developed countries. She is planning to estimate the total economic impact of aflatoxin on a crop-by-crop basis for key US commodities that are affected by this mycotoxin.

Crop Resistance - Conventional Breeding

Among 13 presentations, seven were on corn, four on peanut, one on cotton, and one on almond. New peanut varieties with low aflatoxin have developed by improving nematode resistance and drought tolerance (Holbrook). Tom Gradziel from UC-Davis reported that tannin content was related to aflatoxin accumulation in almond. Corn breeders at Texas A&M University showed that new corn hybrids with improved drought tolerance and earworm resistance had much lower aflatoxin and comparable yield as compared to commercial check hybrids. The teams led by Robert Brown at USDA-ARS, Steve Moore at Louisiana State University, and Georgia Davis at University of Missouri found promising aflatoxin-resistant sources from diverse corn germplasm. Texas A&M scientists mapped QTLs linked to aflatoxin resistance with a population from $B73O_2$ x CML161 (Betrán). Don White at University of Illinois continued marker-assisted selection to transfer aflatoxin-resistance QTLs to elite germplasm.

Baozhu Guo and Meng Luo at USDA-ARS in GA used microarray techniques to find new DNA markers for corn and peanut. They identified 12 unique EST probes in corn and 20 unique probes in peanut for breeder use in variety selection. They try to use a diversity of tolerance genes acquired from microarray analysis to screen probes which can be used in corn germplasm assessment with drought tolerance and disease resistance. Twelve probes were screened from more than 100 genes, and primary tests show that probes can distinguish resistant lines from susceptible lines. However, the probes only affect genetic expression difference, and are developed from limited inbred lines. More inbred lines and hybrid lines will be used for further testing, and the identification of the final probes will be published. The probe information and protocol can be sent to any laboratory where probes are needed. Breeders can follow the protocol and use the probes to select varieties from any breeding section.

Use of Natural Products

The use of natural products for prevention of fungal invasion and/or aflatoxin biosynthesis in

crops continues to be a lively topic encompassing biological control of aflatoxin-producers and natural plant products that interfere with contamination. Molyneux observed that communication might occur between fungal cultures in different culture plates, implying that aflatoxin biosynthesis is influenced by fungal behavior in adjacent plates. The plates were just covered with a standard Petri dish lid since sealing the plates (e.g. with Parafilm) drastically and erratically alters aflatoxin production. Efforts to trap volatiles by solid-phase extraction detected only trace amounts of limonene, which probably comes from the pistachio media, not from the fungus. The plates were arranged in a single layer with "systematic randomization" (Molyneux). Gary Payne's laboratory avoids stacking plates since this produces anomalies in aflatoxin production.

A. flavus strains that do not produce aflatoxin probably handle oxidative stress by utilizing a completely different mechanism. Preliminary experiments in Molyneux's laboratory with a different strain of *A. flavus* from that reported on showed the same response to oxidative stress. Cotty stated that clearly the atoxigenic strains used to competitively exclude aflatoxin producers are very successful at overcoming oxidative stress during crop colonization and infection and this success occurs without accumulation of either aflatoxins or any of the intermediates in the aflatoxin biosynthetic pathway.

How late in the season can applications of atoxigenic strains be made while still retaining adequate efficacy, and does this coincide with the most susceptible period of crop development? Jaime-Garcia's data suggest that late applications have improved sporulation and product persistence and viability. However, canopy closure provides a favorable environment for a rapid and abundant sporulation of the product. At that time there might be an important percentage of cotton bolls already open, and these might receive only a reduced benefit from atoxigenic strain applications. Work is underway to evaluate when biocontrol applications are too late to be effective in displacing the toxigenic fungi.

Crop Management and Handling, Insect Control, and Fungal Relationships

Mycotoxin contamination, regardless of crop, is generally associated with drought stress and is magnified by invasive wounds caused by insects or nematodes which are fungal entry points. Management techniques that reduce incidence of drought stress or pest damage to crops will aid in mitigating fungal invasion and subsequent toxin production. Caterpillar damage to corn ears can greatly influence levels of mycotoxins. Current commercial corn hybrids, including the CryIA(b) Bt protein, provide sufficient control of European corn borers and Southwestern corn borers. New plant transformations using a gene from *Arabidopsis* show a high mortality of fall armyworms and corn earworm. Transformations with an insecticidal protein from the A.c. NPV insect virus are effective against corn earworm and fall armyworm.

Recent studies in peanut show that root infection in the absence of pod infection by the peanut root-knot nematode (M*eloidogyne arenaria*) can lead to greater aflatoxin contamination in the kernels; however, it is not known whether wounding of pods by nematodes can also affect aflatoxin contamination by serving as entry points for toxigenic *Aspergillus* spp. Breeding peanut for resistance to *M. arenaria* has had initial success. Studies comparing aflatoxin contamination in resistant lines with susceptible cultivars have so far been inconclusive.

Managing drought stress is key to preventing mycotoxin contamination. Recent research with furrow diking in the Southeast has shown positive agronomic responses in both irrigated and non-irrigated peanut, cotton, and corn. Furrow dikes improve water capture and enhance the efficient

use of rainfall and irrigation water. Real-time soil water potential data show that furrow diked plots take better advantage of surface applied water. Simulated rainfall studies show that land without furrow dikes had 3 times more runoff and 3.5 times more erosion compared to land with furrow dikes when water was applied at 2 inches per hour. Thus furrow diking could extend water supplied from a 2 inch irrigation from 3.9 days to 7.1 days in the soil type tested. How does soil type affect furrow diking? With a range of heavy and light soils, and even in sandy soils, the dikes are still present at the end of the season. In all of the soils tested, the dikes are able to complete their main purpose of disturbing runoff of water from irrigation or rainfall. Furrow diked systems have produced equal yield with less irrigation requirements in peanut, cotton, and corn. Research to this point has not shown a significant influence on preharvest aflatoxin contamination, however improving soil moisture reserves should provide a threshold buffer in conditions favoring *Aspergillus* spp. growth. (Nuti)

The boring of codling moth (*Cydia (Laspeyresia) pomonella*) larvae into tree nuts provides the primarily invasion pathway for *Aspergillus* spp.. The most effective controls of codling moth are organophosphate insecticides, which are likely to be banned or highly restricted by EPA. The current alternative control materials are cost prohibitive. Through a CRADA, patents and licenses between USDA/ARS and Trécé, Inc., research has now demonstrated four novel control tactics using a kairomone compound found in pear. Trécé, Inc. is currently petitioning the EPA for registration of the codling moth kairomone as an adjuvant for both mating disruption and larval insecticide sprays. The pear ester kairomone adjuvant combined with the pheromone disruptant reduced fruit/nut damage rates from 30% to 90+% below the low damage rates incurred with pheromone–alone dispensers. This kairomone does not attract other pest species in other crops. The key contribution of the kairomone adjuvant to mating disruptants was the reduction in multiple-matings by females. Eliminating the codling moth and disrupting male mating orientation reduces insect damage and *Aspergillus* infection. With the expected EPA registration of pear ester kairomone adjuvant products next year, the efficacy of available kairomone-augmented tactics will be dramatically expanded. (Light)

To avoid nematodes and the associated increased aflatoxin contamination in peanut, a grower can plant the nematode resistant cultivar that will be released by Holbrook. This cultivar has a major gene for resistance that was introgressed from wild peanut species and results in near immunity to the peanut root-knot nematode. Since the cultivar also has excellent resistance to tomato spotted wilt virus and high yield and grade, the use of this enhanced cultivar would be good insurance against the peanut root-knot nematode. How long will resistance last when controlled by a single major gene? Nematode resistance is a hypersensitive response and it is possible that, over time, the nematodes may overcome this resistance. Patty Timper is already looking for additional genes for resistance, in case they are needed. (Holbrook)

Molecular markers for the resistance trait/gene (RFLP markers) were developed by groups in Texas and North Carolina. However they are difficult to use, and in Holbrook's program they were not adequately associated with phenotype. However Juilette Chu, with Peggy Ozias-Akins, has developed a high throughput AFLP marker system that is beginning to be used in our breeding program. (Holbrook)

Kairomone adjuvant (pear ester attractant) is limited to control of codling moths, but it is effective in all three host crops, apple, pear, and walnut. This kairomone does not attract other pest species. New kairomones must be isolated and identified for other key pests, such as, the nut pests of almonds and pistachios, particularly the navel orangeworm. (Light)

Microbial Ecology (Biological Controls)

Strain AF36 has been used effectively in Arizona to reduce aflatoxin contamination in cotton and is now being studied in fig and pistachio orchards. This strain persisted for at least one year following application in suspended drip-irrigated fig orchards, and recovery in areas adjacent to treatment zones was substantial. Similarly, in years following application of strain AF36 and two other non-aflatoxigenic *A. flavus* strains to flood-irrigated pistachio orchards, strain AF36 persisted at higher levels than the other two strains. A separate study in sprinkler-irrigated experimental pistachio orchards showed that strain AF36 incidence in treated soils was higher than in untreated soils following multiple years of application, although the proportion of strain AF36 in leaf samples did not reflect this. Strain AF36 was the predominant non-aflatoxigenic strain in commercial fig and pistachio orchards in California (Doster and Michailides).

Afla-guard®, a commercialized formulation of non-aflatoxgenic *A. flavus*, was applied to corn field test plots, either to soil or to whorl-stage or silking-stage corn plants in 2006. Aflatoxin was significantly reduced in afla-guard®-treated plots in this drought stressed year. The incidence of the non-aflatoxigenic strain was high in all treatments, including untreated plots, indicating the potential for substantial plot to plot movement in the corn environment (Dorner). Using a pin-bar inoculation technique, two non-aflatoxigenic strains were evaluated for their ability to colonize corn kernels. Strain K49 was a better colonizer under these conditions than strain CT3 demonstrating the variability of non-aflatoxigenic *A. flavus* strains as biocontrol agents in corn and the necessity to evaluate each strain (Abbas).

The mechanism of activity of non-aflatoxigenic *A. flavus* strains has been thought to be displacement of toxigenic strains via nutrient competition. Kenneth Damann demonstrated for the first time that a more specific interaction between toxigenic and non-toxigenic strains occurs which was independent of vegetative compatibility group and nutrient availability. Using a filter cup assay with different pore sizes, aflatoxin inhibition required a pore size greater than 3 μm, indicating that physical contact is required. This aflatoxin inhibition by "touching" was strain-specific for both the non-toxigenic strain and the aflatoxin-producing strain used. The distinction between physical interactions between fungi in terms of touching versus large non-diffusible molecules was discussed. In comparisons of live versus dead cells, dead cells do not function the same. Thus some metabolic or physiological activity of the competitor must be involved. Perhaps a slowly diffusible compound, containing a lipid moiety, for example, may be involved, and spore washes or other fractionations might help understand this interaction.(Damann)

The yeast *Pichia anomala* inhibited the frequency and extent of *A. flavus* colonization on artificially wounded pistachio nuts under field conditions.(Hua) Other studies have shown *P. anomala* to be adaptable as a biocontrol agent against a broad range of fungal pathogens in many different crops. Palumbo identified bacterial strains from corn field soil and rhizosphere samples that could be developed as biocontrol agents. The prevalent bacteria in these environments that showed antifungal activities against aflatoxin-producing *A. flavus* and fumonisin-producing *Fusarium verticillioides* were typically *Pseudomonas* and *Bacillus*. The relative abundance of Gram-negative, plant-associated bacteria with antifungal activity suggests that crop-specific rhizosphere samples might be good sources for customized biocontrol organisms.

The primary target is soil in the delivery for bacterial biocontrol agents on corn used to initially reduce the potential inoculum population of *A. flavus* and *F. verticillioides;* secondary applications on corn plants will also be tested. Another strategy, although almost prohibitive in scale, would be to initiate screening for widely distributed antagonists in several crops, then narrow the candidates by crop. (Palumbo)

The distribution and relationship of non-aflatoxigenic *A. flavus* strains to the drip diameter of the irrigation lines for tree nuts was discussed. The drip zones stay relatively wet even between irrigation events, and the population of *A. flavus* presumably varies seasonally, as well as by cyclic wet and dry conditions (Doster). The effect of suspended versus on-ground drip lines could not be compared in a study aimed at demonstrating displacement of toxigenic *A. flavus* in soil, since the growers had installed only the suspended drip lines (Michailides and Doster).

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6TH ANNUAL FUNGAL GENOMICS WORKSHOP
7TH ANNUAL FUMONISIN ELIMINATION WORKSHOP
19TH ANNUAL AFLATOXIN ELIMINATION WORKSHOP

HILTON FORT WORTH
FORT WORTH, TEXAS

SUNDAY, OCTOBER 15, 2006

1:00 – 6:00 WORKSHOP REGISTRATION AND POSTER SETUP

MONDAY, OCTOBER 16, 2006

7:00 – 5:00 WORKSHOP REGISTRATION

8:10 **WELCOME**
David Gibson, Texas Corn Producers Board, Lubbock, TX.

OPENING REMARKS
Jane F. Robens, USDA-ARS, National Program Leader, Beltsville, MD

8:20 **Application of Multiple Approaches toward Reducing Aflatoxin Contamination of Corn Grain**. *John M. Headrick*, Monsanto Co., St. Louis, MO.

FUMONISIN ELIMINATION/ FUNGAL GENOMICS, REGULATION OF AFLATOXIN BIOSYNTHESIS

Moderator: Scott Averhoff, Texas Corn Producers Board, Waxahachie, TX.

9:00 **Biosynthetic Mechanism for Fumonisins, a Model System for Fungal Non-Aromatic Polyketides.** *Liangching Du*, Xiangcheng Zhu, Fengan Yu, Kathia Zaleta-Rivera. Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE.

9:15 **Use of RT-PCR to Detect *Fusarium verticillioides* during Endophytic Colonization of Maize.** *David F. Kendra* and Rex Dyer. USDA-ARS Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Peoria, IL.

9:30 **Identification and Characterization of Regulators of FUM Gene Expression.** *Robert A.E. Butchko*, Daren W. Brown, Mark Busman, and Robert H. Proctor. USDA-ARS Mycotoxin Unit, NCAUR, Peoria, IL.

9:45 **Microarray Analysis of *Fusarium verticillioides*.** *Daren W. Brown*, Robert R.A. Butchko, David Kendra, and Robert H. Proctor. USDA-ARS Mycotoxin Research Unit, NCAUR, Peoria, IL.

10:00 BREAK AND POSTER VIEWING

10:30 **Sequence Analysis of *Aspergillus flavus*.** *Gary A. Payne*. North Carolina State University, Raleigh, NC.

10:45 **The Functional Genomic Basis of Antioxidant Potentiation of Inhibition of Aflatoxin Biosynthesis.** Jong H. Kim[1], Jiujiang Yu[2], Russell J. Molyneux[1], Noreen Mahoney[1], Kathleen L. Chan[1], Deepak Bhatnagar[2], Thomas E. Cleveland[2], William C. Nierman[3], and *Bruce Campbell*[1]. [1]USDA-ARS Western Regional Research Center, Albany, CA; [2]USDA-ARS Southern Regional Research Center, New Orleans, LA; [3] The Institute for Genomic Research, Rockville, MD.

11:00 ***Aspergillus flavus* Genomics for Solving Preharvest Aflatoxin Contamination in Crops.** *Jinjiang Yu*[1], Jeffery R. Wilkinson[2], Gary A. Payne[3], Bruce C. Campbell[4], Deepak Bhatnagar[1], Thomas E. Cleveland[1], and William C. Nierman[5]. [1]USDA-ARS Southern Regional Research Center, New Orleans, LA; [2]Department of Biochemistry and Molecular Biology, Mississippi State University, Mississippi State, MS; [3]North Carolina State University, Raleigh, NC; [4]USDA-ARS Western Regional Research Center; [5]The Institute for Genomic Research, Rockville, MD.

11:15 **Identifying Potential Aflatoxin Gene Regulators by Microarray Analysis.** *Jeffery R. Wilkinson*[1], J. Yu[2], J.M. Bland[2], H.K. Abbas[3], B.E. Scheffler[4], E. Mylroie[1], R. Shivagi[1], W.E. Nierman[5], H.S. Kim[6], D. Bhatnagar[2], T. E. Cleveland[2], and W.P. Williams[7]. [1]Department of Biochemistry and Molecular Biology, Mississippi State University, Mississisippi State, MS; [2]USDA-ARS Southern Regional Research Center, New Orleans, LA; [3]USDA-ARS –MSA National Biological Control Laboratory, Stoneville, MS; [4]USDA-ARS-MSA Jamie Whitten Delta States Research Center, Stoneville, MS; [5]The Institute for Genomic Research, Rockville, MD; [6]Department of Medicine, Korea University, Seoul, South Korea; [7]USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS.

11:30 PANEL DISCUSSION
Jeffery Wilkinson, Leader

12:00 ADJOURN

Lunch will be provided for those participating in the afternoon tour.

1:00 – 5:00 Tour of Morrison Milling and Fort Worth Grain Exchange

TUESDAY, OCTOBER 17, 2006

7:00 – 5:00 REGISTRATION

7:00 – 8:00 BREAKFAST PROVIDED

8:00 **OPENING REMARKS**
David Gibson, Texas Corn Producers Board, Lubbock, TX

8:05 **The True Economic Impact of Aflatoxin in U.S. Crops: Who Wins, Who Loses, and Who Should Care?** *Felicia Wu*, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

CROP RESISTANCE---GENETIC ENGINEERING

Moderator: Phil Wakelyn, National Cotton Council, Washington, DC

8:20 **Secondary Metabolite Biosynthetic Gene Clusters in Sequenced Aspergilli.** *William Nierman*[1], Natalie Fedorova[1], and Jiujiang Yu[2]. [1]The Institute for Genomic Research, Rockville, MD; [2]USDA-ARS Southern Regional Research Center, New Orleans, LA.

8:35 **Expression of an Active Form of Maize *RIP 1* in Transgenic Peanut Inhibits Fungal Infection and Reduces Aflatoxin Contamination.** *Arthur Weissinger*[1], Minsheng Wu[1], Tom Isleib[1], Tom Stalker[1], Barbara Shew[2], Kanniah Rajaesekaran[3], Jeffrey Cary[3], and T.E. Cleveland[3]. [1]Department of Crop Science, North Carolina State University, Raleigh, NC; [2]Department of Plant Pathology, North Carolina State University, Raleigh, NC; [3]USDA-ARS Southern Regional Research Laboratory, New Orleans, LA.

8:50 **Genetic Engineering of Cotton for Resistance to *Aspergillus flavus* and Other Phytopathogens.** *Jeffrey W. Cary*[1], Kanniah Rajasekaran[1], Mauricio Ulloa[2], Jesse M. Jaynes[3], and Thomas E. Cleveland[1]. [1]USDA-ARS Southern Regional Research Center, New Orleans, LA; [2]USDA-ARS-WICS, Shafter, CA; [3]College of Agricultural, Environmental and Natural Sciences, Tuskegee University, Tuskegee, AL.

9:05 **Host Genetic Components for Aflatoxin Reduction Strategies in Peanut.** *Peggy Ozias-Akins*, Ye Chu, Paola Faustinelli, and Laura Ramos. Department of Horticulture, University of Georgia,Tifton, GA.

9:20 **A Maize 9-Lipoxygenase Is Required for Resistance to Aflatoxin Contamination, Insects, and Nematodes.** *Xiquan Gao*[1], Tom Isakeit[1], Javier Betrán[2], Xinzhi Ni[3], James Starr[1], Cornelia Göbel[4], Marion Brodhagen[5], Ivo Feussner[4], Nancy Keller[5], Jürgen Engelberth[6], James Tumlinson[6], and Michael Kolomiets[1]. [1]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX; [2]Department of Soil and Crop Sciences, Texas A&M University, College Station, TX; [3]USDA-ARS, Crop Genetics and Breeding Research, Coastal Plain Experimental Station, Tifton, GA; [4]Department of Plant Biochemistry, Albrecht-von-Haller Institute for Plant Sciences, Georg-August University Göttingen, Göttingen, Germany; [5]Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI; [6]Department of Entomology, The Pennsylvania State University, University Park, PA.

9:35 **The Effect of PR-10 Expression on Host Resistance in Maize against *Aspergillus flavus* Infection and Aflatoxin Production.** *Zhi-Yuan Chen*[1], Robert L. Brown[2], Kenneth E. Damann[1], and Thomas E. Cleveland[2]. [1]Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA; [2]USDA-ARS Southern Regional Research Center, New Orleans, LA.

9:50 **Annotation and Computational Analysis of Maize Proteome and Gene Expression Datasets.** *Susan M. Bridges*[1,2], Rowena Kelley[3], Gregory Bryce Magee[1,2], Nan Wang[1,2], Shane Burgess[2,4], Dawn S. Luthe[2,5], and W. Paul Williams[6]. [1]Department of Computer Science and Engineering, Mississippi State University, Mississippi State, MS; [2]MSU Institute for Digital Biology, Mississippi State University, Mississippi State, MS; [3]Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS; [4]Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS; [5]Department of Crop and Soil Sciences, Pennsylvania State University, University Park, PA;[6]USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS.

10:05 BREAK AND POSTER VIEWING

10:30 PANEL DISCUSSION
Peggy Ozias-Akins, Leader

CROP RESISTANCE---CONVENTIONAL BREEDING

Moderator: Paul Bertels, National Corn Growers, Chesterfield, MO

11:00 **New Approaches to Breeding for Resistance to Preharvest Aflatoxin Contamination in Peanut.** *Corley Holbrook*[1], E. Cantonwine[2], B.Z. Guo[3], P. Timper[3], D. Sullivan[4], and D.M. Wilson[2]. [1]USDA-ARS Crop Genetics and Breeding Research Unit, Tifton, GA; [2]University of Georgia, Tifton, GA; [3]USDA-ARS Crop Protection and Management Research Unit, Tifton, GA; [4]USDA-ARS Southeast Watershed Research Unit, Tifton, GA.

11:15 **Variation Among Cultivars in Pod Water Uptake and Its Effect on Pre-harvest Aflatoxin Contamination.** *Diane Rowland*[1], K. Gray[1], J. Dorner[1], S. Hilton[1], R. Sorensen[1], L. Powell[1], and C. Holbrook[2]. [1]USDA-ARS National Peanut Research Laboratory, Dawson, GA; [2]USDA-ARS Crop Genetics and Breeding Unit, Tifton, GA.

11:30 **Remote Sensing for Rapid Selection of Drought and Aflatoxin Resistant Peanut Genotypes.** *Dana Sullivan*[1] and Corley Holbrook[2]. [1]USDA-ARS Southeast Watershed Research Lab, Tifton, GA; [2]USDA-ARS Crop Genetics and Breeding Unit, Tifton, GA.

11:45 **Development of Peanut EST (Expressed Sequence Tag)-based Genomic Resouces and Tools.** *B.Z. Guo*[1], H. Chen[2], P. Dang[3], M. Luo[2], X. Liang[4], G. He[5], C.C. Holbrook[6], and C.K. Kvien[2]. [1]USDA-ARS Crop Protection and Management Research Unit, Tifton, GA; [2]University of Georgia, Tifton, GA; [3]USDA-ARS National Peanut Research Lab, Dawson, GA; [4]Guangdong Academy of Agricultural Sciences, Guangzhou, China; [5]Tuskegee University, Tuskegee, AL; [6]USDA-ARS Crop Genetics and Breeding Research Unit, Tifton, GA.

12:00 LUNCH PROVIDED

1:00 **Field Based Assessment of Cotton Cultivars for Aflatoxin Contamination.** *Mary Olsen*[1] and Peter J. Cotty[2]. [1]Department of Plant Sciences, University of Arizona, Tucson, AZ; [2]USDA-ARS Southern Regional Research Center, New Orleans, LA.

1:15 **The Development and Characterization of NOW Resistant Almond Variety and Advanced Breeding lines.** *Tom Gradziel* and Abhaya Dandekar. University of California, Davis, CA.

1:30 **The Development of Aflatoxin-Resistant Maize Germplasm and the Identification of Potential Markers.** *Robert L. Brown*[1], Abebe Menkir[2], Ranajit Bandyopadhyay[2], Zhi-Yaun Chen[3], and Thomas E. Cleveland[1]. USDA-ARS-SRRC, New Orleans, LA; [2]International Institute of Tropical

Agriculture, Ibadan, Nigeria; [3]Louisiana State University Agricultural Center, Baton Rouge, LA.

1:45 **Microarray as a Tool for Evaluation and Understanding the Resistance Mechanisms of Preharvest Aflatoxin Contamination in Corn.** *M. Luo*[1], R.D. Lee[1], and B.Z. Guo[2]. [1]University of Georgia, Department of Crop and Soil Sciences, Tifton, GA; [2]USDA-ARS Crop Protection and Management Research Unit, Tifton, GA.

2:00 **Diverse Maize Germplasm Aflatoxin Levels Survey.** *Dana Bush*[1], Teresa Musket[1], Doug Davis[1], Paul Williams[2], Gary Windham[2], Matthew Krakowsky[3], Tom Brooks[2] and Georgia Davis[1]. [1]University of Missouri,Columbia, MO; [2] USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS; [3]USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, GA.

2:15 **Improving Drought-Tolerance and Earworm Resistance to Reduce Aflatoxin in Corn.** *Wenwei Xu*[1], Gary Odvody[2], and W. Paul Williams[3]. [1] Texas A&M University Agricultural Research and Extension Center, Lubbock, TX; [2] Texas A&M University Agricultural Research and Extension Center, Corpus Christi, TX; [3]USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS.

2:30 **Breeding Corn Germplasm for Agronomic Performance and Reduced Aflatoxin Contamination.** Javier Betrán[1], Kerry Mayfield[1], *Tom Isakeit*[1], and Gary Odvody[2]. [1]Texas A&M University, College Station, TX; [2]Texas A&M University Agricultural Research and Extension Center, Corpus Christi, TX.

2:45 **Searching for New Resistance to Reduce Aflatoxin in Corn.** *Steve Moore*[1], Hamed K. Abbas[2] and Mark Millard[3]. [1]Louisiana State University Agricultural Center, Alexandria, LA, [2]USDA-ARS Crop Genetics and Production Research, Stoneville, MS; [3]USDA-ARS North Central Regional Plant Introduction Station, Ames, IA.

3:00 BREAK AND POSTER VIEWING

3:25 **Progress on Creating Commercially Usable Corn Hybrids with Low Aflatoxin in Grain.** *Don White*, University of Illinois, Urbana, IL.

3:40 PANEL DISCUSSION
Wenwei Xu, Leader

4:15 – 5:15 COMMODITY BREAKOUTS

5:30 – 7:00 RECEPTION WITH HORS'OEUVRES

WEDNESDAY, OCTOBER 18, 2006

7:00 – 1:00 REGISTRATION

7:00 – 8:00 BREAKFAST PROVIDED

USE OF NATURAL PRODUCTS FOR PREVENTION OF FUNGAL INVASION AND/OR AFLATOXIN BIOSYNTHESIS IN CROPS

Moderator: Howard Valentine, American Peanut Council, Jasper, GA

8:10 Opening Remarks/ Announcements

8:15 **Aflatoxin Management in Arizona and Texas.** *Peter J. Cotty*[1] and Larry Antilla[2]. [1]USDA-ARS Southern Regional Research Center, New Orleans, LA, and University of Arizona, Tucson, AZ; [2]Arizona Cotton Research and Protection Council, Phoenix, AZ.

8:30 **Persistence and Sporulation of an Aflatoxin Biocontrol Product Are Influenced by Timing of Application.** *Ramon Jaime-Garcia*[1] and Peter J. Cotty[2]. [1]Department of Plant Sciences, University of Arizona, Tucson, AZ; [2]USDA-ARS Southern Regional Research Center, New Orleans, LA.

8:45 **Nonaflatoxigenic *Aspergillus flavus* TX9-8 Competitively Prevents Aflatoxin Production by Toxigenic *A. flavus* Isolates of Large and Small Sclerotial Morphotypes.** *Perng-Kuang Chang*[1] and Sui-Sheng T. Hua[2]. [1]USDA-ARS Southern Regional Research Center, New Orleans, LA; [2]USDA-ARS Western Regional Research Center, Albany, CA.

9:00 **Inhibition of Direct and Indirect Stress-Induced Aflatoxin Biosynthesis.** *Russell J. Molyneux*, Noreen Mahoney, Bruce C. Campbell, and Jong H. Kim. USDA-ARS Western Regional Research Center, Albany, CA.

9:15 **Tissue Specific Inhibitors of Aflatoxin Production from Maize Seeds.** *Gary A. Payne*[1], R.A. Holmes[2], and R.S. Boston[2]. [1]Department of Plant Pathology, North Carolina State University, Raleigh, NC; [2]Department of Botany, North Carolina State University, Raleigh, NC.

9:30 PANEL DISCUSSION
Peter Cotty, Leader

10:00 BREAK AND POSTER VIEWING

CROP MANAGEMENT AND HANDLING, INSECT CONTROL AND FUNGAL RELATIONSHIPS

Moderator: Emory Murphy, Georgia Peanut Commission, Tifton, GA

10:30 **Efficacy of New Genes Introduced into Plants for Control of Corn Ear Feeding Insects**. *Patrick F. Dowd*, Eric T. Johnson, and T. Scott Pinkerton. USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL.

10:45 **Root-Knot Nematodes and Aflatoxin Contamination in Peanut.** *Patricia Timper*[1] and C. Corley Holbrook[2]. [1]USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA; [2]USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, GA.

11:00 **Furrow Diking for Improved Water Use Concerning Preharvest Aflatoxin Contamination in Peanut and Corn.** *Russell Nuti*, Joe Dorner, Ron Sorenson, Marshall Lamb. USDA-ARS, National Peanut Research Laboratory, Dawson, GA.

11:15 **Development of Kairomone – Augmented Control Tactics for Codling Moth and Aspergillus in Walnuts.** *Douglas M. Light***,** James Baker, and Bruce C. Campbell. USDA-ARS Western Regional Research Center, Albany, CA.

11:30 PANEL DISCUSSION
Russell Nuti, Leader

12:00 LUNCH PROVIDED

MICROBIAL ECOLOGY

Moderator: Roy Cantrell, Cotton Incorporated, Cary, NC

1:00 **Biological Control of *Aspergillus flavus* by *Pichia anomala*: Efficacy and Practical Application.** *Sui Sheng Hua*. USDA-ARS, Western Regional Research Center, Albany, CA.

1:15 **Aflatoxin Control in Figs: Biocontrol using Atoxigenic Strains.** *Mark Doster* and Themis Michailides. University of California, Davis/Kearney Agricultural Center, Davis, CA.

1:30 **Aflatoxin Control in Pistachios: Biocontrol using Atoxigenic Strains.** *Themis Michailides* and Mark Doster. University of California, Davis/Kearney Agricultural Center, Davis, CA.

1:45 **Potential for Reducing Aflatoxin in Corn with Field Application of a Nontoxigenic Strain of *Aspergillus flavus*.** *Joe Dorner*, USDA-ARS National Peanut Research Laboratory, Dawson, GA.

2:00 **Isolation of Bacterial Antagonists of *Aspergillus flavus* and *Fusarium verticillioides* from Mississippi Corn Soils.** *Jeffrey D. Palumbo*[1], Teresa L. O'Keefe[1], and Hamed K. Abbas[2]. [1]USDA-ARS, WRRC, Albany, CA; [2]USDA-ARS Crop Genetics and Production Research, Stoneville, MS.

2:15 **Studies on Aflatoxin Inhibition by Intraspecific "Competition"?** *Kenneth Damann*, Changwei Huang, Archana Jha, Rebecca Sweany, and Catherine DeRobertis. Department of Plant Pathology and Crop Physiology; Lousiana State University Agricultural Center, Baton Rouge, LA.

2:30 **Characterization of Colonization and Aflatoxin Accumulation of Corn Following Pin Bar Inoculation with Various *Aspergillus flavus* strains.** *Hamed K. Abbas*[1], Robert Zablotowicz[2], and H. Arnold Bruns[1]. [1]USDA-ARS Crop Genetics and Production Research Unit, and [2]Southern Weed Science Research Unit, Stoneville, MS.

2:45 PANEL DISCUSSION
Jeffery Palumbo, Leader

3:15 BREAK

3:45 COMMON INTEREST MEETINGS—POSTER VIEWING

5:00 RECEPTION

6:00 BANQUET

POSTER PRESENTATIONS

A. FUMONISIN ELIMINATION AND FUNGAL GENOMICS

A-1 ESTs Provide Unique Insight to Fumonisin Regulatory Gene *FUM21*. Daren W. Brown, Robert R.A. Butchko, Mark Busman, and Robert H. Proctor. USDA-ARS Mycotoxin Research Unit, NCAUR, Peoria, IL.

A-2 SSR Fingerprinting Analysis of *Aspergillus flavus* Vegetative Compatibility Groups. Changwei Huang, Rebecca Sweany, Catherine DeRoberts, and Ken Damann. Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA.

A-3 Identification and Characterization of Differentially Expressed Genes in Two Inbred Maize Lines Using Microarray Technology. Rowena Y. Kelley[1], Debbie L. Boykin[2], Susan M. Bridges[3], Thomas D. Brooks[4], and W. Paul Williams[4]. [1]Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS; [2]USDA-ARS Mid South Area Statistics Office, Stoneville, MS; [3]Department of Computer Science and Engineering, Mississippi State University, Mississippi State, MS; [4]USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS.

A-4 Fumonisin B1 Is Necessary for Corn Seedling Disease But Is Minimally Translocated from Roots to Shoots. Anne Marie Zimeri, Lonnie D. Williams, Ronald T. Riley, and Anthony E. Glenn. USDA-ARS Russell Research Center, Toxicology and Mycotoxin Research Unit, Athens, GA.

A-5 Corn Seedling Disease, Fusaric Acid as the Wilt Toxin and the Need for Biocontrol of *Fusarium verticillioides* and other *Fusarium* species. Charles W. Bacon, M.E. Snook, and D.M. Hinton. USDA-ARS Russell Research Center. Toxicology and Mycotoxin Research Unit, Athens, GA.

B. MICROBIAL ECOLOGY

B-1 Antimicrobial Activity of Pyrrocidines from *Acremonium zeae* against Endophytes and Pathogens of Maize. Donald T. Wicklow and Stephen M. Poling. USDA-ARS National Center for Agricultural Utilization Research, Peoria, IL.

B-2 Movement of *Fusarium verticillioides* between Inoculated and Non-inoculated Ears in Field-grown Corn Plants. Ida E. Yates[1] and Darrell Sparks[2]. [1]USDA-ARS Toxicology and Mycotoxin Research Unit, Richard B. Russell Agricultural Research Center, Athens, GA; [2]Department of Horticulture, University of Georgia, Athens, GA.

B-3 Molecular and Microscopic Studies on the Interactions of *Pichia amomala* and *Aspergillus flavus*. Sui Sheng Hua, Maria Brandl, Jeffrey G. Eng, Siov Bouy Ly, and Henry Shih. USDA-ARS Western Regional Research Center, Albany, CA.

B-4 Intracellular Sugar Alcohol and Sugar Accumulation by the Biocontrol Yeast, *Pichia anomala*. Sui Sheng Hua, Siov Buoy Ly, and Wallace Yokoyama. USDA-ARS Western Regional Research Center, Albany, CA.

B-5 Development of Non-toxigenic Strains of *Aspergillus flavus* for Control of Aflatoxin in Maize. Hamed K. Abbas[1], Robert M. Zablotowicz[2], H. Arnold Bruns[1], and Craig A. Abel[3]. [1]USDA-ARS Crop Genetics and Production Research Unit, [2]Southern Weed Science Research Unit, and [3]Southern Insect Management Research Unit, Stoneville, MS.

B-6 Population Ecology of *Aspergillus flavus* and other fungi associated with Mississippi Delta Soils. Robert M. Zablotowicz[1], Hamed K. Abbas[1], Martin A. Locke[2]. [1]USDA-ARS Southern Weed Science Research Unit, Crop Genetics and Production Research Unit, Stoneville, MS; [2]USDA-ARS Water Quality and Ecology Research Unit, Oxford, MS.

B-7 Systemic Movement of *Aspergillus parasiticus* in maize stalks and ears. Gary L. Windham and W. Paul Williams. USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS.

C. CROP RESISTANCE---CONVENTIONAL BREEDING

C-1 Evaluation of Epidermal Conductance as Potential Drought Tolerant Trait of Peanut. E. Cantowine[1], S. Maddie[1], B. Buchanan[1], C.C. Holbrook[2], and C.K. Kvien[1]. [1]University of Georgia, Tifton, GA; [2]USDA-ARS Crop Genetics and Breeding Research Unit, Tifton, GA.

C-2 Genetic Response to Seed Colonization by *Aspergillus flavus* in Peanut. E. Cantowine[1], C. C. Holbrook[2], D.M. Wilson[1], P. Songsri[3], S. Pimratch[3], and B.Z. Guo[4]. [1]University of Georgia, Tifton, GA; [2]USDA-ARS Crop Genetics and Breeding; [3]Khon Kaen University, Khon Kaen, Thailand; [4]USDA-ARS Crop Protection and Management Research Unit, Tifton, GA.

C-3 Production of Defensive Stilbenoids by Commercial Peanut Cultivars. Victor S. Sobolev[1], Baozhu Z. Guo[2], Corley C. Holbrook[3], and Robert E. Lynch[2]. [1]USDA-ARS National Peanut Research Laboratory, Dawson, GA; [2]USDA-ARS Crop Protection and Management Research Unit,

Tifton, GA; [3]USDA-ARS Crop Genetics and Breeding Research Unit, Tifton, GA.

C-4 **Walnuts of Cultivar Tulare Show Similar Aflatoxin Contamination Levels as Those of Two Other Cultivars.** Themis J. Michailides and Mark A. Doster. University of California, Davis/Kearney Agricultural Center, Davis, CA.

C-5 **Multi-location Evaluation of Aflatoxin Accumulation and Agronomic Performance of Maize Hybrids in Texas.** Cody McKee, Kerry Mayfield, Tom Isakeit, Gary Odvody, and Javier Betrán. Texas A&M University.

C-6 **Southern East Regional Aflatoxin Test (SERAT).** Matthew Krakowsky[3], Baozhu Guo[3], Don White[4], Wenwei Xu[5], W. Paul Williams[1], Thomas Brooks[1], Gary Windham[1], Steve Moore[2], Hamed Abbas[7], James Perkins[8], Daniel Gorman[9], Quinton Raab[10], Keith Arnold[10], David Smith[11], Tom Isakeit[6], Kerry Mayfield[6], Javier Betran[6]. [1]USDA-ARS, Mississippi State, MS; [2]Louisiana State University, Alexandria, LA; [3]USDA-ARS, Tifton, GA; [4]University of Illinois, Urbana, IL; [5]Texas A&M University, Lubbock, TX; [6]Texas A&M University, College Station, TX; [7]USDA-ARS, Stoneville, MS; [8]Monsanto Company Crop Protection, Waterman, IL; [9]Pioneer-Dupont, Cairo, GA; [10]BH Genetics, Moulton, TX; [11]Zea Sage, Sycamore, IL.

C-7 **Response to Aflatoxin of Exotic (CIMMYT and LAMA) Germplasm in Southern USA.** Rebecca Corn[1], Matthew Krakowsky[2], Paul Williams[3], David Bergvinson[4], Kerry Mayfield[4], and Javier Betrán[4]. [1]CIMMYT, Mexico; [2]USDA-ARS, Tifton, GA; [3]USDA-ARS, Mississippi State, MS; [4]Texas A&M University, College Station, TX.

C-8 **QTL Mapping of Genetic Factors Associated with Response to Aflatoxin Accumulation in a RIL Maize Mapping Population.** Halima Atta, Andres Gutierrez, Monica Menz, Tom Isakeit, Kerry Mayfield, Javier Betrán. Texas A&M University, College Station, TX.

C-9 **Jasmonic Acid Biosynthesis Pathway Confers Resistance to Maize Embryos against *Aspergillus flavus*.** Alberto Camas[1,] Zenaida Magbanua[2], C. DeMoraes[3], Jeffery Wilkinson[1], Gary Windham[4], Paul Williams[4], and Dawn Luthe[5]. [1]Department of Biochemistry and Molecular Biology, Mississippi State University; [2]Departmento of Plant and Soil Sciences, Mississippi State University; [3]Department of Entomology, Penn State University; [4]USDA-ARS, Mississippi State, MS; [5]Department of Crop and Soil Sciences, Penn State University.

C-10 **Is Rachis Lignification a Deterrent to *Aspergillus flavus* Movement through the Developing Maize Ear?** Lindsay Spangler[1], Olga

Pechanova[2], Zenaida Magbanua[3], Paul Williams[4], and Dawn Luthe[1]. [1]Department of Crop and Soil Sciences, Pennsylvania State University; [2]Department of Forestry, Mississippi State University; [3]Department of Plant and Soil Sciences, Mississippi State University; [4]USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS.

D. CROP MANAGEMENT AND HANDLING, INSECT CONTROL AND FUNGAL RELATIONSHIPS

D-1 Correlations between Biotic Stresses and Aflatoxin Contamination in Maize. Matthew Krakowsky[1], Xinzhi Ni[1], and Richard Davis[2]. [1]USDA-ARS Crop Genetics and Breeding Research Unit, Tifton, GA; [2]USDA-ARS Crop Protection and Management Research Unit, Tifton, GA.

D-2 Spatial Correlation between Aflatoxin Level and Ear-feeding Insect Damage in Pre-harvest Corn. Xinzhi Ni[1], Kedong Da[2], Matthew D. Krakowsky[1], G. David Buntin[3], R. Dewey Lee[4], and Steven L. Brown[2]. [1]USDA-ARS Crop Genetics and Breeding Research Unit, Tifton, GA; [2]Department of Entomology, University of Georgia, Tifton, GA; [3]Department of Entomology, University of Georgia, Griffin, GA; [4]Department of Plant Soil Sciences, University of Georgia, Tifton, GA.

D-3 Effect of Glufosinate on Aflatoxin in Corn. Steve Moore[1], Kenneth Damann[2] , Rick Mascagni[1], and Hamed Abbas[3]. [1]Louisiana State University Agricultural Center, Alexandria, LA; [2]Louisiana State University, Baton Rouge, LA; [3]USDA-ARS, Stoneville, MS.

D-4 Effect of Exogenous Jasmonic Acid Application on *Aspergillus flavus* Kernel Infection and Aflatoxin Production in Two Maize Hybrids (*Zea mays* L.) Leigh K. Hawkins[1], Dawn S. Luthe[2] Gary L. Windham[1], and W. Paul Williams[1]. [1]USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS; [2]Department of Crop and Soil Sciences, Pennsylvania State University,University Park, PA.

E. DETECTION, ANALYSIS, AND EXTRACTION OF AFLATOXINS.

E-1 Investigating the Bright Greenish-Yellow Fluorescence (BGYF) Properties of Corn Kernels with an Imaging Spectrometer. Zuzana Hruska[1], Haibo Yao[1], Robert L. Brown[2], Thomas E. Cleveland[2]. [1]Institute for Technology Development, Stennis Space Center, MS; [2]USDA-ARS, SRRC, New Orleans, LA,

E-2 Antibody Detection of *Aspergillus* in Peanuts. Nicholas Hill[1], Corley Holbrook[2]. [1]University of Georgia, Athens, GA; [2]USDA-ARS, Tifton, GA.

E-3 Is There More Value in Quantifying Mycotoxins or Fungus for Plant Breeding? N.S. Hill[1], S. Neate[2], B. Cooper[3], R. Horsley[4], P. Schwarz[4], L.S. Dahleen[5], K.P. Smith[6], R. Dil-Macky[7,] K. O'Donnell[8], J. Reeves[9]. [1]University of Georgia, Athens, GA; [3]Busch Agricultural Research Inc, Ft. Collins, CO; [4]Department of Plant and Soil Sciences, North Dakota State University, Fargo, ND; [5]USDA-ARS Cereal Crops Research Unit, Fargo, ND; [6]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN; [7]Department of Plant Pathology, University of Minnesota, St. Paul, MN; [8]USDA-ARS NCAUR, Peoria, IL; [9]Department of Statistics, University of Georgia, Athens, GA.

Application of Multiple Approaches toward Reducing Aflatoxin Contamination of Corn Grain

J.M. Headrick
Monsanto Co., St. Louis, MO 63167

Monsanto Company is committed to improving the quality and value of corn grain, including reducing contamination by mycotoxins. In the absence of major genes in corn for resistance to the fungi that produce mycotoxins, a combination of approaches is being pursued in an effort to lessen the problem. These include selection for enhanced resistance to ear rot fungi through conventional breeding, biotech traits for insect resistance, and particularly for the reduction of infection by *Aspergillus flavus*, enhanced tolerance to drought. The relationship between ear feeding by the European corn borer (*Ostrinia nubilalis*) and fumonisin levels in Corn Belt grain has been well established. Corn hybrids expressing Bt proteins for the control of corn borers, including YieldGard® Corn Borer and YieldGard® Plus, typically produce grain with reduced levels of fumonisins compared to conventional hybrids when corn borers are present. Monsanto has in advanced development trials a second-generation product for the control of lepidopteran insects. This product, which will be marketed under the name YieldGard VT™ Pro, provides superior control of corn earworm (*Helicoverpa zea*) and fall armyworm (*Spodoptera frugiperda*) compared to first-generation products. Preliminary results from 2005 indicated that aflatoxin levels may be significantly reduced in grain from YieldGard VT™ Pro compared to conventional controls under heavy pressure from corn earworm and fall armyworm. The potential to further reduce aflatoxin through deployment of YieldGard VT™ Pro in selected germplasm and in combination with biotech traits for drought tolerance will be discussed.

Biosynthetic Mechanism for Fumonisins, a Model System for Fungal Non-Aromatic Polyketides

L. Du, X. Zhu, F. Yu, K. Zaleta-Rivera
Department of Chemistry, University of Nebraska, Lincoln, NE 68588

Fumonisins are polyketide-derived mycotoxins produced by the widespread pathogen *Fusarium verticillioides*. We have chosen to use fumonisin polyketide synthase gene, *FUM1*, as a model system to study the biosynthetic mechanism for fungal non-aromatic polyketides because it encodes a PKS with a domain organization very similar to two other PKS genes involving in the biosynthesis of T-toxins (*PKS1*) in *Cochliobolus heterostrophus* and lovastatin (*LovF*) in *Aspergillus terreus*. While the metabolites have very different structure, these PKS have the same seven domains, KS-AT-DH-(MT)-ER-KR-ACP. These features are ideal for the domain swapping experiments. Our results showed that both the KS domain and the specific interaction between the synthase and polyketide chain-releasing enzyme (Fum8p) play an important role in producing polyketide products with a distinct structure. The results shed lights on the biosynthetic mechanism for fungal non-aromatic polyketides, which is the least understood group of polyketides so far.

Use of RT-PCR to Detect *Fusarium verticillioides* During Endophytic Colonization of Maize

D.F. Kendra[1] and R. Dyer
Mycotoxin Research Unit, National Center for Agricultural Utilization Research, USDA, ARS, 1815 N. University St., Peoria, Illinois 61604, USA

Fusarium verticillioides is one of the most important world-wide pathogens of maize causing yield loss as well as health problems for livestock and humans through the ingestion of fumonisin contaminated grain. Of particular concern is the ability of *F. verticillioides* to establish an asymptomatic endophytic relationship with the host plant and the potential contamination of visually healthy corn with fumonisins. The lack of understanding the dynamics of the endophytic relationship is a limiting factor in implementing effective control strategies. Current methods for studying the host-pathogen relationship are tedious, time consuming and not very sensitive. We describe a reproducible and highly sensitive real-time PCR method to the detect and quantify *F. verticillioides* in various tissues of corn during plant development from the seedling stage to ear development. Four independent standard curves had a reproducible titration of *F. verticillioides* genomic DNA from 40 ng to 2.56 pg. In some cases, the sensitivity could be increased 5-fold to 0.512 pg. Using our method, we confirm the results of others on the ability of *F. verticillioides* to colonize root, stem and leaf tissue of seedlings. In addition we show that seed infection can be a route to colonization of stalk and ear tissue of more mature plants.

Identification and Characterization of Regulators of FUM Gene Expression

R.A.E. Butchko, D.W. Brown, M. Busman, R.H. Proctor
USDA-ARS Mycotoxin Research Unit, NCAUR, Peoria, IL

Fumonisins are polyketide derived mycotoxins produced by the maize pathogen Fusarium verticillioides. The genes required for fumonisin production are clustered on chromosome I. Previous analysis showed that the expression of the FUM genes is coordinately regulated and expression of the FUM gene cluster first becomes detectable at approximated 48 hours after inoculation of a liquid medium and peaks approximately at 96 hours. None of the FUM cluster genes was predicted to encode a pathway specific regulatory gene, however. In an effort to identify genes that have an affect on the expression of the FUM gene cluster, we hypothesized that, similar to other secondary metabolite pathways, a specific regulatory gene might have a similar expression pattern as the genes required for toxin biosynthesis. Two Expressed Sequence Tag (EST) libraries were compared, one from a 24 hour culture (FUM genes off) and the other from a 96 hour culture (FUM genes on). Twenty ESTs with homology to DNA binding proteins, transcription factors, regulatory genes, etc., were selected that are present in the 96 hour library, but absent in the 24 hour library. These candidate genes were disrupted in a strain containing a FUM1p::GUS fusion construct and fungal transformants were screened initially for the lost of GUS expression. Disruption of six of the twenty ESTs that resulted in the loss of GUS expression also resulted in the loss of FUM gene expression and toxin production. With the release of the first draft of F. verticillioides genomic DNA sequence, sequences flanking the six ESTs were identified and used to create gene deletion mutants. Characterization of deletion mutants confirms the affect on the expression of the FUM genes and on toxin production.

Microarray Analysis of *Fusarium verticillioides*

D.W. Brown, R.A.E. Butchko, D.F. Kendra, and R.H. Proctor
USDA-ARS Mycotoxin Research Unit, NCAUR, Peoria, IL

Fusarium verticillioides can cause a variety of diseases of maize and, concomitantly, contaminate maize and maize products with fumonisins. Ingestion of fumonisins is associated with a variety of diseases in humans and animals, including cancer and neural tube defects. A major goal of our research is to develop new strategies to limit the negative impact *F. verticillioides* has on maize due to disease and mycotoxin contamination. Microarrays provide a powerful tool to examine genome wide patterns of transcription. We are using microarrays to identify *F. verticillioides* structural and regulatory genes involved in the biosynthesis of fungal toxins, virulence factors and other elements involved in plant pathogenesis. In collaboration with The Institute for Genomics Research (TIGR), we developed a NimbleGen Systems, Inc. (Madison, WI) microarray chip containing 15,844 probe sets that represent as many as 11,126 different genes. Each probe set consists of up to twelve 24-mer oligonucleotides. The gene sequences represent approximately 81% of the coding capacity of *F. verticillioides* and were generated from analysis of over 87,000 Expressed Sequence Tags (ESTs). The main set of microarray data described in this paper was generated from 12, 24, 48, 72, 96 and 120-hour cultures of wild-type *F. verticillioides* strain M-3125 in the liquid fumonisin production medium, GYAM. Mycelium was harvested from duplicate cultures at each time point and RNA was extracted and analyzed for quality. We are using the microarray data generated in this experiment for three different goals. First, we are attempting to identify genes that are involved in fumonisin biosynthesis by determining which genes represented on the microarray are co-regulated with genes in the fumonisin biosynthetic gene cluster. Transcriptional co-regulation has facilitated identification of genes involved in the same biological process in other organisms. Second, we are using the microarray data to study the role of alternative splice forms (ASFs) of transcripts of the fumonisin biosynthetic regulatory gene *FUM21*. Data presented provide further support for our hypothesis that ASFs play a role in regulating fumonisin biosynthesis. Third, we are using the microarray data to identify polyketide biosynthetic gene clusters. Analysis of co-regulated genes flanking polyketide synthase (PKS) genes should facilitate elucidation of the biochemical pathways and functions of polyketide metabolites produced by *F. verticillioides*. Together, these results demonstrate the power of microarray technology to provide a wealth of information on expression of genes involved in a large number of different biological processes.

Sequence Analysis of *Aspergillus flavus*

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A genome sequence for *Aspergillus flavus* strain NRRL 3357 is now complete and awaiting final gene annotation. There are many advantages to having a whole genome sequence. One advantage is the ability to construct whole genome DNA microarrays. Gene expression analysis using DNA microarrays is a powerful tool to dissect complex regulatory circuits as one can simultaneously measure the expression of thousands of genes at once. As the result of funding from USDA/CSREES/NRI to Drs. Payne, Keller, Woloshuk, and Yu, a whole genome Affymetrix GeneChip was developed for *A. flavus*. The array contains 12,834 predicted genes and 397 predicted antisense transcripts of *A. flavus*. No biological function of any of the antisense transcripts is yet known, although one of the antisense transcripts overlaps the 5' end of the pathway regulatory gene, *aflR*. All of the putative antisense transcripts have ESTs in either the *A. flavus* or *A. oryzae* libraries. Because this array will be employed in plant and animal pathogenicity studies, we included 8,895 maize seed genes and 25 human and mouse innate response genes. To determine if there are transcriptionally active regions within the aflatoxin biosynthetic cluster that we have not predicted, we has also tiled across the 26 intergenic regions of the cluster (both strands) with one probe approximately every 25bp. This array will be a powerful tool to help understand the pathogenicity of *A. flavus* and the regulatory elements involved in secondary metabolism. We also included over 300 genes from *A. oryzae* that appear to be absent in the sequenced strain of *A. flavus*. Our initial experiments with these multiple species arrays indicate that they are reliable for measuring gene expression in pure cultures and in host parasite interactions.

The Functional Genomic Basis of Antioxidant Potentiation of Inhibition of Aflatoxin Biosynthesis

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Natural chemical compounds used in combination with genomic tools are powerful resources for "dissecting" functional genomic processes involved in mycotoxin biosynthesis. We found that exposing *Aspergillus flavus* to oxidative stress induces aflatoxin biosynthesis. When *A. flavus* is treated with *tert*-butyl hydroperoxide, aflatoxin production significantly increases compared to controls. Alternatively, we found that certain natural phenolic compounds having antioxidative properties can completely inhibit aflatoxin biosynthesis, while having no effect on fungal growth. We next sought to determine the functional genomic interrelationship between aflatoxin biosynthesis and the oxidative stress response of the fungus.

In order to identify the genes involved in the antioxidative/anti-aflatoxigenic interaction, we initially used gene-deletion mutants of *Saccharomyces cerevisiae*. This yeast has been completely sequenced and its genes annotated. Thus, it serves as a useful model fungus. Initial assays with this yeast showed us that phenolics and reactive oxygen species modulated the antioxidative stress-response system. We were able to identify a number of candidate genes involved in this modulation and surmised that orthologous genetic components in *A. flavus* might play key roles in aflatoxin biosynthesis.

We next performed comparative gene expression analysis of *A. flavus* treated with caffeic acid, an anti-aflatoxigenic phenolic. We were able to "key-in" on certain genes based on the yeast assays. Using microarrays and qRT-PCR, for verification, we examined the functional genomics associated with antioxidant potentiation of anti-aflatoxigenesis. Firstly, there were significant down-regulations of genes in the aflatoxin biosynthetic gene cluster, starting with *verB* and *omtB*, encoding a desaturase and an *O*-methyltransferase, respectively. Interestingly, the results also showed there was no change in expression of *laeA* (Bok and Keller, 2004), a gene previously identified as regulating production of secondary metabolites in fungi. However, we identified a number of genes in the antioxidative stress response pathway that showed significant changes in expression. We found there was an almost 1 to 2.6 (log2 ratio; qRT-PCR) up-regulation of *ahpC1* and *ahpC2* genes, encoding alkyl-hydroperoxide reductases. Our findings strongly support the view that antioxidative stress-response genes are key to signaling and modulating expression of the aflatoxin biosynthetic gene cluster. Moreover, it appears *ahpC* genes play a pivotal role in potentiating inhibition of aflatoxin biosynthesis.

Aspergillus flavus Genomics for Solving Preharvest Aflatoxin Contamination in Crops

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Aspergillus flavus can infect crops of corn, cotton, peanuts and treenuts at preharvest and thereby contaminate them with aflatoxins. Aflatoxins are toxic and the most potent carcinogenic of the mycotoxins. Due to the significant health and economic impact of aflatoxin contamination on food and feed, tremendous research effort has been undertaken to better understand aflatoxin biosynthesis and its genetic regulation in *A. flavus* and *A. parasiticus*. Identification and elucidation of genes involved in aflatoxin biosynthesis through genomic strategy has been actively pursued at SRRC in order to decipher the molecular mechanisms that control or regulate aflatoxin formation. This information is to be used to devise strategies for eliminating aflatoxin contamination of food and feed commodities. A total of 19,618 *A. flavus* expressed sequence tags (EST) were generated through an EST project, from which 7,218 unique EST sequences were assembled. These ESTs have been released to the public in NCBI GenBank database (http://www.ncbi.nlm.nih.gov) and the functional classification has been presented in the form of Gene Index at the website of The Institute for Genomic Research (TIGR, http://www.tigr.org). The 5031-element *A. flavus* amplicon microarray has been constructed at TIGR. Microarray profiling for identification of genes potentially involved in aflatoxin formation and its gene regulatory network has been performed under different culture media conditions: yeast extract (YE, non-aflatoxin-producing) vs. yeast extract sucrose (YES, aflatoxin-producing), YES vs. YES supplemented with tryptophan or tyrosine. Groups of genes that are potentially involved in aflatoxin production have been identified in response to the medium conditions. Further investigations on the functions of these genes by gene knockout experiments are underway. In collaboration with Professor Gary A. Payne, NCSU and Dr. William C. Nierman at TIGR, the *A. flavus* whole genome sequencing has been completed and the genome sequences have been released to NCBI GenBank database (http://www.ncbi.nlm.nih.gov). Sequence assembly and gene annotation have resulted in the identification of about 12,000 genes in the *A. flavus* genome. The *A. flavus* whole genome microarray is under construction at TIGR. This 70mer oligo array contains a total of 12,094 putative unique genes consisting of 11,813 genes from *A. flavus*, 278 from *A. oryzae*, and additional sequences of interest. Comparative genome hybridization studies and genome-wide gene profiling using the newly constructed microarrays are expected to generate significant amounts of data for understanding the mechanism of aflatoxin biosynthesis and thus aid in solving aflatoxin contamination of crops.

Identifying Potential Aflatoxin Gene Regulators by Microarray Analysis

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Aflatoxins are extremely toxic and carcinogenic compounds produced primarily by the fungi *Aspergillus flavus* and *A. parasiticus*. Molecular studies on the genetics of aflatoxin biosynthesis have established a well organized aflatoxin pathway gene cluster consisting of 25 genes within 70 kb DNA region, though few regulatory factors have been identified. To investigate mechanisms that contribute to the regulation of aflatoxin biosynthesis two experiments using a 5031-element *A. flavus* amplicon microarrays were performed: a time course study of *A. parasiticus* shifted between two media and a comparison of tryptophan supplementation in *A. flavus* and *A. parasticus.* Aflatoxin examination of *A. parasiticus* during a shift from Yeast Extract (YE), to a similar medium with sucrose, Yeast Extract Sucrose (YES) showed aflatoxins (B_1, B_2, G_1, and G_2) concentration in fungal mycelia to be 50 ppb pre-shift, this level reduced to below 10 ppb at 3, 6, and 12 hours post-shifting, followed by a dramatic rise to 942 ppb by 24 hours post-shift. When aflatoxin levels of *A. parasiticus* and *A. flavus* grown 72 hours in Yeast extract sucrose media supplemented with tryptophan were compared to YES grown samples, a significant reduction in aflatoxins B_1 and B_2 were detected for *A. flavus;* while a significant increase in B_1 and G_1 was detected for *A. parasiticus*. Microarray analysis comparing the *A. parasiticus* RNA samples from the 48 hr YE culture to the post-shift YES samples identified 56 genes that were expressed with significant variation across all time points, included among these are three genes responsible for the conversion of norsolorinic acid to averantin in the aflatoxin biosynthetic pathway; *aflD, aflE, aflF* (*nor-1, norA* and *norB*). Likewise microarray analysis comparisons of the tryptophan supplemented samples revealed seventy seven genes to be significant, including the aflatoxin biosynthetic genes *aflD*, *aflE*, and *aflO (nor-1, norA, and omtB).* In addition to the aflatoxin biosynthetic gene *aflD (nor-1)* four other genes were found to occur significantly in both experiments. Creation of *A. flavus* deletion mutants of these four genes and qRT-PCR analysis of these genes during *A. flavus* pathogenesis of maize is expected to generate significant information regarding aflatoxin regulation.

ESTs Provide Unique Insight to Fumonisin Regulatory Gene *FUM21*

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Mycotoxins are natural products that can negatively affect animal and plant health. Genes involved in mycotoxin synthesis are often located adjacent to each other in clusters and encode structural enzymes, regulatory proteins, and/or proteins that provide self protection. Fumonisins are mycotoxins synthesized by *Fusarium* species that can contaminate maize or maize products, are associated with several animal diseases and linked with cancer in animals and humans. The current fumonisin biosynthetic gene cluster includes 16 genes of which none appear to play a regulatory role. We identified a new gene, based on analysis of Expressed Sequence Tags (ESTs), located adjacent to the fumonisin polyketide synthase gene, *FUM1*. The predicted protein of the new gene, designated *FUM21*, includes a Zn(II)2Cys6 DNA binding domain and a second domain also associated with fungal transcription factors. *fum21* deletion mutants produce no fumonisin on cracked maize and transcription of the fumonisin structural genes *FUM1* and *FUM8* is significantly reduced in liquid medium. Over-expression of a wild-type copy of *FUM21* in the *fum21* mutant restored fumonisin production. Analysis of *FUM21* ESTs identified an unusually large number of alternative splice forms (ASFs) where one or more introns had not been excised or, in the case of the 1st intron, an alternative 3' border maybe utilized. In all ASFs, a stop codon is introduced into the *FUM21* ORF. To explore whether *FUM21* ASFs play a role in fumonisin biosynthesis, microarray analysis of some *FUM21* ASFs were conducted. We found that the expression of ASFs that retain the 7th intron increase over time, ASFs that retain the 2nd intron decrease over time, and ASFs that retain the 3rd or 4th intron do not appear to change over time. We present a model depicting how ASFs may be involved in regulating fumonisin biosynthesis.

SSR Fingerprinting Analysis of Vegetative Compatibility Groups in *Aspergillus flavus*

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The use of Simple Sequence Repeats (SSR) as a fingerprinting method to distinguish different Vegetative Compatibility Groups (VCGs) in *Aspergillus flavus* was investigated. Eight loci composed of three (TTC)n repeats, one (AC)n repeat, one (ACT)n repeat, two (TTA)n repeats, and one (TTTC)n repeat were selected from the SSR sequence of *A. flavus* NRRL 3357 provided by William Nierman from the Institute for Genomic Research – TIGR in Rockville Maryland. Primers flanking these repeats were designed to amplify each locus. Eighty-two isolates from Horn's VCG tester collection and 24 isolates from Louisiana were used. PCR products were analyzed on MetaPhor agarose gels. A single band was detected for each primer pair and a given isolate except that some isolates from Louisiana gave multiple bands. Digital pictures of agarose gels were taken by BioRad Gel Doc 2000 using Quantity One 1-D Analysis Software (version 4.4) and then transported to the BioNumeircs software (version 3.0) for analysis. Each band of different size was assigned as an allele. The result showed that there is clustering for the same VCG except VCG 18 from Horn's collection.

Identification and Characterization of Differentially Expressed Genes in Two Inbred Maize Lines Using Microarray Technology

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Aspergillus flavus Link:Fr is a saprophytic fungus that can produce aflatoxin during pathogenesis of maize and other oilseed crops. Aflatoxin B_1 is the most potent natural occurring carcinogen known for livestock and humans. Efforts to reduce afaltoxin accumulation have focused on bio-control, agronomic practices, and enhancing host resistance. Though a number of historically important aflatoxin resistant maize lines exist, traditional breeding programs have failed to integrate this resistance into elite lines. Despite a number of Quantitative Trait Loci (QTL) that correspond to resistance having been identified, few individual genes known to contribute to resistance in maize have been characterized. With the availability of maize microarrays we can now begin to identify specific genes within these QTLs that are involved in resistance. Recent microarray studies have identified genes differentially expressed in *A. flavus* during aflatoxin biosynthesis and during maize acclimation response to ultraviolet radiation. However, no work using microarrays has been done examining gene expression related to *A. flavus* infection and aflatoxin production. Thus, the objectives of this study were (1) to evaluate differential gene expression levels for resistance to *A. flavus* kernel infection in susceptible (Va35) and resistant (Mp313E) maize lines using cDNA microarray analysis, (2) to evaluate differences in *A. flavus* accumulation between the resistant and susceptible maize lines and between inoculation treatments using ears harvested at physiological maturity, and (3) to identify genetic locations of any significantly expressed ESTs. Of the 5065 ESTs represented on the maize microarrays, 234 were found to be significantly up-regulated in response to *A. flavus* inoculation ($P \leq 0.05$). Among these significantly up-regulated genes, 123 were up-regulated in the susceptible line Va35; 95, in the resistant Mp313E; and 16, in both lines. These up-regulated genes were classified into three functional categories: biological process, molecular functions, and cellular components. Using the Maize Genetic and Genomics Database to compare these identified gene sequences with Quantitative Trait Loci (QTL) from Mp313E x B73 and Mp313E x Va35 mapping studies, 64 genes can be mapped to specific locations within the genome. Five of the 64 mapped genes are located on chromosome 1, 3, 4 or 6 and are in close proximity to four previously published QTLs shown to be linked to aflatoxin resistance. As these findings demonstrate, using cDNA microarray experiments and QTL data allows for a more accurate identification of candidate aflatoxin resistant genes in maize. Future coupling of microarray data with available QTL analysis will facilitate understanding of pathways that lead to aflatoxin resistance and identify ESTs/candidate genes that have the largest impact on resistance.

Fumonisin B_1 is Necessary for Corn Seedling Disease but is Minimally Translocated from Roots to Shoots

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Fusarium verticillioides, the causative agent of corn seedling blight and ear rot, produces the mycotoxin fumonisin B_1 (FB1). The toxicity of FB1 is due to its inhibition of ceramide synthase, a key enzyme necessary for sphingolipid metabolism. Such inhibition occurs in both animals and plants. For example, FB1 is known to inhibit ceramide synthase in tomato, causing and increase in free sphinganine and phytosphingosine, which are ceramide precursors. We recently reported that roots of corn seedlings inoculated with *F. verticillioides* had elevated free sphinganine and phytosphingosine as well as their respective 1-phosphates. Thus, FB1 produced by *F. verticillioides* can inhibit ceramide synthase in corn. Additionally, FB1 production by *F. verticillioides* strains was tightly linked to their pathogenicity. FB1-producing strains caused necrotic leaf lesions, developmental abnormalities, severe stunting, and even death of 'Silver Queen' seedlings. Non-producing strains were able to systemically infect seedlings but did not cause the foliar disease symptoms. Exposing seedlings to FB1 in the absence of *F. verticillioides* caused some leaf lesions, though the full spectrum of symptoms was not as severe as when the fungus was present. Corn lines were assessed for their sensitivity to FB1, and B73, FR1064, CG1, Mo17, and CQ201 were unable to germinate on media containing 100 μM FB1. 'Silver Queen' had a lower germination rate (12%) and stunted growth (44.1% of the control for root weight, 58.3% of the control for shoot weight, and 75% of the control for shoot height). Of the lines evaluated, only W23 was truly insensitive with 100% germination and equal or better growth compared to control plants (98.8% of the control for root weight; 128.6% of the control for shoot weight; and 124% of the control for shoot height). Plants were also exposed to extracts of fungal cultures grown on autoclaved corn kernels. Extracts containing FB1 exhibited the full suite of symptoms as occurs on plants infected with FB1 producing strains. In addition, we found that aerial portions of plants subjected to this treatment contained FB1 (0.0044 ppm), suggesting the metabolite was taken up by roots and translocated by the plant vasculature. However, active uptake and translocation of FB1 into leaf tissue was minimal (60-90 fold less in leaves than in roots). The insensitivity of W23 to FB1 compared to sensitive lines B73 and 'Silver Queen' was not due to differential translocation and accumulation of FB1 in leaf tissues since they all passively accumulated FB1 (1.3 to 2.6 ppm) in a detached leaf assay. Accumulation of fumonisin in kernels via translocation was assessed by injecting fungal culture extracts containing FB1 into stalks of 'Tom Thumb' below developing ears prior to pollination. Resulting kernels were collected and sampled for fungal infection as well as for FB1 contamination. Translocation and accumulation of the injected FB1 was not supported. However, the injected fungal extracts appeared to confer resistance because the extract treatments had less *F. verticillioides* infection and only 0.7 ppm total fumonisin contamination compared to 3.1 ppm in the control kernels. Thus, while FB1 production by *F. verticillioides* was necessary for development of foliar disease symptoms on seedlings, the mycotoxin apparently was not translocated or accumulated to high levels in the leaves, suggesting the disease development may not involve localized ceramide synthase inhibition in the leaves but may result from another mobile signal or pathway. Furthermore, the apparent lack of FB1 accumulation in the kernels via translocation is encouraging from the standpoint of control strategies, and additional studies are planned to more closely evaluate our initial data on suppression of *F. verticillioides* infection and FB1 contamination of kernels by fungal culture extracts.

Corn Seedling Disease, Fusaric Acid as the Wilt Toxin and the Need for Biocontrol of *Fusarium verticillioides* and other *Fusarium* Species

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Fusaric acid (5-butylpicolinic acid) was first discovered during the laboratory culture of *Fusarium heterosporum*, and was one of the first fungal metabolites implicated in the pathogenesis of wilt symptoms of plants especially under adverse conditions. In addition to a primary role in plant pathogenesis, fusaric acid is mildly toxic to mice and has other pharmacological properties. During tests for control of the fungus *F. verticillioides* and reduction of the mycotoxin fumonisin B1, it was determined that fusaric acid was produced *in planta* and appears to control the growth of the biocontrol endophytic bacterium, *Bacillus mojavensis*, without any apparent symptoms of a disease. Since fusaric acid is considered a wilt toxin, we examined its *in planta* production and role in the wilt of field maize. Using plants infected with fusaric acid producing and non-producing strains of *F. verticillioides,* we isolated, identified, and measured fusaric acid in roots of seedlings grown with and without drought stress. It was determined that fusaric acid was produced *in planta* at the same concentrations regardless of drought stress, and there were no symptoms of wilt disease in the one field maize cultivar tested. Perhaps its major importance is as an antibiotic against *B. mojavensis,* other biocontrol species and endophytic competing bacteria that co-occur with *Fusarium* species and in soil, suggesting that fusaric acid does not function solely as a wilt toxin in maize.

PANEL DISCUSSION: FUMONISIN ELIMINATION/FUNGAL GENOMICS, REGULATION OF AFLATOXIN BIOSYNTHESIS

Panel Chair – Jeffery Wilkinson

An excellent overview of Monsanto's' work on improving resistance to insect predation and aflatoxin accumulation in maize was given by John Hendrick. As he highlighted in his brief overview maize is a staple crop that a vast majority of the world depends on for nutrition, in spite of increased yearly outputs maize stores have been steadily declining due to increased demand. Thus, it is becoming increasingly important to minimize losses of this commodity resulting from insect or fungi damage and Monsanto's has focused their efforts on increasing resistance to insects and on improving drought tolerance. Yield Guard Bt Pro is a second generation Bt plant Monsanto is preparing for release. With proprietary genes that improve resistance above the first generation Bt plants it is expected to further reduce insect damage caused by cornborer. As showed by Williams and Windham reductions in fungal infections and mycotoxin accumulations associated with these insect vectors is reduced by elimination or reduction of the corn borer. However, with the complex problems of fumonsin and aflatoxin contamination a complex solution is required. To that end Monsanto is also testing lines for drought tolerance, examining such traits as leaf rolling and leaf temp in their selection process aimed at stacking these and other genes to increase drought tolerance. As with all of Monsanto's work regular test of aflatoxin levels is used to assist screening efforts. These mycotoxin tests are one of the many traits used during screens of hybrid lines.

Fungal Genomics

Many advances and promising discoveries of the genetic basis and functional genomics of fumonisin and aflatoxin production were made over the past year.

In efforts to reduce time and cost of identification of *Fusarium verticillioides* during maize infection a simple, reproducible and highly sensitive real-time PCR method was presented (Kendra). It was shown that this in real-time PCR method was sensitive enough to detect and quantify *F. verticillioides* infection from the seedling stage to ear development I. Tracking of seed infection revealed that this can be a route to colonization of stalk and ear tissue of more mature plants as revealed by the detection of micro spores at node plates (Kendra). Studies of the mechanisms of polyketide synthases in fumonisin production demonstrated that ketosynthase are not the key enzyme for generating Fumonisins and that by substituting ketosynthases new non-aromatic polyketides can be generated (Du).

To identify what genes may control the regulation of the FUM cluster genes twenty ESTs with homology to DNA binding proteins, transcription factors, regulatory genes, etc., were selected from a comparison of two Expressed Sequence Tag (EST) libraries. These candidate genes were disrupted in a strain containing a FUM1p:GUS fusion construct, and the fungal transformants were screened for the loss of GUS expression. Disruption of six of the 20 ESTs that resulted in the loss of GUS expression also resulted in the loss of FUM gene expression and toxin production (Butchko). One gene known to be a specific differtial regulator of the FUM genes, FUM21 was examined to determine the effects alternative splice forms have on fumonisin biosynthesis. By RT-PCR it was determined that the OE plasmid made transcripts earlier than wild-type but not all make fumonsins. Perhaps this is an indication that alternative splice forms are controlling regulation. The next generation of arrays will contain sequence to allow studies of alternative splice forms (Brown).

Advances in studies of aflatoxins and Aspergillus have also made great strides since last year. The whole genome sequence of Aspergillus flavus has been completed and is estimated to contain 13,800 genes, larger than either A. nidulans or A. fumigatus. The 33.1 Mb of the preliminary draft consist of 17 scaffolds on 2995 contigs. Manual annotation of this genome is coordinated through North Carolina State University and will be made available at www.Aspergillusflavus.org. This genome sequence will be the basis of two *A. flavus* whole genome array platforms (Payne). An affymetrix based array containing the 13,800 *A. flavus* genes probes and an additional 10,000 probes from maize to yield a comprehensive *A. flavus/*maize kernel expression array (Payne) and a 70mer oligo array containing 11813 genes from *A. flavus* and 278 genes from *A. oryzae*, and additional interest sequences (Yu). Though we look forward to these future arrays several interesting experiments are ongoing with the 5031-element *A. flavus* amplicon microarray (Yu).

Investigations of *A. flavus* and *A. parasiticus* have reveled several candidate genes that may function the regulate aflatoxin (Yu, Campbell, Wilkinson). Several avenues of research seem to be converging in support of oxidative stress as a major inducer of aflatoxin biosynthesis. In a screen of compounds that have antioxidative properties and inhibit aflatoxin biosynthesis using gene-deletion mutants of *Saccharomyces cerevisiae* it was determined that caffeic acid modulated the antioxidative stress-response system (Campbell). Using gene expression analysis of *A. flavus* treated with caffeic acid it was shown that significant down-regulations of genes in the aflatoxin biosynthetic gene cluster occured, including *verB* and *omtB*, encoding a desaturase and an *O*-methyltransferase, respectively (Campbell). Likewise microarray analysis comparisons of tryptophan supplemented *A. flavus* revealed a suppression of the aflatoxin biosynthetic genes *nor-1, norA, and omtB*, two norsolorinic acid ketoreductase and *O*-methyltransferase. *A. parasiticus* samples do not show the same reduction in transcipts seen in *A. flavus* (Wilkinson).

The True Economic Impact of Aflatoxin in U.S. Crops

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Aflatoxin, a toxic and carcinogenic mycotoxin produced by *Aspergillus flavus*, colonizes a variety of crops including corn, cottonseed, peanuts, and tree nuts, lowering their economic value and occasionally also jeopardizing animal health in the United States. The economic impacts of these health and market risks associated with aflatoxin should be measured, so that policy decision-makers have accurate quantitative data by which to begin addressing the risks. Previous work to estimate the economic impact of aflatoxin considered only market rejection costs, not other losses to a variety of industry sectors and indirect losses.

This work addresses the gaps in previous studies by building a comprehensive economic model to estimate total aflatoxin costs to a food commodity group. In the model, five categories of stakeholders are represented: growers, handlers, food and feed processors, distributors, and consumers. Each of these stakeholder groups experiences different forms of economic loss including: product disposal before reaching market, sampling (analytical costs and product lost through testing), market rejection at food- and feed-grade levels, geographic complications, export-related losses, and livestock losses. Inputs vary considerably depending on the commodity (e.g., export-related losses are proportionately larger for tree nuts than for other commodities); thus, calculations are made separately for each commodity group.

The model is implemented in Analytica™, a software tool in which model parameters can be specified as probability distributions, and values from these distributions are sampled using Monte Carlo techniques in each model run. Sensitivity analyses calculated by the model can estimate what total and individual sector costs will be in a year of severe aflatoxin contamination, as well as in a "normal" year. Moreover, certain inputs can be adjusted to account for improvements to preharvest or postharvest aflatoxin control, to estimate the economic benefits provided by improved aflatoxin control methods and technologies.

A case study in corn estimates that the total measurable annual loss to the U.S. corn industry is $192 million (95% C.I. = $84 million to $591 million). Of this amount, on average, market losses make up $163 million, livestock health losses $4 million, and sampling and testing costs $25 million. In addition, there are "intangible" economic losses, including the costs of grower disposal of contaminated corn, sampling errors, losses on a geographical basis, and losses associated with aflatoxin concentration in the production of ethanol. Inclusion of these costs would increase the average annual loss due to aflatoxin in corn to over $200 million.

The benefits of this work to food industries are threefold. 1) The economic model will allow industry to calculate the *true* economic impact of aflatoxin, which is likely to be several orders of magnitude higher than previous, more narrowly focused studies have indicated. This information can be used to acquaint policymakers with the true magnitude of the aflatoxin problem. 2) Understanding which stakeholders bear the burden of aflatoxin cost, and identifying where the greatest costs are incurred, will offer implications for which aflatoxin control strategies to pursue. 3) This model is also useful for providing institutional memory. Industries will likely run into a variety of contamination events in the future, regarding aflatoxin as well as other mycotoxins or contaminants. In these cases, they will want an accurate estimate of the extent of costs, as well as models by which to calculate such costs and to aid in decision-making.

Secondary Metabolite Biosynthetic Gene Clusters in Sequenced Aspergilli

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Comparative analysis of the mycotoxin and other secondary metabolite biosynthetic gene clusters has provided a new perspective on the evolution and regulation of these clusters. I will review the genome sequences and comparative analysis of secondary metabolite biosynthetic clusters in an important human pathogen, *Aspergillus fumigatus* CEA10, a very closely related sexual species, *Neosartorya fischeri* NRRL181 (*A. fischerianus*), and a more distantly related asexual species, *A. clavatus* NRRL1. The comparative analysis of these three genomes along with recently sequenced genome of *A. fumigatus* Af293 revealed a large number of species-specific genes, which represent up to 25% of each genome. A substantial genetic variability was observed among putative metabolic adaptation and secondary metabolism clusters. Each genome encodes 23-30 putative secondary metabolism clusters, but only nine of them are orthologous in all three species. Both strains of *A. fumigatus* also have unique secondary metabolism clusters. Genes in the nine orthologous clusters appear to be under negative Darwinian selection like most primary metabolism genes. About 200 genes in *A. fumigatus* Af293 and CEA10 strains appear to be strain-specific and located predominantly in subtelomeric and intrasyntenic blocks. Similar to prokaryotic "genomic islands", these nonsyntenic regions often contain genes with different GC content and codon adaptation indexes and are enriched for transposable elements and secondary metabolite clusters. No evidence however was obtained to support their origin by acquisition through horizontal gene transfer from other species as suggested previously. An alternative mechanism is proposed here that accelerated differentiation of duplicates translocated into the non-syntenic chromosomal domains. All these findings highlight the plasticity of the aspergilli genome organization that can accommodate a variety of lifestyles and different evolutionary modes for the core and 'dispensable' genes particularly those encoding genes for secondary metabolite biosynthetic pathways. The results of experiments supporting a role for these mycotoxins in soil competitiveness will be presented.

Expression of an Active Form of Maize RIP 1 in Transgenic Peanut Inhibits Fungal Infection and Reduces Aflatoxin Contamination

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RIP 1 is a ribosome inactivating protein from maize which has been shown to inhibit hyphal growth of *Aspergillus flavus in vitro*. RIP 1 is expressed in maize as an inactive pro-RIP that must be activated by proteolytic cleavage. Mod 1 is a synthetic gene that encodes an active RIP 1 identical to the proteolysis-activated form of the protein found in maize. We have transferred this gene into peanut in an attempt to control aflatoxin contamination by retarding fungal growth.

Peanuts cv. 'Georgia Green' and 'NCV 11' were transformed by co-bombardment of mature embryos with two plasmids, one containing a gene encoding the active RIP, Mod 1, the other carrying a hygromycin phosphotransferase gene, a selectable marker. A total of 28 transgenic events were recovered that have been shown to express the Mod 1 protein. Efficacy tests of Mod 1 transgenic peanuts indicated that detached cotyledons from transgenic lines expressing Mod 1 retarded growth of *Aspergillus flavus* isolate "32-8". Further, resistance against the fungus was proportional to the amount of Mod 1 protein present, as indicated by comparative immuno-blots of the expressing lines.

Leaflets from transgenic lines known to express Mod 1 protein were inoculated with mycelium of *Sclerotinia minor* (Sclerotinia blight) or *Sclerotium rolfsii* (Southern Blight) to test for resistance against these fungi. Three of the lines tested, including two from Georgia Green and one from NCV 11, showed a statistically significant reduction in growth of both *S. rolfsii* and *S. minor*.

Seeds from twelve R_2 lines, selected for high levels of expression of Mod 1, were re-tested against *A. flavus*. All transgenic cotyledons showed enhanced resistance relative to inoculated, un-transformed controls. Cotyledons inoculated with *A. flavus* were tested by LC-MS/MS for the presence of aflatoxins. Control seeds of Georgia green and NCV 11 without inoculation contained no detectable aflatoxin. Inoculated control seeds of both varieties produced large amounts of aflatoxin B1. Aflatoxin B1 in all transgenic lines tested were lower than that observed in inoculated, un-transformed controls. In some lines, this reduction in aflatoxin B1 contamination was statistically significant compared with controls, and there were statistically significant differences in aflatoxin contamination among transgenic lines within a parent genotype. In all, four independent transformants from Georgia Green, and five independent transformants from NCV 11 showed a statistically significant reduction in aflatoxin B1 contamination, compared with inoculated controls.

All lines have now been advanced to R_3 generation. Three lines are of special interest, since they have shown statistically significant resistance against *S. rolfsii*, *S. minor*, and *A. flavus* and also showed significantly reduced aflatoxin contamination. These lines are now being hybridized with other peanut accessions in order to move the Mod 1 transgene into those materials. In some cases, the conventional parent was chosen for inclusion in the crossing program because the line naturally exhibits lower aflatoxin contamination, and therefore is assumed to be carrying one or more genes affecting this trait. The goal in these crosses is to combine the transgenic and native sources of resistance to achieve more consistent and perhaps more significant resistance in progeny.

Other crosses are being made with conventional lines that are high in oleic acid. While these lines show improved keeping quality because of the high oleic acid content, they have also been shown to be especially susceptible to aflatoxin contamination. We hypothesize that by transferring the Mod 1 gene into the high oleic acid lines, it may be possible to retain their desirable qualities, while reducing their susceptibility to *Aspergillus* infection, and thus reducing their tendency to become contaminated with aflatoxins.

Genetic Engineering of Cotton for Resistance to *Aspergillus flavus* and other Phytopathogens

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Fertile transgenic cotton plants expressing the synthetic antimicrobial peptide, D4E1, were produced through *Agrobacterium*-mediated transformation (Rajasekaran et al. 2005). *In vitro* assays with crude leaf protein extracts from T0 and T1 plants confirmed that D4E1 was expressed at sufficient levels to inhibit the growth of *Fusarium verticillioides* and *Verticillium dahliae* compared to extracts from negative control plants transformed with pBI-d35SΩ-*uid*A-nos (CGUS). Although *in vitro* assays did not show control of pre-germinated spores of *Aspergillus flavus*, bioassays with cotton seeds *in situ* or *in planta*, inoculated with a GFP-expressing *A. flavus*, indicated that the transgenic cotton seeds inhibited extensive colonization and spread by the fungus in cotyledons and seed coats. *In planta* assays with the fungal pathogen, *Thielaviopsis basicola*, which causes black root rot in cotton, showed typical symptoms such as black discoloration and constriction on hypocotyls, reduced branching of roots in CGUS negative control T1 seedlings, while transgenic T1 seedlings showed a significant reduction in disease symptoms and increased seedling fresh weight, demonstrating tolerance to the fungal pathogen. 2005 field evaluations of T2 progeny for Fusarium wilt (*Fusarium oxysporum* f.sp. *vasinfectum* (FOV) Atk. Sny & Hans) race 1 was carried out in sandy soil that also exhibited presence of root-knot nematodes (*Meloidogyne incognita*). R2 progenies of four independent transformation events expressing the antifungal peptide D4E1, a transgenic control entry with the GUS marker gene and the original non-transgenic variety (Coker 312), along with commercial Acala (*G. hirsutum*) and Pima (*G. barbadense*) cultivars were included in the field evaluation. Preliminary observations indicated that the transgenic D4E1 entries showed a healthy, higher germination stand (up to 68%) than the controls (43%). Preliminary observations from 2006 field trials did not show as strong FOV resistance as the 2005 trials. However this may be due to the use of seed in 2006 obtained from open pollinated plants from the 2005 trials which might have resulted in dilution of the D4E1 transgene and hence reduced efficacy against FOV. Experimental tobacco and cotton transgenic lines that were lost due to Hurricane Katrina include cotton lines expressing barely hordothionin and tobacco lines expressing the D4E1 transgene integrated into the plastid genome. These experiments are being repeated. We have also generated a binary vector for expression of a D4E1 dimer peptide in tobacco and cotton in order to increase the efficacy of D4E1 against *A. flavus*.

Host Genetic Components for Aflatoxin Reduction Strategies in Peanut

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Multiple genetic approaches to reduce aflatoxin contamination of peanut are being explored. Putative antifungal genes being tested include a chloroperoxidase gene (CPO-P) from *Pseudomonas pyrrocinia* and an anti-apoptotic gene (*Bcl-xl*) from human. The CPO-P gene under the control of the CaMV35S promoter was introduced into peanut by microprojectile bombardment, and multiple transgenic lines were tested for expression of the transgene. Expression was detected for both RNA and protein. One line has been advanced for several generations and the transgene insertion is stable and homozygous. This line also has shown a consistent 50% reduction in *Aspergillus flavus* growth when seeds are inoculated in vitro. The CPO-P line was planted in two aflatoxin tests in 2006. Each test contained 10 plots of the CPO-P line that were subjected to drought stress and will be assayed for aflatoxin production. For *Bcl-xl*, 309 plants from 60 hygromycin-resistant lines were regenerated and greenhouse-grown materials were screened by PCR. Most (87.1%) were PCR positive for *Bcl-xl*. Seeds (2,131) from 11 transgenic lines were collected. All lines tested showed expression at the RNA level by RT-PCR; however, protein has been detected in only a few lines. The strongest expressing line showed tolerance to paraquat (2 and 5 μM) as measured by chlorophyll retention of treated leaf discs. This line was not vigorous, set very few seeds, and could not be maintained. Other lines showed tolerance to 50 and 100 mM NaCl when T1 seeds were germinated in vitro on salt containing medium; however, this tolerance was not demonstrated with T2 seeds. Lines with stable protein expression currently are being sought since it is expected that the levels of biotic and abiotic stress tolerance will be positively correlated with the level of protein expression.

A non-transgenic method being used to alter the host's resistance to *Aspergillus* or aflatoxin production is based on a screen for mutations in specific genes. TILLING (Targeting Induced Local Lesions IN Genomes) requires that sequence for a particular gene of interest be known. A mutant population is most frequently produced by mutagens such as ethyl methanesulfonate that induce single nucleotide changes at random in the genome. Alternatively, wild relative accessions are a good source of natural allelic variants for inbred plants such as peanut and "EcoTILLING" is a useful method to detect natural polymorphism. We have initiated both TILLING and EcoTILLING in peanut. Since peanut is a tetraploid crop, and each gene should have at least two copies (one from each sub-genome), we designed gene-specific primers to distinguish between the two homoeologs of *ara h 2*, an allergen gene with trypsin inhibitor activity. The A-genome-specific primer pair was used to screen the putative A-genome progenitor, *A. duranensis* for polymorphisms in the *ara h 2* gene. Several allelic variants were identified, some with predicted amino acid changes in antigenic epitopes and also in secondary structure. A small population (~1400 M2 plants) of peanut currently is being screened to determine the mutation frequency. Preliminary results indicate the mutation frequency to be $\sim 10^{-5}$ similar to what has been found in *Arabidopsis*. A primary TILLING target for aflatoxin reduction is lipoxygenase genes. These gene products are involved in the synthesis of hydroperoxide fatty acids, which have been shown to either promote or inhibit aflatoxin biosynthesis. Before TILLING for gene knockouts can be initiated, the genomic sequence for lipoxygenase genes must be determined. We have sequenced ~50% of the 3' end of lipoxygenase genes amplified from genomic DNA of tetraploid peanut and its putative A- and B-genome progenitors. Primers for amplification were designed to regions conserved between published peanut cDNA sequences and soybean genomic DNA sequence. Putative pseudogenes containing deletions have been identified and a second set of primers has been designed to eliminate the amplification of these pseudogenes.

Analysis of *ara h 2* genes has led to the recovery of transgenic lines partially silenced for Ara h 2 expression. These lines will be tested for a potential increase in fungal susceptibility since trypsin inhibitor activity has been associated with fungal resistance in maize. Segments of the upstream regulatory region of *ara h 2* have been fused with the *gus* gene in order to identify the promoter elements required for seed specific expression. These experiments are being carried out in *Arabidopsis*.

A Maize 9-Lipoxygenase is Required for Resistance to Aflatoxin Contamination, Insects, and Nematodes

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Lipoxygenases (LOXs) are dioxygenases that catalyze the addition of molecular oxygen to polyunssturated fatty acids including linoleic (LA) and linolenic acid (LeA), either at position 9 or 13 of their carbon chains, to produce a group of acyclic and cyclic hydropexides compounds called oxylipins. The resulting two distinct fatty acid hydroperoxides then enter separate biosynthetic pathways that lead the accumulation of oxylipin compounds that have diverse physiological functions in plants. Previous reports showed that fungal oxylipins, so-called psi factor, play import roles in regulating both asexual and sexual spore development in *A. nidulans*. On the other hand, exogenously applied plant 9-LOX derived oxylipin 9*S*-HPODE stimulated aflatoxin production and sexual spore development rather than conidial development in *A. nidulans*, whereas 13-LOX derived oxylipins had opposite effects. Moreover, a maize 9-LOX, *ZmLOX3* (formerly *cssap92*) was induced by *A. flavus*, accompanied by increased levels of 9*S*-HPODE. These data suggest an essential role for 9-LOXs signaling pathways in *Aspergillus*-host interaction. To provide genetic evidence, by reverse genetics strategy we have generated *Mu*-insertional *lox3-4* mutant and its near-isogenic lines (NILs) at BC4F5 stage. Field infection data from two years at two different locations showed consistently that *lox3-4* mutants accumulated significantly more aflatoxin than wild type. Further, kernel assay indicated on *lox3-4* mutants conidiation of *A. flavus* was significantly increased, compared to NIL wild types, suggesting that ZmLOX3 is required for resistance of maize to aflatoxin contamination. *lox3-4* mutants also displayed increased attractiveness to RKN, increased number of nematodes and eggs, suggesting that *lox3* mutants are more susceptible to RKN. Moreover, opening field evaluation and controlled green house infestation showed *lox3-4* mutants are significantly susceptible to insects damage. Taken together, our data suggest a role for 9-LOX playing in resistance of maize against diverse pathogens and insects.

The Effect of PR-10 Expression on Host Resistance in Maize Against *Aspergillus flavus* Infection and Aflatoxin Production

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Aflatoxins are carcinogens produced mainly by *Aspergillus flavus* during infection of susceptible crops such as maize (*Zea mays* L.). Although resistant maize genotypes have been identified, the incorporation of resistance into commercial lines has been slow due to the lack of selectable markers. Recently, resistance-associated proteins (RAPs) have been identified by comparing constitutive protein profiles between resistant and susceptible maize genotypes using proteomics. Preliminary characterization of some of these RAPs suggests that they play a direct role in host resistance, such as the pathogenesis-related protein 10 (PR-10). However, direct evidence for the involvement of these proteins in kernel resistance was missing. In the present study, an RNA interference (RNAi) gene silencing approach was used to silence the expression of a maize *pr-10* gene. RNAi silencing is a posttranscriptional, sequence-specific RNA degradation process. It is triggered by a double stranded (ds) RNA, leading to the degradation of homologous RNA encoded by endogenous genes, and transgenes. A binary vector containing all the key elements needed to generate a dsRNA structure was constructed using the Gateway technology. Two inverted repeats containing parts of the coding region of *pr-10* gene were integrated into the vector through the site-specific recombination. The resulting construct, PR-10 RNAi vector, was then transformed into immature maize embryos using both bombardment and *Agrobacterium* infection.
Eleven of 15 callus clones, representing 15 independent transformation events of *pr-10*, were confirmed to be positive for transformation through PCR. The extent of gene silencing in transgenic callus tissues varies from one to another ranging from 65% to over 99% based on real-time PCR. The PR10-RNAi silenced transgenic maize seeds also have been produced from plants regenerated from *Agrobacterium* transformed callus clones. The number of kernels per ear varies significantly, from as few as 2 to as many as 196. Kernels from 8 ears of transgenic plants were germinated and 7 of them were confirmed positive for transformation using genomic DNA isolated from these young leaves. Two of these *pr-10* RNAi silenced lines also showed significant increase in aflatoxin production compared to the non-silenced controls as determined through the Kernel Screening Assay. However, the *pr-10* does not appear to express in leaf tissues of maize seedlings, therefore, the extent of gene silencing for *pr-10* in transgenic kernels was determined using proteomics. It was found that the two lines that showed significant increase in aflatoxin production also showed significant reduction in the level of PR-10 protein. Data from the RNAi studies indicate a direct involvement of PR-10 in maize aflatoxin resistance.

Annotation and Computational Analysis of Maize Proteome and Gene Expression Datasets

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Characterization of the transcriptome and proteome of the developing maize ear under different conditions has the potential to reveal the fundamental processes that confer resistance in some cell lines. We have previously reported the development of the PepIdent database of translated ESTs for maize for identification of proteins using mass spectroscopy, the PepSort tool for analysis of multi-dimensional protein identification technology (MudPIT) datasets, and the AgBase suite of tools for functional analysis of protein datasets. During the past year, the AgBase suite of tools has been extended for annotation of gene expression data, a new method has been developed for validation of peptide identifications, a semi-quantitation method has been developed for MudPIT proteomics data, and methods for characterizing changes in expression of both RNA and protein have been investigated.

Protein identification from Sequest analysis of MudPIT data depends on accurate peptide identifications from trypsin digested mixtures. Several Sequest scoring outputs are provided for each peptide that allow a scientist assess the quality of the peptide identifications, but the standard practice of using fixed thresholds for the scores can reduce the number of peptide identifications or admit an unacceptable number of false positive identifications. We have developed a new method that compares identifications from a random database and real database for different scoring values and uses an outlier detection approach to determine which identifications should be accepted.

Relative quantification of proteins in MudPIT datasets has typically been done using differential labeling with agents such as ICAT. We have implemented tool for relative quantification of proteins (reported by Nanduri et al, *Proteomics*, 2006) that does not require labeling.

The MaizeGO is part of Agbase www.agbase.msstate.edu, a curated, open-source, Web-accessible resource for functional analysis of maize gene products. The AgBase Goanna tool has been extended to provide functional annotations for gene expression data. The parent sequences of the probes of interest from the array (those that are differentially regulated) are provided as input in fasta format. A blastn search is performed against the AgBase database and a user-specified number of top hits (above a user-specified E-value) are returned along with their GO annotations. For each hit, a link is provided to the alignment that produced the hit to allow the scientist to evaluate the quality of the alignment. After review, the GO categories from the accepted alignments can be analyzed. The GO Slim Viewer tool provides an overview of the membership in GO categories of a gene expression data set using categories defined in a GO Slim. Output is in a form that can be easily imported into Excel for formatting as a pie chart. When this tool was used with a gene expression study of the differences in *Aspergillus*-inoculated and un-inoculated plants of two different varieties, a few of the GO terms came from annotated maize proteins, but most came from homology with rice or Arabidopsis proteins. Current efforts are underway to integrate these results with systems biology tools such as Cytoscape for modeling biological networks.

PANEL DISCUSSION: CROP RESISTANCE – GENETIC ENGINEERING
Panel Chair - Peggy Ozias-Akins

A question was addressed to Jeff Cary regarding the expression of the peptide D4E1 and the evidence that fungal resistance was a consequence of peptide expression. It is not possible to detect the peptide on a western due to technical reasons; therefore, expression in transgenic plants has been assayed solely by RT-PCR to detect transcription. In vitro evidence does support the mode of action for the peptide to be disruption of the fungal membrane rather than an indirect effect. It is hoped that a D4E1 dimer will be more antigenic and retain antifungal activity. Dr. Gao responded to a question about jasmonic acid (JA) levels in the maize *lox* mutants. The *Zmlox3* mutant does produce higher levels of JA. Some additional points were addressed by Arthur Weissinger for peanut *mod1* transgenics. The transgenics are being used as pollen parents rather than female parents just to increase the probability of successful crosses even though there is no apparent reduction in seed set on the transgenic plants. Chloroplast engineering is preferable to nuclear engineering when high levels of stable gene expression are desired along with a reduced probability of transgene transmission through pollen. Chloroplast engineering has not become standard practice, however, because it is difficult to accomplish chloroplast transformation in plants other than tobacco. Peanut chloroplast transformation has not yet been achieved. The expression pattern of *mod1* in peanut seeds was established to be in the cotyledonary epidermis, but it has not yet been determined whether there is an expression difference between abaxial and adaxial epidermis. Dr. Wu was asked if she had published any of her work on the economics of aflatoxin contamination. Her most recent publication on Bt corn can be found in *Transgenic Research* 15:277 (2006) and others are in the *Journal of Toxicology and Toxin Review* and *Environmental Science and Technology*. Her publications include US and international data but not health-related costs since aflatoxin levels are below the threshold for concern in the diets of developed countries. She also has not included the costs of research or crop insurance in her analyses but is beginning to do some work on fumonisin in addition to aflatoxin. With regard to RNA silencing of PR-10 in maize, Dr. Chen responded that the level of silencing as detected by protein assay was positively correlated with the level of aflatoxin production. The panel discussion did not focus on any key issue, but instead dealt with specific follow-up questions.

New Approaches to Breeding for Resistance to Preharvest Aflatoxin Contamination in Peanut

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On average, preharvest aflatoxin contamination (PAC) costs the U.S. peanut industry over $20 million annually. The long-term objective of this research program is to develop peanut cultivars which have less aflatoxin contamination. During the coarse of this project we have developed field screening techniques that can measure genetic differences in aflatoxin contamination. We have also identified several sources of resistance. These sources of resistance have been entered into a hybridization program to attempt to combine this resistance with high yield, acceptable grade, and resistance to tomato spotted wilt virus (TSWV). This has resulted in the development of breeding lines with relatively high yield and relatively low aflatoxin when grown under heat and drought stress. We are releasing C76-16 as peanut germplasm with improved resistance to drought and aflatoxin contamination. We continue to looks at new approaches which could be used to accelerate our breeding progress. During the past year we evaluated epidermal conductance as a potential drought tolerance trait, and evaluated genetic response to *in vitro* seed colonization by *Aspergillus flavus* in peanut. The genetic variation in epidermal conductance does not appear to be large enough to be useful in our breeding program. We did observe some interesting genetic difference in *in vitro* seed colonization and we plan to conduct some additional studies on this approach. The most promising approach to accelerate the development of PAC resistant peanut may be the use of nematode resistance. Previously, we have documented an association between root-knot nematode damage and increase PAC. We plan to release C724-19-15 as a nematode resistant cultivar this winter, and hope that this cultivar can be used as a tool to reduce PAC on the farm level.

Variation Among Peanut Cultivars in Pod Water Uptake and Its Effect on Pre-harvest Aflatoxin Contamination

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The ability to withstand drought stress appears to be a critical crop physiological characteristic impacting pre-harvest aflatoxin contamination. This is especially true in peanut production where dryland peanuts exposed to late season drought are extremely vulnerable to aflatoxin contamination. However, very little is known about the link between peanut physiological drought tolerance and aflatoxin resistance. One key physiological response may be the ability to maintain the water status of the kernel, either through adequate water flow from the plant or direct uptake of water through the hull during pod development. The objectives of this research were to determine the differences in pod water uptake between aflatoxin resistant and susceptible genotypes by: 1) quantifying the relative contribution of water to the developing kernel both from the plant and from the soil directly; 2) determining differences in pod water uptake patterns between genotypes during late season drought; and 3) determining the correlation between water movement through the hull and eventual aflatoxin contamination at harvest. These questions were addressed utilizing labeled water applied to the soil surrounding developing peanuts in enclosed pans, thereby isolating the labeled pod soil from non-labeled rooting soil. These pans were installed next to the developing crop and deuterium was applied at 90 days after planting, just prior to subjecting the crop to complete drought until harvest. The deuterium signal was quantified in pods, attached pegs, and attached stems as the drought progressed. Evidence was found of passive movement of water across the hull during drought as well as differences between cultivars in the amount of water movement. Aflatoxin contamination at harvest was correlated with water movement in a positive direction such that the more water movement, the greater the aflatoxin contamination. This suggests that the movement of water across peanut hulls may indicate a more permeable hull that allows for greater fungal invasion of intact pods, or allows for a moist microclimate within the pods that is conducive to fungal growth and eventual aflatoxin production.

Remote Sensing for Rapid Selection of Drought and Aflatoxin Resistant Peanut Genotypes

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Drought stress and aflatoxin contamination continue to challenge peanut (*Arachis hypogaea* L.) producers across the U.S. As a result, researchers have continued to investigate new varieties with enhanced resistance to drought and aflatoxin contamination. Current methods of in-season crop evaluations are qualitative and laboratory screening for aflatoxin contamination can be cost-prohibitive. However, ground-based remote sensing may be used to quantify crop response to induced drought stress and aflatoxin inoculation. The objectives of our study were twofold: 1) to evaluate canopy reflectance as a means to quantify crop response to induced drought and 2) evaluate the utility of remotely sensed estimates of drought tolerance as an indirect indicator of aflatoxin resistance. In May 2004 and 2005 several small plots (2 m x 2 m) were established at the Gibbs Farm research facilities in Tifton, GA. Treatments consisted five peanut genotypes encompassing a range of drought tolerance and yield characteristics arranged in a completely randomized block design. Drought conditions were simulated beginning 90 days after planting and maintained through harvest. Once drought conditions were established, a handheld radiometer was used to acquire twice weekly reflectance measurements in the visible and near infrared regions of the spectrum. Coincident with remotely sensed data collection standard visual ratings and soil water content (0-15cm) were acquired. Seasonal measurements included aflatoxin and yield measurements. Data are promising and indicate that remote sensing may be used to facilitate the selection of drought and aflatoxin resistant varieties of peanut. Remotely sensed vegetative indices were significantly different for drought tolerant and intolerant varieties of peanut throughout the growing season in both years. Moreover, vegetative indices were also highly correlated with yield ($r = -0.50 - -0.75$, alpha = 0.05) and aflatoxin contamination ($r = 0.45 – 0.75$, alpha = 0.05).

Development of Peanut EST (Expressed Sequence Tag)-Based Genomic Resources and Tools

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U.S. Peanut Genome Initiative (PGI) has widely recognized the need for peanut genome tools and resources development for mitigating peanut allergens and food safety. Genomics such as Expressed Sequence Tag (EST), microarray technologies, and whole genome sequencing provides robotic tools for profiling genes. In spite of continuous decrease in DNA sequencing costs, it is improbable that many large plant genomes, such as peanut, will be sequenced in the near future. However, partially sequencing of large numbers of expressed genes (ESTs) can deliver substantial amounts of genetic information that will allow comparative and functional studies. The target of PGI is to have at least 150,000 ESTs by 2008. Notable research progress has been made recently in development of peanut ESTs. Up to today, total 44,064 cDNA clones from ten peanut cDNA libraries have been sequenced. After comparison and assembly of overlapping sequences, about 10,096 unique sequences have been identified. These sequence data will be available to the community in order to develop genomic tools and resources for deciphering the chromosomal location and biological function of genes in the peanut genome and mitigating peanut food safety issues. A proposed peanut 70-mer oligo microarray consisting over 10,000 gene-elements is under discussion in collaboration with TIGR (the Institute for Genomic Research). The peanut oligo array will be available by 2008 for peanut international community.

Field Based Assessment of Cotton Cultivars for Aflatoxin Contamination

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In Arizona, southern Texas and the Imperial Valley of California, aflatoxins are a concern in cottonseed used for animal feed. At present there are no assays available to compare susceptibility of cotton cultivars to aflatoxin contamination under field conditions. A field assay that gives reproducible results for aflatoxin contamination will enable us to determine if varietal characteristics such as seed coat hardness and timing of boll opening are related to aflatoxin contamination. Development of a reliable, flexible field assay is the objective of this project.

In 2005 trials, two different experiments were conducted at The University of Arizona Maricopa Agricultural Center (MAC) near Maricopa, Arizona using a variation of the misting system first developed in 2004. The first experiment was a continuation from 2004 of investigations of the timing of wetting on aflatoxin contamination. There were four treatments with eight replications each: no wetting, wetting early at first boll opening, wetting late when most bolls were open, and wetting both early and late. Plots were all planted with DP449BR. The second experiment was a variety trial in which eight replications of four varieties, Hammer (CPCSD high yielding, thin-coated seed variety), DP455BR, ST5599BR and PHY470WR were wetted both early and late. Humidity was increased over non-treated controls, but not to an acceptable level. Aflatoxin contamination of seed was very low or non-detectable in all treatments.

In 2006 trials, field plots were established at MAC and at the Campus Agricultural Center (CAC) in Tucson, Arizona. A new technique was introduced using commercially available portable greenhouses to increase both temperature and humidity in the cotton canopy. These covers are $6ft^2$ and about 6 ft high, and they covered 6ft of each of 2 rows. At MAC, two experiments were conducted: (1) comparison of environmental parameters created by misting and greenhouse covers in cultivar DP449BR, and (2) a variety trial using greenhouse covers to compare aflatoxin contamination in cultivars Hammer, DP449BR, ST4892 and ST 5599. At CAC a time trial was conducted for 2, 4, 6, and 8 and 10 days on two cultivars, Hammer and DP449BR, to determine the length of time the covers need to stay in place. Relative humidity and temperature of treated (misted, covered) and untreated plots were monitored using Hobo data loggers. All plots were inoculated and there were four replications of each treatment.

Results in the MAC variety experiment indicate that in the lower canopy, cotton under the covers was exposed to sustained relative humidity of 90 to 100% and temperatures of 27 to 43^{o}C; cotton outside the covers was exposed to radical daily variations in relative humidity of 60 to 100% and temperatures of 21 to 32^{o}C. Preliminary results show that cotton lint and fuzzy seed from cotton covered for one week in the variety trial (30% boll opening) have BGFP, with more in cotton from partially open bolls than from fully open bolls in those observed; while cotton outside the covers has no BGFP. Assays are underway to determine if the cotton under covers is contaminated with aflatoxins and if there are significant differences in cultivars of cotton. Early results indicate that the use of the covers will be a reliable and efficient way to test for aflatoxin contamination in cotton cultivars.

The Development and Characterization of Navel Orangeworm and *Aspergillus flavus* Resistance in Almond

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In almond seedcoats, condensed tannins have been found to predominate and appear to represent an alternative class of toxin-suppressing antioxidant. Independent MALDI-TOF MS analysis has demonstrated considerable variability in seedcoats among California almond varieties for flavanol glycosides, a type of condensed tannin, which our analysis has shown to be negatively correlated with aflatoxin production levels following controlled inoculations. A much wider variability in condensed tannin content has now been identified in resistance breeding lines derived from interspecies hybridizations. Condensed tannins have also been implicated in the seedcoat ink-staining disorder observed on many almond varieties infected by nontoxigenic *Aspergillus spp*., suggesting that any future AF36-based bio-control may depend upon the identification/development of ink-stain resistant almond varieties. Key biochemical pathways during the crucial seed-coat maturation phase are being characterized and transformation/regeneration strategies are being developed to allow gene knock-out testing of putative resistance factors. Transformation and regeneration protocols based on the walnut repetitively embryogenic regeneration system have recently achieved the recovery of viable somatic embryos from almond breeding selections.

In field trials, virtually all infections by *Aspergillus flavus* were found to be associated with navel orangeworm (*Amyelois transitella*) damage to the seedcoat and seed. Navel orangeworm was found to enter only through pre-existing gaps in mesocarp/endocarp tissue. Control of insect damage to the critical seedcoat barrier has been achieved through high shell-seal integrity. A unique highly-sealed yet high kernel crack-out shell trait has been transferred to resistance lines from the related species, *Prunus webbii*. Large-scale field-testing of advanced resistance selections from 2000 through 2006 in northern, central and southern California growing regions has shown effective aflatoxin control in navel orangeworm resistant lines. Breeding selection *UCD36-52* has shown exceptional promise as an insect resistant and premium kernel quality selection and will be released in 2007 as the variety *Sweetheart*. Resistance appears to result from a combination of good shell seal with suppressed larval development when feeding on hull and seedcoat tissue. The suppression of larval development is highest when feeding on the mature, desiccated hull. While this trait confers very high levels of postharvest resistance when kernels are stored in-hull, its contribution to preharvest insect control has not yet been tested.

The Development of Aflatoxin-Resistant Maize Germplasm and the Identification of Potential Markers

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For the last 6 years, the Southern Regional Research Center (SRRC) and the International Institute of Tropical Agriculture (IITA) – Ibadan, Nigeria, have been collaborating on the development of aflatoxin-resistant maize inbred lines and on the identification of breeding markers in those lines. Two breeding populations were originally developed from crosses between U.S. aflatoxin-resistant lines and ear rot resistant lines selected in Central and West Africa that accumulated low aflatoxin in the kernel screening assay (KSA). One population contained 50% U.S/ 50% African germplasm, while the other was a backcross population containing 75% U.S. germplasm. To the S_4 generation, lines were selected on ear rot and foliar resistance and agronomic qualities. S_4 lines and after have been tested by the KSA for aflatoxin accumulation and the best performers, field tested in Nigeria for resistance and agronomic performance. Presently, over 50 S_7 lines are being evaluated in confirmation field trials. Also, a number of hybrids generated from inbreds that supported low toxin have produced excellent yields and demonstrated good combining ability. Comparative proteomics is being used to identify potential markers in IITA-SRRC lines. This approach is based on the belief that tracking protein expression under relevant conditions may provide a quicker way of identifying genes involved in resistance. Also, it is believed that a subtractive approach, based on side-by-side comparisons of aflatoxin-resistant with -susceptible lines, is an efficient way of isolating proteins potentially involved in resistance. The recent availability of near-isogenic lines has enhanced the search for resistance genes, rendering unnecessary the development of composite profiles for homogenizing nonresistance-related differences. Most proteins associated with resistance (RAPs), thus far identified, have comprised the storage, stress-related, and antifungal categories; a few are putatively involved in metabolism or regulation. Protein investigation has, thus far, focused on those that are constitutively expressed. The appearance of several stress-related proteins among RAPs is interesting given the enhancing effect of drought on aflatoxin accumulation in maize. Several RAP genes have been cloned and characterized; PR 10, glyoxalase (GLXI), and trypsin inhibitor (TI) have been inserted in RNAi gene silencing vectors which were used to transform maize plants to determine gene function. A 2 year field study involving 3 resistant and 3 susceptible maize genotypes and *Aspergillus flavus* infection was performed; nine different time points were used for sampling. The expression of TI, GLXI, PR 10, peroxiredoxin (PER1) and a protein kinase was studied using RT-PCR and data is being summarized. These discoveries have enhanced our understanding of the complexities involved in maize kernel response to fungal infection and aflatoxin formation. It is expected that inbred lines produced through the IITA-SRRC collaboration may be released by 2008. Released germplasm will go to Central and West African national programs, and to U.S. breeding programs. Further investigation is needed to understand the actions of plant stress proteins in the host plant environment following fungal infection. Also, it is important to investigate the corresponding actions of invading fungi (possible self-protection mechanisms) in a "foreign" environment. Comparative proteomics will be used next to identify kernel proteins induced as a result of fungal infection.

Microarray as a Tool for Understanding Mechanisms of Pre-harvest Aflatoxin Contamination in Corn

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Maize oligonuleotide microarray was used to analyze the temporal patterns of gene expression in late developmental maize kernels after 25 days after pollination (DAP). There are a total of 57,452 70-mer oligonucleotides on the array. We analyzed gene expression profiles in the developing kernels of Tex6, reported with drought tolerance and reduced aflatoxin contamination, at 25, 30, 35, 40 and 45 DAP. There were a total of 8621 positive array spots with unique IDs and 4247 cross-talking genes identified in all sampling stages. There were averaged at 6218 array spots expressed in each sampling stage. Expression patterns of key genes in several metabolic pathways, including starch, lipid and storage proteins, were analyzed. Among the storage proteins, the expression patterns of defense related genes (2676 unique IDs) were identified as up-regulated, down-regulated, or no-change based on hierarchical analysis. Some defense related genes were expressed highly throughout the late kernel development. Twenty genes with different expression trends from microarray were selected for validation using quantitative real-time PCR. The real-time PCR reproduced the expression patterns of up- and down-regulated genes, but did not completely reproduce the genes without change as in microarray study. Gene ontology (GO) annotation provided a global overview of the molecular biological function of these genes differentially expressed during maize kernel development. This study was able to investigate the gene categories at various stages of kernel development, therefore providing further insights into the molecular importance of the gene expression associated with the reduction of aflatoxin contamination in Tex6.

The gene expression profiles of Tex6 kernel under drought stress from 25 DAP to 45 DAP were compared with expression profiles in normal condition using oligo microarray. The result indicated that kernels had a significant response of gene expression at 35 DAP to drought stress. Key genes in JA, ABA and PAL pathways were monitored, and the key genes in all three pathways were up-regulated at 35 DAP. More than 100 genes related with defense and stress tolerance from microarray analysis were selected for real-time PCR. These gene expressions were compared between tex6 and B73 using real-time PCR. The results indicated that the differences of gene expression in responding to drought stress were clearly documented in Tex6 and B73 from both aspects of genetic expression and response expression.

Several inbred lines, difference in drought tolerance or resistance to *A. flavus,* A638, Lo964, Mp313E, Tex6, and susceptible lines B73, Mo17, Lo1016 were chosen to perform clustering analysis based on the relative expression quantitation of 93 gene probes of adversity tolerance. The result indicated the resistant lines A638, Mp313E, Tex6 could be clustered together. Although 12 universal probes were screened from the 96 probes, the primary test suggested the usefulness of these probes and more work needs to be done in order to have practical application.

Diverse Maize Germplasm Aflatoxin Levels Survey

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Aflatoxin infects a wide range of hosts and causes devastating economic losses to producers. Aflatoxin is produced by soil-borne fungi *Aspergillus flavus*, *Aspergillus nomius*, and *Aspergillus paraciticus*. A number of environmental factors can increase accumulation such as drought stress, high temperature, and insect damage. The FDA legislates the allowable toxin level to be 20 parts per billion (ppb) in grain for human consumption and 0.5 ppb in milk products. The optimum strategy against aflatoxin accumulation is the development of aflatoxin resistant inbreds. The objective of this experiment was to conduct a survey of aflatoxin levels in maize germplasm lines. A subset of 86 lines, from the Maize Diversity Project, was examined for aflatoxin accumulation. Two replications of each line were grown over two years in one location. To further examine this material, Mississippi and Georgia locations were added to the experiment in year 2. Inoculation of *A. flavus* NRRL 3357 by the nonwounding silk channel technique was performed 19 days after pollination in Missouri and 7 days after mid-silk in Mississippi. Ears in Georgia were inoculated 20 days after mid-silk with a paring knife dipped in inoculum. Ears were harvested at maturity, shelled, bulked, and ground for aflatoxin analysis. Aflatoxin analysis was conducted using a competitive binding ELISA or Vicam Aflatest. Lines CML277 and M162W had consistently low aflatoxin accumulation while lines IL101 and F7 had high aflatoxin accumulation. Year, line, replication, line*rep, line*year, year*rep, line*year*rep, and year*rep*subsample were all highly significant variables in the analysis of variance for the combined years at the Missouri location. This germplasm survey will set the stage for associative analysis of candidate genes for aflatoxin reduction in maize.

Improving Drought-Tolerance and Earworm Resistance to Reduce Aflatoxin in Corn

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Aflatoxin contamination of corn by *A. flavus* is a chronic problem in the southern United States where a hot and dry environment favors aflatoxin production, stresses the plants, increases aflatoxin production. Producers need new hybrids that are adapted to the southern environments and resistant to aflatoxin contamination. The corn breeding program at the Texas Agricultural Experiment Station (TAES) at Lubbock has been developing drought tolerant corn hybrids by introgressing tropical corn germplasm. We have observed that under drought conditions, drought tolerant corn hybrids produced a higher yield and had much less grain mold than susceptible hybrids. The objective of this study was to identify lines and hybrids with improved stress tolerance and low aflatoxin.

Twenty-one experimental and four commercial hybrids (Pioneer hybrid 31B13, Garst 8285, Triumph 1416, and DKC66-80) were grown under replicated trials in Lubbock, Halfway, Dumas, Corpus Christi, and Beeville in Texas and Mississippi State, MS in 2005 and 2006. The Lubbock and Halfway locations had optimum and limited irrigation treatments. The experimental hybrids are developed by the corn-breeding program of the Texas Agricultural Experiment Station (TAES) in Lubbock, TX. They were chosen for improved drought tolerance and earworm resistance. In Lubbock and Halfway, plants were inoculated one week after silking by injecting 3-ml *A. flavus* conidia (1.5×10^6 conidia/ml) into silk channels. In Corpus Christi, Beeville, and Mississippi State, corn kernels colonized by *A. flavus* were distributed between all rows when the first hybrid was at the mid-silking stage to provide the increased and uniform aerial dissemination of conidia. In all cases, the inocolum was from a high aflatoxin-producing *A. flavus* strain (NRRL3357). A limited late planting date was used in Corpus Christi, Beeville and Mississippi State to encourage severe drought stress at later stages of maturity. The tests used a randomized complete block design with nine replications at Corpus Christi and Beeville, three replications in other locations. Ears from each plot were hand-harvested. All ears were threshed and agronomic data were recorded including grain yield. Corn earworm feeding damages were measured on 10 ears. All grain from each composite replicate was initially ground in a Romer mill at the coarse grind setting. After thoroughly mixing the ground kernels, a 200 g sub-sample was ground again at the finest grind setting on the mill. Aflatoxin B1 assay was done on 50 g sub-samples of the finely ground material for each composite replication using the Vicam immunoassay/ fluorometer system. Standard ANOVA was performed to test the differences among hybrids. Natural log transformation of aflatoxin data was made before data analysis.

The aflatoxin level was low and not significantly different among hybrids in Lubbock and Halfway, but it was high and different among hybrids at Corpus Christi, Beeville, and Mississippi States. At the three locations, the average aflatoxin of six TAES hybrids (C2A554-1 x B110, S2B73BC x NC300, CUBA117:S15-1A x Tx205, WQ22W x S1W, Tx202 x CML343, and S1W x CML343) was at least 50% lower than the mean of four check hybrids. The aflatoxin S2B73BC x NC300 and S1W x CML343 was 501 and 1060 ppb, equivalent to 13% and 28% of check means (3806 ppb). The samples from 2006 trials have not analyzed for aflatoxin. The average grain yield in eight environments in 2005 and 2006 was 156 bu/a for S2B73BC x NC300 (ranging 78 to 204 bu/a) and 137 bu/a for S1W x CML343 (ranging from 40 to 191 bu/a) as compared to the 154 bu/a average yield of commercial checks (ranging from 71 to 209 bu/a). These results indicate that new hybrids such as S2B73Bc x NC300 have comparable yield yet significantly low aflatoxin in comparison to the commercial hybrids.

Breeding Corn Germplasm for Agronomic Performance and Reduced Aflatoxin Contamination

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Our program has identified and developed corn germplasm with resistant factors that can reduce the risk of aflatoxin and have a good agronomic performance in hybrids. We have used inoculation with *Aspergillus flavus* in field experiments and quantify aflatoxin with monoclonal antibody. The experimental techniques have facilitated the display of genetic differences among inbreds and hybrids. Traits selected for lowering aflatoxin contamination include kernel texture, kernel integrity, environmental adaptation, husk tightness and length among others. Following are the most relevant research highlights accomplished during year 2005-6. Yellow and white inbred lines were evaluated in hybrid combinations for aflatoxin contamination at College Station, Weslaco and Corpus Christi, TX. Recycled lines derived from combination of most consistent aflatoxin resistant sources and elite susceptible lines (Tx772, CML269, CML 78, and Tx130 derived lines) showed reduced aflatoxin accumulation at all three locations along with competitive grain yields. New additional tropical lines and hybrids selected under inoculation by CIMMYT were evaluated for aflatoxin accumulation at College Station, TX, Tifton, GA and Starkville, MS. At the College Station hybrid trial, several entries had lower aflatoxin than commercial checks. Other tropical lines developed in Texas, LAMA lines, also exhibited traits related to reducing aflatoxin accumulations. Multilocation testing for aflatoxin evaluations is critical to estimating correctly the response of corn to aflatoxin contamination. Three sets of germplasm of 25 hybrids each were evaluated under inoculation at eight locations across South-Central Texas. High genotype by environment interaction for response to aflatoxin accumulation was observed. These evaluations supported further the need of multilocation testing and collaboration among programs to validate selected material. We have also participated in the SERAT trial where research groups across southern states and Illinois have collaborated to better estimate the response to aflatoxin, agronomic performance and yield potential of selected germplasm. Recombinant inbred line (RIL) populations from resistance sources (e.g., CML176 and CML161) have been used to map potential genomic regions or QTLs associated with response to aflatoxin and other secondary traits. Several QTLs with major effects were detected in these populations. With the purpose of combining resistant factors from different sources, Texas lines were crossed with lines from Illinois. Aflatoxin accumulations at College Station showed several combinations with reduced aflatoxin. Further hybridization of resistance sources from different breeding programs can reduce susceptibility to aflatoxin accumulations and increase agronomic performance.

Searching for New Resistance to Reduce Aflatoxin in Corn

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Aflatoxin contaminated grain is a major production concern for Louisiana corn growers and can be devastating throughout the Southern U.S. Although resistant lines have been identified, resistance may not be sufficient to keep aflatoxin at sufficiently low levels in years of very high stress. The primary objective of this research is to identify new genes that provide superior resistance to aflatoxin and incorporate them into commercial corn cultivars. Corn accessions from the collection at the North Central Regional Plant Introduction Station in Ames, Iowa, are being systematically screened for resistance at the Dean Lee Research Station in Alexandria Louisiana. During the first season the lines are cross-pollinated with the inbred 'B73" to produce F1 seed. In the second season, the F1 hybrids are screened in conventional 38-inch row spacing on a Norwood silt loam soil using a randomized complete block design with two replications. Each replication now includes resistant checks of B73:Mp313e and B73:Tex6. The targeted experimental unit has been ten ears from a single row. Ears were inoculated with *A. flavus* spores after anthesis using a hand-held pin bar apparatus dipped in a container containing spores in liquid suspension (about or equal to 90 million spores/ml). Ears are harvested after physiological maturity, dried, ground to a fine meal and sent to the USDA-ARS facility at Stoneville, Mississippi for aflatoxin and fumonisin analyses. The first 300 accessions were planted in 2003, followed by 500 in 2004, 300 in 2005, and 500 in 2006. Eleven corn lines have been selected from the first two sets planted in 2003 and 2004: Nigerian Composite B, Manio-PI490411, Lancaster Surecrop-PI280061, Haiti 33–PI483902, Saint Croix1–PI484036, TZI18–PI506253, Tx81, Tx807 and CML43–PI595535. These lines are now being advanced in a breeding program to stabilize resistance in an improved agronomic background. Twenty-one lines from the third set planted in 2005 had below 300 ppb aflatoxin in 2006. The lowest resistant check B73*Tex6 had 287 ng g^{-1}. The lines selected in 2006 (followed by aflatoxin concentration) were CML91-Ames27079 0.2 ng g^{-1}; Brazil 1135-AF-PI511446 83 ng g^{-1}, Brazil 1088-AF-PI511399 84 ng $g^{-1,}$ BENZ 745-Ames19933 89 ng g^{-1}, Veracruz 59-PI515533 90 ng g^{-1}, Jalisco 38-PI490890 110 ng g^{-1}, BENZ 755-Ames19941 150 ng g^{-1}, SE 028-PI583916 167 ng g^{-1}, 31116 G M37W2T/A-PI583905 181 ng g^{-1}, Veracruz 200-PI484942 190 ng g^{-1}, Chihuahua 230-PI620780 199 ng g^{-1}, Brazil 1483-PI511523 220 ng g^{-1}, Durango 84-PI620787 222 ng g^{-1}, Brazil 2797-PI498460 223 ng g^{-1}, Hidalgo 17-PI629180 225 ng g^{-1}, Veracruz 119-PI515540 238 ng g^{-1}, Sao Paulo Group 9-PI490821 260 ng g^{-1}, Chihuahua 75-PI515169 265 ng g^{-1}, Veracruz 130-PI515542 270 ng g^{-1}, Brazil 1519-PI511525 275 ng g^{-1}, Coahuila 21-PI629147 293 ng g^{-1}**.** Crosses of the twenty-one selections with B73 and Mo17 are planned to be initiated in 2007. It seems that mass screening of germplasm is providing helpful and promising results. Some lines seem to deserve more breeding effort to put resistance in better agronomic backgrounds.

Creating Commercial Corn Hybrids with Low Aflatoxin in Grain

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This project is creating high yielding, commercially acceptable, corn hybrids with high levels of resistance to Aspergillus ear rot and low levels of aflatoxin in grain. This is being accomplished by using molecular marker assisted backcrossing to move chromosome regions associated with resistance from resistant inbreds Tex6 and Mp313E into the commercially elite, but susceptible, inbred lines FR1064 and LH195RR. With FR1064 we have crossed FR1064 with Mp313E and backcrossed three times to FR1064 while selecting for the chromosome four regions from MP313E that have been associated with resistance. I now have five inbred lines at the self eight level of inbreeding that have the chromosome region four from Mp313E. I also am pyramiding the chromosome region from Mp313E with chromosome regions from Tex6 associated with resistance. These lines are much different than those with just the Mp313E chromosome four crossed into FR1064. For those lines I took a version of MP313E crossed with FR1064 and backcrossed twice to FR1064 with chromosome four from Mp313E and crossed it with a line that was developed from the cross of B73xTex6 then backcrossed to B73 and selfed that had resistance from Tex6 on chromosomes 8,10,2, and 5. Therefore, the pyramid lines have both B73 and FR1064 which contribute yield and agronomic characters and both Mp313E and Tex6 chromosome regions associated with resistance. The resulting three lines are later in maturity than resistant versions of FR1064. In years and locations where differences are significant, testcross hybrids with genes from just Mp313E or the pyramid lines have 50% or less aflatoxin than crosses with the original FR1064. The resistant lines are related to stiff stalk synthetic and are best used as female parents of hybrids because of seed quality and rapid emergence. In testcross hybrids, sometimes the pyramid lines have even lower aflatoxin than the FR1064 resistant lines but many times they do not.

In the summer of 2006, I had 15 locations in southern areas in cooperation with three different seed companies where 18 different resistant hybrids were evaluated for yield, and in many cases aflatoxin, in replicated plots. Hybrids were created by crossing three different commercial male lines with selected resistant lines. B-H genetics had plots at five locations in Texas (two locations near Ganado, and one at Garwood, Granger, Malone, and Uvale), Warner Seeds had plots at two locations in Texas (Temple and Waco), and Terrel Seed had six plots in Mississippi, Alabama, and Louisiana. This is in addition to SERAT evaluations. B-H genetics also had various resistant hybrids in strip tests in Texas. In general, the resistant hybrids compared favorably with respect to yield compared to commercially used hybrids when the same male parents were used. In many cases they had yields superior to or equal to commercial hybrids where parentage was unknown. I hope to reduce the number of resistant inbreds to a more manageable number prior to release and sales.

In Urbana, I had a 7 acre seed production field to make even greater volumes of hybrid seed for testing next year. Also, at Urbana, the resistant inbreds were self pollinated in order to produce large volumes of seed to make additional commercially usable hybrids for planting over numerous locations next summer. Producing commercial bag quantities of hybrid seed is taking considerable time especially considering I am not well equipped for such production.

I also am making good progress in crossing resistance into the widely used line LH195RR. I currently am at backcross five. Backcrossing will continue to backcross seven in order to completely recover the agronomic characteristics of LH195. I also am working on obtaining breeding rights to a triple stack version of LH195. I also am crossing the triple stack traits of RR, CRW and BT from Monsanto and traits from Dow into the resistant versions of FR1064 and the pyramid lines.

I also am creating lines with various segments of chromosome four from the resistant line Mp313E in both LH195 and FR1064 backgrounds. Those lines will be very helpful in determining how much of the chromosome four is required for resistance. I hope we will not need as large a segment of the chromosome four from Mp313E in order to have resistance. A smaller area of the chromosome would speed backcrossing. It also opens the possibility of getting a better understanding of the genes involved. I also am crossing resistance from the resistant lines Oh516 and MI82 into a inbred usually used as a male parent. We have good information on the chromosome location of resistance from Oh516. It could be very useful in improving male lines.

PANEL DISCUSSION: CROP RESISTANCE – CONVENTIONAL BREEDING
Panel Chair - Wenwei Xu

Summary of Presentation: Paul Bertels of the National Corn Growers served as moderator of the session. Among 13 presentations, seven were on corn, four on peanut, one on cotton, and one on almond. Corley Holbrook and his colleagues at USDA-ARS in GA have developed new peanut varieties with low aflatoxin by improving nematode resistance and drought tolerance. Corn breeders at Texas A&M University showed that new corn hybrids with improved drought tolerance and earworm resistance had much lower aflatoxin and comparable yield as compared to commercial check hybrids. The teams led by Robert Brown at USDA-ARS in LA, Steve Moore at Louisiana State University, and Georgia Davis at University of Missouri found promising aflatoxin-resistant sources from diverse corn germplasm. Betran and his colleagues at Texas A&M mapped QTLs linked to aflatoxin resistance with a population from $B73O_2$ x CML161. Don White at University of Illinois continued marker-assisted selection to transfer aflatoxin-resistance QTLs to elite germplasm. The team lead by Baozhu Guo at USDA-ARS in GA found new DNA markers for corn and peanut by using microarray technique. Tom Gradziel from UC-Davis reported that tannin content was related to aflatoxin accumulation in almond. Other topics included the effect of soil moisture and canopy humidity on aflatoxin accumulation in peanut and cotton.

Summary of Panel Discussion: The panel discussed various issues related to their presentations. Baozhu Guo and his colleague Meng Luo reported that they identified 12 unique EST probes in corn and 20 unique probes in peanut primarily through microarray analysis. In response to the question how the breeders can use the EST probes from microarray in the variety selection, Dr. Meng Luo answered: we try to use a diversity of tolerance genes acquired from microarray analysis to screen probes which can be used in corn germplasm assessment with drought tolerance and disease resistance. We screened 12 probes from more than 100 genes, and primary test shows the probes can distinguish resistant lines from susceptible lines. But the result is not final because they just affect genetic expression difference, and developed from limited inbred lines. We try to use more inbred lines and hybrid lines to test them. When we identify the final probes, we would like to publish the result. We can send the probe information and protocol to any lab which the probes are needed. Breeders can follow our protocol to use the probes in their lab to select their varieties from any breeding section.

Evaluation of Epidermal Conductance as a Potential Drought Tolerant Trait of Peanut

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Epidermal conductance (ge) is the loss of water vapor from leaves when stomata are closed. An experiment was conducted to compare epidermal conductance values of peanut genotypes with varied levels of field resistance to drought to assess ge as a potential drought response trait in peanut. Epidermal conductance was computed using the equation ge = (ΔFW/*t*)*(1/A)*1/(el-ea), where ΔFW is change in fresh weight of a detached leaf over time *t*, A is the area of the leaf, and (el-ea) is the absolute humidity gradient between the leaf and the ambient air inside the measuring chamber. Water loss from leaves occurred linearly after 1 hr. No differences in ge were detected among genotypes or drought response groups. Based on these preliminary results, ge does not appear to vary much across peanut genotypes with different responses to drought.

Genetic Response to Seed Colonization by *Aspergillus flavus* in Peanut

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Previous studies to evaluate peanut genotypes for *in vitro* resistance to seed colonization by *Aspergillus* flavus have not resulted in the development of cultivars with resistance to aflatoxin contamination in the field. New breeding lines showing pre-harvest field resistance to aflatoxin contamination were compared to field susceptible genotypes and genotypes showing *in vitro* resistance to colonization in an *in vitro* seed assay to determine whether seed colonization might be a resistance trait associated with the field resistant lines. Seed of each genotype were inoculated with spores of *A. flavus* and maintained at 25°C and 98% RH for 7 days. The percentage of seeds with sporulating colonies and severity was recorded. Due to contamination, data of only 10 genotypes are compared. The field resistant lines (511-CC and 522-CC) showed intermediate levels of percent incidence and severity, which were comparable to most of the susceptible lines. Based on this experiment, it does not appear that seed colonization by *A. flavus* is a significant trait affecting the field resistance to aflatoxin contamination by 511-CC or 522-CC.

Production of Defensive Stilbenoids by Selected Peanut Genotypes

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In peanut, the mechanism of resistance to fungal infection is reportedly due to the synthesis of stilbene phytoalexins, which are antibiotic, low molecular weight metabolites. The phytoalexin-associated response of different peanut genotypes to exogenous invasion in the field has not been investigated and may be useful for breeding resistant peanut cultivars. Five peanut genotypes, Georgia Green, Tifton 8, C-99R, GK-7 high oleic, and MARC I, that differ in resistance to major peanut diseases were investigated for their ability to produce phytoalexins under field conditions in Southwest Georgia in 2001 and 2002. Five known peanut phytoalexins, *trans*-resveratrol, *trans*-arachidin-1, *trans*-arachidin-2, *trans*-arachidin-3, and *trans*-3-isopentadienyl-4,3',5'-trihydroxystilbene (IPD), were quantitated. The phytoalexins were measured in peanuts of different pod maturity (yellow, orange, brown, and black) with or without insect pod damage (externally scarified or penetrated). Kernels from insect-damaged pods of C-99R and Tifton 8 genotypes had significantly higher concentrations of phytoalexins than other genotypes. The same genotypes were the most resistant to tomato spotted wilt virus and late leafspot, while MARC I, which is highly susceptible to these diseases, produced very low concentrations of phytoalexins. However, there was no significant difference in phytoalexin production by undamaged peanut pods of all tested genotypes. *Trans*-arachidin-3 and *trans*-resveratrol were the major phytoalexins produced by insect-damaged peanuts. In damaged seeds the concentrations of *trans*-IPD were significantly higher in Tifton 8 compared to other genotypes. There was an association between total phytoalexin production and published genotype resistance to major peanut diseases. Stilbene phytoalexins may be considered potential chemical markers in breeding programs for disease-resistant peanuts.

Walnuts of Cultivar Tulare Show Similar Aflatoxin Contamination Levels as Those of Two Other Cultivars

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Although aflatoxin contamination of walnuts is not as frequent as in other nuts (pistachio and almonds), it can still be a problem under some circumstances. Studies done *in vitro* by other scientists showed that after grinding the seed coats of the walnut cultivar Tulare and amending them in media, *A. flavus* produced very little aflatoxin. These researchers discovered that the release of gallic acid after grinding the seed coats was responsible for preventing *A. flavus* from producing high levels of aflatoxin. Based on these *in vitro* studies it was claimed that "a commercial walnut variety, Tulare, is remarkably resistant to being contaminated by the fungus" (*A. flavus* or *A. parasiticus*). However, the kernels of cv. Tulare were not tested as intact nuts or in the orchard. We wanted to determine whether the kernels of walnut cultivars were resistant to infection by *Aspergillus flavus* or *A. parasiticus* and whether aflatoxin contamination of Tulare was significantly reduced in comparison with two other commonly used walnut cultivars in California.

Walnuts of Tulare, Chico, and Chandler cultivars were harvested at commercial harvest time. In a factorial experiment, kernels (Tulare and Chico) and intact nuts (Tulare and Chandler) were inoculated with a drop (50 µl) or by injecting 1 ml of a 10^6 spores/ml suspension of either *A. flavus* or *A. parasiticus.* In the case of the intact nuts, the kernels were inoculated through the stem end and were either wounded (by inserting a needle into the kernel) or not wounded (also avoiding touching the kernel with the syringe needle). All inoculated and noninoculated nuts were incubated at 30°C for 2 and 4 weeks and infection and aflatoxin levels were determined.

The Tulare and Chico nut kernels without shells inoculated with either *A. flavus* or *A. parasiticus* were all (100%) infected and had similar amounts of aflatoxins. Inshell kernels of fresh Tulare or Chandler walnuts inoculated after wounding with a needle or without wounding also developed similar large amounts of aflatoxins. Wounding did not have a consistent effect in the infection rate and the amounts of aflatoxins produced. Similarly, dried nuts of Tulare and Chandler were infected after inoculation with either *A. flavus* or *A. parasiticus* and had similar amounts of aflatoxins after 2 and 4 weeks incubation at 30°C. In general, *A. parasiticus* produced greater amounts of aflatoxins than *A. flavus*.

Regardless of wounding, 100% of inoculated inshell Tulare and Chandler nuts were infected. With no inoculation, intact inshell Tulare nuts had a range of 116.8 to 927.4 ppb aflatoxins while those with naturally cracked shells had a mean of 292.8 ppb. In contrast, intact inshell Chandler showed a range of 39.9 to 85.1 ppb, while cracked nuts a mean of 101.7 ppb. Interestingly, noninoculated, nonwounded Tulare nuts after 4 weeks incubation at 30°C were colonized at higher levels with *A. niger* (100%) or *Aspergillus* Section *Flavi* (13.8%) while nonwounded Chandler had 37.8% *A. niger* and only 3.8% *Aspergillus* Section *Flavi.* All the inoculated walnuts (fresh or dried) of either the Tulare or Chandler cultivars were infected, colonized by either *A. flavus* or *A. parasiticus,* and covered with abundant sporulation of these fungi.

The results from three years of experiments indicate that the seed coat of the walnuts did not provide any protection from infection. Most importantly, the Tulare cv. that was shown to have significantly higher levels of gallic acid (GA) than the cultivars Chico and Chandler did not result in any prevention of infection by *A. flavus* or *A. parasiticus* nor of aflatoxin contamination. In conclusion, the results of this study suggest that Tulare's seed coat's higher content of gallic acid does not confer any resistance to infection by *Aspergillus flavus or* A. *parasiticus* and to aflatoxin contamination.

Multi-location Evaluation of Aflatoxin Accumulation and Agronomic Performance of Maize Hybrids in Texas

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A major obstacle in maize production across the Southern U.S. and other parts of the world is accumulation of aflatoxin, a known carcinogen in both humans and livestock produced by *Aspergillus flavus*. Aflatoxin contamination is difficult to evaluate in the field because of varying amounts of source inoculum and dependency on favorable environmental conditions. Texas is an excellent area for examining aflatoxin accumulation because abiotic stresses such as drought and high temperatures are common. Our objectives were to 1) estimate the responses of these hybrids to aflatoxin across a range of environments; 2) identify the hybrids within each group that exhibited the lowest levels of contamination; 3) analyze the relationship between agronomic performance and aflatoxin accumulation; and 4) determine how much genotype x environment interaction (GEI) affect these traits. During 2005 three groups of twenty five hybrids, 20 or 21 experimental testcrosses with inbreds LH195 and LH210 and 4 or 5 commercial yellow, white, and Quality Protein Maize hybrids, were evaluated under inoculation with *A. flavus* in several locations representing the maize producing regions of Texas. Eight locations were used for the yellow experiment, and all locations but College Station were used for the white and QPM experiments. For the yellow experiment, aflatoxin means were as follows: Weslaco, 86 ng g^{-1}; Castroville, 131 ng g^{-1}; Bardwell, 209 ng g^{-1}; Prosper, 274 ng g^{-1}; Wharton, 315 ng g^{-1}; College Station and Granger, 407 ng g^{-1}; Corpus Christi, 1094 ng g^{-1}. For the white experiment, aflatoxin means were: Weslaco, 103 ng g^{-1}; Castroville, 170 ng g^{-1}; Granger, 348 ng g^{-1}; Bardwell, 513 ng g^{-1}; Wharton, 698 ng g^{-1}; Prosper, 945 ng g^{-1}; Corpus Christi, 1338 ng g^{-1}. For the QPM experiment, aflatoxin means were: Weslaco, 155 ng g^{-1}; Castroville, 248 ng g^{-1}; Bardwell, 364 ng g^{-1}; Granger, 407 ng g^{-1}; Prosper, 445 ng g^{-1}; Wharton, 809 ng g^{-1}; Corpus Christi, 996 ng g^{-1}. Overall, we found that the commercial hybrids had higher grain yields than the experimental hybrids. The highest yielding yellow experimental hybrid was ((B104/NC300)x(CML 415/B104))/LH210. It averaged 6.1 Mg ha^{-1} and ranked fifth for yield overall. The highest yielding white experimental hybrid was TxX150 (CML269/TX114)/LH210. It averaged 6.5 Mg ha^{-1} and ranked first for yield. The highest yielding QPM experimental hybrid was TxX817 (CML184/CML176)/LH210. It averaged 6.2 Mg ha^{-1} and ranked fifth for yield overall. However, experimental hybrids, especially testcrosses with LH195, were less susceptible to aflatoxin accumulation than commercial hybrids. The yellow experimental hybrid with the lowest mean aflatoxin accumulation was LAMA2002-42-B-B/LH195, at 154 ng g^{-1} across all environments. The white experimental hybrid with the lowest mean accumulation was TxX819 (Tx811 x CML176)/LH210, at 174 ng g^{-1} across environments. The QPM experimental hybrid with the lowest mean accumulation was CML176/LH195 at 93 ng g^{-1} across environments. Aflatoxin concentration was positively correlated with test weights and negatively correlated with grain yield and 1000 kernel weight. We observed a significant GEI for both aflatoxin concentration and grain yield. Therefore, multiple locations are necessary for estimating agronomic performance and response to aflatoxin of maize hybrids.

Southern East Regional Aflatoxin Test (SERAT)

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Aflatoxin contamination is a chronic problem of corn producers in the southeast United States. For several years, research groups from Georgia, Mississippi, Louisiana, Texas and Illinois have been screening corn germplasm under specific local environments. Several sources of resistance to aflatoxin accumulation have been identified and released, however; currently none is used directly in commercial hybrids. Aflatoxin accumulation is severely affected by the environment. Genotype by environment interaction for aflatoxin, different relative genetic response across environments, is normally significant. A testing network of environments across major growing areas affected by aflatoxin has been established to identify the most consistent stable sources of resistance. SERAT is a multilocation and multistate regional test of the most promising germplasm from each breeding program. Participants provide seed of a few hybrids and a testing location. Evaluations are conducted under inoculation with *A. flavus* following the protocols commonly used by each research group. In addition to aflatoxin, grain yield and other agronomic traits such as maturity, lodging, plant height, grain moisture, test weights, etc. are recorded. In 2006, SERAT tests were conducted at 9 locations: Alexandria, LA; Tifton, GA; Starkville, MS; Urbana, IL; Halfway, TX; College Station, TX; Ganado, TX; Sasser, GA and Bainbridge, GA. Tests at Halfway, TX; Bainbridge, GA and Sasser, GA were not inoculated. Average aflatoxin was 778 ng g^{-1} at Alexandria, 605 ng g^{-1} at Starkville, 226 ng g^{-1} at College Station, and 326 ng g^{-1} at Ganado. Grain yield was variable across environments. Average grain yield was 124 bu a^{-1} at College Station; 167 bu a^{-1} at Tifton; 145 bu a^{-1} for Sasser; 56 bu a^{-1} at Ganado; 167 bu a^{-1} at Bainbridge and 132 bu a^{-1} at Halfway. Principle component analysis of aflatoxin concentrations and grain yield suggest differing responses of the hybrids to the different locations. Most of the experimental hybrids accumulated lower aflatoxin than commercial checks and several experimental hybrids had similar grain yields to commercial checks. SERAT has fostered collaboration between researchers working to lower susceptibility aflatoxin accumulations in corn. Germplasm from each program has been identified which has lowered susceptibility to aflatoxin accumulation and comparable grain yields to commercial checks. With this collaborative regional test, we expect to identify germplasm with the most stable responses for lowered aflatoxin accumulations, along with other beneficial agronomic traits, and increase collaboration among researcher groups from different states.

Response of Exotic (CIMMYT and LAMA) Germplasm to Aflatoxin in Southern USA

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Pre-harvest aflatoxin contamination is a chronic problem and major limiting factor for maize production in the Southern USA. Current commercial maize hybrids lack adequate resistance to *Aspergillus flavus* infection and subsequent aflatoxin accumulation. Tropical maize germplasm may be a source of needed resistance. Our objective is to evaluate exotic germplasm from two sources, CIMMYT and LAMA, for response to *A. flavus* across environments in Texas and the Southern USA. Two experiments were conducted, one with exotic CIMMYT material grown in three locations across the Southern USA including College Station, TX, Tifton, GA, and Mississippi State, MS, and the other with exotic LAMA material grown in three locations across Southern Texas including College Station, Corpus Christi, and Weslaco. An alpha-lattice field experimental design was used for replicated trials in all locations. Trials in College Station and Weslaco were inoculated using the non-injuring silk channel inoculation method and were grown without induced stress. The trial at Corpus Christi was inoculated by broadcast colonized kernels and was planted a month late to induce heat and drought stress. All locations were inoculated with *A. flavus* isolate NRRL 3357. Aflatoxin was quantified using Vicam Aflatest columns. Data analysis was completed using SAS. Significant differences were detected for aflatoxin in both the white and the yellow CIMMYT hybrid tests in College Station. In general, the CIMMYT hybrids accumulated less aflatoxin than the commercial checks. White CIMMYT hybrids accumulated an average of 27.03 ng g^{-1} aflatoxin compared to 38.21 ng g^{-1} aflatoxin accumulated by the commercial hybrids. Yellow CIMMYT hybrids accumulated an average of 47.71 ng g^{-1} aflatoxin while the checks accumulated 92.59 ng g^{-1} aflatoxin. Sixteen white hybrids and 6 yellow hybrids accumulated less than 10 ng g^{-1} aflatoxin. This germplasm source has potential to provide effective genetic factors for resistance to aflatoxin. Significant differences were detected for aflatoxin accumulation in each environment in the LAMA experiment. In general, the LAMA lines accumulated less aflatoxin (mean 131.6 ng g^{-1}) than the commercial checks (mean 298.9 ng g^{-1}) at single locations and across locations. The hybrids LAMA-20-3-B/LH195, LAMA-7-2-B/LH195, and LAMA-20-5-B/LH195 accumulated the least amount of aflatoxin across locations. Introgression of exotic germplasm can contribute factors for resistance to aflatoxin.

QTL Mapping for Response to Aflatoxin

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Aflatoxin is a potent toxin and carcinogen produced by the fungus *Aspergillus flavus* that can cause aflatoxin and liver cancer in animals and humans. The objective of this research was to use a mapping population comprising 146 recombinant inbred lines from a cross between CML161 and B73*o2* to identify QTL associated with aflatoxin resistance. This population was evaluated in replicated trials in two Texas locations, Weslaco and College Station under inoculation. Secondary traits such as maturity, endosperm texture, percentage of rotten ears and grain yield were also measured. Heritability for each trait and genotypic and phenotypic correlations of each pf this trait to aflatoxin was estimated from variance components. Narrow sense heritability for aflatoxin concentration was 0.53 and 0.44 for Wesalco and College Station, respectively. The higher heritability estimates in Weslaco could be due to more favorable environmental conditions inductive for aflatoxin production when compared to College Station. Aflatoxin was significantly positively correlated to percentage of rotten ears and negatively correlated to grain yield. The population was genotyped using simple sequence repeat (SSR) markers. Composite interval mapping method was applied for the QTL analysis using 1000 permutations to determine QTL significance. The mapping population showed significant differences and broad range for aflatoxin, with the offspring showing transgressive segregation. Results of analysis of variance indicated that genotypic effects were highly significant in both locations. A total number of three QTL's were detected in chromosome 1 for aflatoxin concentration (ng g^{-1}) in Weslaco and across locations explaining 41% and 40% of the total phenotypic variation, respectively. For the logarithmic transformation of aflatoxin concentration, seven QTLs explaining 38%, 55% and 38% of the total phenotypic variation were identified in Weslaco, College station and across locations, respectively. Epistatic interactions were detected and explained significant proportion of the phenotypic variance for aflatoxin. The alleles for reducing aflatoxin contamination came from the both parents. The QTLs reported in this study are promising and need to be validated in other environments and genetic background for further use in marker assisted selection.

Jasmonic Acid Biosynthesis Pathway Confers Resistance to Maize Embryos against *Aspergillus flavus*

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Much evidence suggests that lipid-derived secondary metabolites (oxylipins) produced by plants are mediating plant host-pathogen interactions. It has been hypothesized that several host plant and fungal genes are involved in determining the degree of aflatoxin contamination of maize kernels. For example, it has been reported that corn oxylipins could have a sporogenic effect on *Aspergillus* species. Jasmonic acid (JA), is a naturally occurring oxylipin in higher plants and it has been proposed as potent inhibitor of aflatoxin biosynthesis. JA is a cyclopentanone derivative synthesized from linolenic acid via the octadecanoic branch of the lipoxygenase (LOX) pathway. Lipoxygenases initiate the octadecanoic branch in response to fungal attack. JA synthesis begins with the conversion of α-linolenic acid to HPOTE (13-hydroperoxy octadecatrienoic acid) by a 13-LOX. HPOTE is further metabolized by Allene oxide Synthase (AOS) and cyclized by Allene oxide cyclase (AOC) to produce 12-oxo-pytodienoic acid (12-OPDA), the natural precursor for JA biosynthesis. OPDA is reduced by Oxophytodienoic acid reductases (OPR) enzymes. However, it has been shown that OPDA by itself is an oxylipin involved in plant defense response in the absence of JA. Another branch of the LOX pathway involved in the inhibition of the sporogenesis and aflatoxin synthesis is the HPL (hydroperoxide lyase) branch. HPL branch produces volatile aldehydes and alcohols that also inhibit fungal growth and aflatoxin synthesis. In this work, we investigated the gene expression of two branches of the LOX pathway, Hidroxyperoxide Lyase (aldehydes synthesis pathway) and the Octadecanoic branch (jasmonic acid synthesis pathway) in relation to corn embryos resistance to *A. flavus* infection in the field. For this purpose, specific primers were designed to detect the expression pattern of several LOX pathway genes such as two 9-lipoxygenases genes (*ZmLOX1* and *ZmLOX3*), one Allene oxide synthase (AOS), two Oxophytodioenoic acid reductases (*OPR7* and *OPR-2*), and one Hydroperoxide Lyase (HPL), as well. We compared the gene expression patterns between the embryos of one resistant (Mp313E) and one susceptible (Sc212M) maize genotypes to aflatoxin contamination. We sampled the embryos 6 days after ears inoculation with *A. flavus* spores. The ears were inoculated 14 days after pollination. (*ZmLOX1, ZmLOX3*, *OPR7*, *OPR2,* and *HPL* transcript levels were up-regulated in corn embryos during *A. flavus* infection in both genotypes, although higher levels of transcripts were already present in Mp313E before fungal inoculation. Surprinsingly, we found out that the *AOS* gene expression was repressed in Sc212M inoculated embryos compared to control uninoculated embryos. It might be possible that some metabolites produced during *A. flavus* infection may inhibit AOS expression in the embryos of the susceptible genotype, contributing to the susceptibility of Sc212M corn inbred line. Furthermore, we also compared the JA levels produced in embryos of both genotypes. Although the differences in JA concentracion were not significantly different between inoculated and control embryos, JA levels increased in the embryos of Mp313E genotype while they decreased in the embryos of Sc212M the susceptible genotype, after the inoculation with *A. flavus*. Taken together this results suggest that the expression of the LOX pathway is required to activate JA and another different oxylipins produced by the action of ZmLOX3 and HPL pathway to confer resistance to the embryos of Mp313E against *Apergillus flavus* to inhibit either the fungal growth and/or the aflatoxin synthesis. We conclude that LOX pathway has to be considered in maize breeding programs to enhance corn resistance against *A. flavus*.

Is Rachis Lignification a Deterrent to *Aspergillus flavus* Movement through the Developing Maize Ear?

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In previous research, the proteomes of the immature rachis of *Aspergillus flavus* resistant and susceptible maize inbreds were compared using differential-in-gel electrophoresis (DIGE). One of the proteins that was 66-fold more abundant in the resistant inbred MP313E than in the susceptible inbred SC212M was caffeoyl-CoA-O-methyl transferase (CCoAOMT). CCoAOMT is one of the key enzymes in the lignin biosynthesis pathway. Other studies with GUS and GFP-labeled *A. flavus* strains indicated that fungal growth in the cob was restricted in the resistant lines. Taken together these results suggested that the lignin content of the rachis may be a factor in retarding fungal growth or movement. In this study, cross-sectioned slices of MP313E and SC212M collected 21 days after silking were stained with phloroglucinol to observe the differences in lignin patterns. Preliminary tests show that MP313E appears to have more lignin and a different lignification pattern in the rachis than SC212M.

Aflatoxin Management in Arizona and Texas

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Abstract not submitted.

Persistence and Sporulation of an Aflatoxin Biocontrol Product Are Influenced by Timing of Application

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Aflatoxins are toxic, carcinogenic metabolites produced by certain fungi in the genus *Aspergillus*. Atoxigenic *A. flavus* strains are used as biocontrol to reduce aflatoxin contamination. The current recommendations for using the atoxigenic strain technology *A. flavus* AF36 were developed in Western Arizona. Currently, treatments have expanded to central Arizona where cropping time, cost of water, and field topologies are different and no recommendation data is available. Applications of the biocontrol are considered successful if 80% or more of the *A. flavus* in the treated field is displaced by a single application of 10 lb/acre. However, a percentage of cotton fields applied in central Arizona have less than 80% displacement of toxigenic strains. For the applications to be effective, the atoxigenic strains must reproduce during crop development when environmental conditions are conducive to aflatoxin contamination. This requires applying the biocontrol agent at the appropriate time. However, optimal timing of atoxigenic strain applications in Central Arizona has not been determined. The objectives of the present study were to determine the optimal application time for the atoxigenic *A. flavus* AF36 biocontrol in cotton fields in order to avoid loss of both product and product viability and to allow optimal sporulation of the product.

Commercial *A. flavus* AF36 biocontrol product was applied biweekly from crop emergence in five cotton fields in each of two locations (Casa Grande, Gila Bend) in 2004 and three locations (Casa Grande, Gila Bend, Cosmos) in 2005. For the sporulation and viability tests, product was applied at a high rate (~10 fold more than normal) on top of a single row. To determine spore production, 10 sporulating product seeds from each sampling time and application time per field were taken and washed individually in 0.01% Tween 80 in 1.5 ml microfuge tubes. The number of colony forming units (CFU) per gram of product was determined by dilution plate technique. This technique detected the number of spores already formed prior to sampling. For the viability test, 20 non-sporulating product-seeds from each sampling time and application time per each field were taken and incubated at 100% RH in individual cells of a multi-well plate. The number of sporulating seeds was recorded and the percentage of viable seeds was obtained by dividing the sporulating seeds by the total number of seeds incubated. For the persistence tests, the applications consisted of five plots of 1 m at the normal rate of 10 lb/acre for each field and application time. Incidence of product sporulation, quantity of spores produced, product persistence and product viability were determined weekly for four weeks after each application.

Results indicate significant differences among application dates. Applications before June when crop canopy is still open had little sporulation. Applications during June had good sporulation, but only two to three weeks after application. Optimal sporulation within one week occurred for applications following canopy closure during July and August. Product that does not sporulate within the first week of application is more susceptible to lost viability or to being taken off by animal feeding. Less than 50 % of the product applied in early season prior to July was viable after one week and over 80% of product applied in July and August was viable after one week. Product persistence was frequently low. Product loss is greater for applications made before canopy closure (late May, June) when no product is found two weeks after application, except for the very early applications at crop emergency. Very early applications persist longer, but lose viability fast. Low percentages (10-20 %) of the product applied during July and August persisted in the field for at least three weeks with high viability and sporulation. Prior to application, an environment favorable for fungal growth is required for optimal dispersal from the biocontrol formulations.

Nonaflatoxigenic *Aspergillus flavus* TX9-8 Competitively Prevents Aflatoxin Production by *A. flavus* Isolates of Large and Small Sclerotial Morphotypes

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Toxigenic *Aspergillus flavus* is the main etiological agent for aflatoxin contamination of crops. Using nonaflatoxigenic *A. flavus* isolates to competitively exclude toxigenic *A. flavus* isolates in agricultural fields has become an adopted approach to reduce aflatoxin contamination. We determined the phylogeny of toxigenic L- and S-strain sclerotial isolates and nonaflatoxigenic L-strain isolates of *A. flavus.* The genetic criterion included single nucleotide polymorphisms in the *omtA* gene and deletions in and distal to the *norB-cypA* intergenic region. Phenotypical traits such as aflatoxin production and sclerotial size also were weighted in the analysis. *A. flavus* isolates are genetically diverse and were categorized into different groups. From screening subgroups of L-strain nonaflatoxigenic *A. flavus*, we identified an isolate, TX9-8, which competed well with three *A. flavus* isolates producing low, intermediate, and high levels of aflatoxins, respectively. TX9-8, like *A. flavus* AF36, has a defective polyketide synthase gene (*pksA*), which is necessary for aflatoxin production. Coinoculating TX9-8 at the same time with L-strain *A. flavus* isolates at a ratio of 1:1 or 1:10 (TX9-8:toxigenic) prevented aflatoxin accumulation. The intervention of TX9-8 on S-strain *A. flavus* isolates varied and depended on isolate and ratio of coinoculation. At a ratio of 1:1 TX9-8 prevented aflatoxin accumulation by *A. flavus* CA28 and decreased aflatoxin accumulation 10-fold by *A. flavus* CA43. No decrease in aflatoxin accumulation was apparent when TX9-8 was inoculated 24 h after toxigenic L- or S-strain *A. flavus* isolates started growing. The competitive effect likely is due to TX9-8 outgrowing toxigenic *A. flavus* isolates.

Inhibition of Direct and Indirect Stress-Induced Aflatoxin Biosynthesis

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Previous research has shown that aflatoxin production *in vitro* is markedly suppressed by the presence of natural antioxidants occurring in tree nuts, including hydrolysable tannins, flavonoids and phenolic acids. This lead to the hypothesis that aflatoxin biosynthesis is stimulated by oxidative stress on the fungus and that compounds capable of relieving oxidative stress should therefore suppress or eliminate aflatoxin biosynthesis.

Aspergillus flavus strain 4212, a pistachio isolate, showed greatly enhanced aflatoxin production relative to control when incubated separately under oxidative stress induced by addition of *tert*-butyl hydroperoxide to the media. Furthermore, stressed samples maintained high levels of aflatoxin in contrast to the control samples, which showed gradual loss of aflatoxin over time. However, when control and stressed samples were incubated together the levels and patterns of aflatoxin production were virtually identical at the enhanced level, indicating that intercommunication must be bringing the aflatoxin biosynthesis of the control into synchronicity with the stressed sample. The addition of tannic acid, a commercial hydrolysable tannin containing only gallic acid moieties, to stressed samples reduced aflatoxin levels well below those observed without induced oxidative stress. The addition of tannic acid also eliminated the ability of the stressed samples to influence aflatoxin production in control samples when both were incubated together.

There was no effect of oxidative stress on fungal weights during the growth phase of the fungus up to 5 days. Thereafter, fungal weights in stressed samples were lower compared with the control, probably due to increased stress-induced autolysis versus growth. When grown in the presence of oxidatively stressed cultures, the fungal weights of the control cultures were similar to those with direct exposure to oxidative stress, having lower fungal weights from days 6 to 9 compared with the controls grown in isolation. This is an additional indicator that the control samples were exhibiting conditions of stress from proximity to cultures exposed to direct oxidative stress.

Drought stress in plants produces reactive oxygen species and therefore may exacerbate aflatoxin contamination by:

- Stimulation and retention of aflatoxins when *A. flavus* is grown on drought stressed plant material.
- Stimulation and retention of aflatoxin levels from *A. flavus* growing on healthy plant material, but within the environment of *A. flavus* on drought stressed plant material.

Antioxidant natural products can mitigate the aflatoxin intensifying effects of drought stress by:

- Alleviating oxidative stress in *A. flavus* growing on drought stressed plant material, resulting in significant inhibition of aflatoxin production.
- Eliminating the ability of *A. flavus* growing on stressed plant material to transfer the symptoms of stress to *A. flavus* growing on healthy plant material.

Maize Kernel Inhibitors of Aflatoxin Production in *Aspergillus flavus*

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The goal of our research project is to identify maize kernel compounds that perturb aflatoxin biosynthesis and development in *Aspergillus flavus*. Previously we have reported the partial purification of two separate inhibitory activities from mature Tex6 kernels, which we have termed ABI-1 and ABI-2 (Aflatoxin Biosynthesis Inhibitor).

ABI-1 is sensitive to boiling and its activity is growth inhibitory. Trypsin digestion and mass spectrometry analysis of the two major protein bands present in partially purified ABI-1 fractions identifies them as Chitinase A (Uniprot ID Q6JBL4) and Zeamatin-like Protein (Uniprot ID Q2XXJ3). Which of these two proteins is primarily responsible for the antifungal activity remains unresolved and they may act synergistically as has been shown for other PR proteins. We are currently working to assess the individual and combinatorial effects of these two proteins on *A. flavus* growth and aflatoxin biosynthesis.

ABI-2 is resistant to boiling and has characteristics of a small molecule. It acts as an inhibitor of aflatoxin biosynthesis with little effect on fungal growth. We have shown that ABI-2 activity is localized to the kernel embryo. ABI-2 activity is reduced in embryo extracts of *low phytic acid 1* (*lpa1*) mutants compared to wild-type, suggesting that ABI-2 may be related to the inositol polyphosphate class of compounds. Comparisons of the metabolic profile of *lpa1* and wild-type embryo extracts by LC-MS showed large reductions the amount of inositol monophosphate (IP) and inositol bisphosphate (IP_2) in the *lpa1* background. We then examined a partially purified fraction from Tex6 that contained ABI-2 activity. This fraction contained IP_2 but no IP. We are conducting additional experiments to determine if IP_2 has ABI-2 activity, and if so, if a specific IP_2 isomer(s) is active.

PANEL DISCUSSION: USE OF NATURAL PRODUCTS FOR PREVENTION OF FUNGAL INVASION AND/OR AFLATOXIN BIOSYNTHESIS IN CROPS
Panel Chair – Peter Cotty

The session consisted of threes talks on biological control of aflatoxin-producers and two talks on natural plant products that interfere with contamination. The biocontrol talks were by Chang, Jaime-Garcia, and Cotty on the use of isolates of *Aspergillus flavus* that do not produce aflatoxins for the prevention of aflatoxin contamination of crops. Molyneux covered natural plant products with anti-oxidative properties originally investigated as potential aflatoxin resistance factors in tree nuts and Payne discussed characteristics of two inhibitors of aflatoxin biosynthesis from maize.

The discussion initially addressed Molyneux's observations that communication might be occurring between fungal cultures in different culture plates. Thus aflatoxin biosynthesis is influenced by fungal behavior in adjacent plates. Arthur Weissinger asked about the experimental set up and if the plates were sealed. Molyneux indicated that the plates were just covered with a standard Petri dish lid and that sealing the plates (e.g. with Parafilm) drastically and erratically alters aflatoxin production. Ken Damann asked if they had considered doing the experiments in a single plate with membrane separation. Molyneux responded that his group was not aware of the existence of such plates. (Damann described his experiments with these plates in a later session). Arthur Weissinger asked if plates with *tert*-butyl hydroperoxide alone were tested (i.e. no *Aspergillus flavus*) for their effects on stressed and unstressed fungal cultures? Molyneux replied that they had not but that it was an excellent suggestion and that they would do that experiment as a control. Jeff Cary asked if the headspace volatiles had been trapped and *A. flavus* grown in that atmosphere? Molyneux indicated that his group has tried to trap volatiles by solid-phase extraction and detected only trace amounts of limonene, which probably comes from the pistachio media, not from the fungus. He indicated that the suggestion was a good one but that he needed to consider how the experiment could be done in a controlled manner. Molyneux was also asked how the plates were arranged in his experiment and he indicated that the plates were arranged in a single layer with "systematic randomization". Gary Payne commented that his lab has found that stacking plates produces anomalies in aflatoxin production and his lab avoids this.

Jane Robens asked Molyneux and Cotty how *A. flavus* strains that do not produce aflatoxin handle oxidative stress? Molyneux replied that this is not known but it is probably a completely different mechanism. His lab has done some preliminary experiments with a different strain of *A. flavus* from that he reported on and it showed the same response to oxidative stress. His group plans to test more strains, including a non-aflatoxigenic strain, but the experiments are logistically difficult and time-consuming. Cotty replied that clearly the atoxigenic strains used to competitively exclude aflatoxin producers are very successful at overcoming oxidative stress during crop colonization and infection and this success occurs without accumulation of either aflatoxins or any of the intermediates in the aflatoxin biosynthetic pathway.

Themis Michailides asked Jaime-Garcia how late applications of atoxigenic strains may be made while still retaining adequate efficacy and if this coincided with the most susceptible period of crop development. Jaime-Garcia indicated that data presented in his lecture suggest that late applications have a better sporulation and better product persistence and viability. However, canopy closure provides a favorable environment for a rapid and abundant sporulation of the product. At that time there might be an important percentage of cotton bolls already open, and these might receive reduced benefit from atoxigenic strain applications. He indicated that work underway is evaluating when biocontrol applications are too late to be effective in displacing the toxigenic fungi.

Sylvia Hua asked Jaime-Garcia and Cotty how many propagules/gram of wheat seed can the product yield? Jaime-Garcia indicated that the official biopesticide label states that the product contains a minimum of 3,000 CFU/g. However, when optimal conditions are met in the field, the product yields an average of over 40 million CFU per gram.

Chang was asked the mechanism of action of the atoxigenic strain he reported on and he indicated it was similar to the other strains used for biocontrol. The atoxigenic strain competes for resources in the same niches as the aflatoxin-producers.

Mark Doster asked Cotty the status of the biopesticide registration for the atoxigenic strain *Aspergillus flavus* AF36 on corn. Cotty indicated that an application for an experimental use permit for corn would be submitted soon by the IR-4 project and that an application for pistachios had already been submitted.

Examination of Genes Newly Introduced Into Plants for Control of Corn Insects Associated with Mycotoxin Problems

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Corn ear damage by insects can greatly enhance the levels of mycotoxins. Currently, only commercial corn hybrids with the CryIA(b) Bt protein provide sufficient control to reliably reduce levels of mycotoxins when European corn borers or Southwestern corn borers are the principal insect pests. Appropriate combinations of plant-derived genes, preferably from corn, may lead to effective, and stable broad-spectrum control of corn insects that can result in consistent mycotoxin reductions and thus provide a more acceptable material for consumer use and export.

We are exploring new genes and gene combinations for corn insect control using plant-derived or readily available models for gene analogs that occur in plants. Previously, *in vitro* assays of plant-derived hexosaminidase demonstrated high activity against the fall armyworm. Mortality of fall armyworms fed tassels of Hi-II plants that expressed an *Arabidopsis* gene putatively coding for the enzyme died at a significantly greater rate than those fed tassels from GUS transformants. The mortality level was inversely correlated to levels of enzyme expression. Leaves of some Oh43 plants that expressed the *Arabidopsis* enzyme killed significant numbers of fall armyworms and corn earworms as compared with essentially no mortality for these insects when fed leaves from negative transformants. Mean fall armyworm feeding on positive transformant leaves were significantly lower than for negative transformants.

Newly emergent silks of HI-II corn plants that expressed a putative corn silk promoter regulated P_1 gene (which can enhance levels of secondary metabolites that defend against insects) caused significantly higher mortality of corn earworms compared to wild-type Hi-II plants. Maysin levels were significantly higher in silks that browned when cut, but additional factor(s) appear to have contributed to activity against insects in newly emergent nonbrowning silks. Silks approximately 3 weeks old did not cause any mortality above 5%, but weights of survivors were significantly less when compared to those of larvae fed wild-type silks.
The P35 protein that is produced by A.c. NPV insect virus significantly retarded larval growth rates and enhanced activity of other virus species tested during *in vitro* assays. Some Oh43 plants that expressed the gene caused significant mortality of corn earworms and fall armyworms compared to negative transformants in the absence of applied virus. Overall, T1 plants that expressed the gene caused significantly higher mortality of corn earworms and fall armyworms when A.g. virus (which does not contain the P35 gene) was added to leaves compared to negative transformants.

Related studies in progress include examination of a potential corn-derived selectable marker gene, determining functionally compatible combinations of resistance genes using gene introductions, array based detection of potentially useful directly active or regulatory genes producing products active against insects, and pathway genes that may produce novel secondary resistance compounds when introduced/upregulated into corn.

Root-Knot Nematodes and Aflatoxin Contamination in Peanut

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Infection of peanut by root-knot nematodes (*Meloidogyne arenaria*) can lead to an increase in aflatoxin contamination of kernels when the plants are subjected to drought stress during pod maturation. The nematode can infect both the roots and peanut pods. We recently showed that root infection in the absence of pod infection by *M. arenaria* can lead to greater aflatoxin contamination in the kernels; however, it is not known whether wounding of pods by nematodes can also play a role in aflatoxin contamination by serving as entry points for toxigenic *Aspergillus* spp. Our objectives were: 1) to determine the contribution of pod galling caused by root-knot nematodes to the increase in aflatoxin contamination, and 2) to determine whether nematode-resistant peanut genotypes reduce the risk of preharvest aflatoxin contamination in soil infested with root-knot nematodes.

A greenhouse experiment was conducted in which pods and roots were physically separated. Pod set was restricted to soil-filled pans (41 cm dia. x 10 cm depth), while the roots grew underneath the pan into a pot. The experiment had a factorial arrangement of treatments: pod zone with and without nematodes, and root zone with and without nematodes. The four treatment combinations were replicated 10 to 13 times. Grain infested with *Aspergillus flavus/A. parasiticus* was added to the soil surface (pods zone) at mid bloom. Plants were subjected to drought stress 40 days before harvest. In both 2004 and 2005, there was very little pod galling from *M. arenaria*, and there was no difference in aflatoxin concentrations between treatments with and without nematodes. In 2006, there was heavy pod galling, but we have not yet determined aflatoxin concentrations in the kernels.

A field microplot study was conducted in 2004 and 2005 to determine whether there was lower aflatoxin concentrations in nematode-resistant than in susceptible peanut when exposed to *M. arenaria*. In 2004, a moderately resistant peanut (C209-6-60) was used and in 2005, a highly resistant peanut (C725-25) was used. In both years GA 02C was the susceptible peanut. Half of the 24 plots were inoculated with nematodes at two different times (at plant and after pegging) and the other half were not inoculated with nematodes. There were six replicates of each treatment combination. All plots were inoculated with *A. flavus*/*A. parasiticus*. Drought was induced 5 to 6 weeks before digging. In 2004, root-galling from *M. arenaria* was so severe that only 3 of 240 susceptible peanut plants survived to crop maturity and no peanuts were present on the dead vines. The gall index (0 – 10 scale) on the moderately resistant peanut was 6.7. However, despite this moderately high galling, there was no difference in aflatoxin concentrations between plots with and without nematode inoculum. In 2005, root galling was mild with indices of 1.9 on the susceptible peanut GA 02C. Nematode inoculation had no effect on aflatoxin concentrations; however, GA 02C had lower ($P = 0.005$) concentrations of aflatoxin in sound, mature kernels than did C725-25 (77 vs 886 ppb). Further studies are need to confirm whether GA 02C has resistance to preharvest aflatoxin contamination.

Furrow Diking for Improved Water Use Concerning Preharvest Aflatoxin Contamination in Peanut and Corn

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Water is the single most limiting resource in crop production. Irrigation improves production stability, but it's efficiency can always be improved upon. In traditional rainfed regions, water capture is essential for maintaining soil moisture levels to support crop growth. Furrow diking is a tillage operation that creates a series of basins and dams in the furrow to catch and absorb water delivered by either rainfall or irrigation. Improving water capture for field crops in the Southeast would improve irrigation efficiency while reducing input costs and have the potential to affect preharvest aflatoxin contamination by relieving water stress. A series of field experiments was conducted in 2005 and 2006 near Dawson, Georgia using furrow dikes in irrigated and non-irrigated peanut, cotton, and corn. The objectives included monitoring soil moisture levels to determine if water can be saved in irrigated systems with furrow dikes compared to those in conventionally tilled systems. Irrigator Pro, a decision based computer software program, was used to for irrigation recommendations. In non-irrigated experiments, soil moisture and yield parameters were monitored to determine affect of furrow dikes. Large samples were processed to quantify the presence of aflatoxin at harvest in peanut and corn. The 2005 growing season had abundant rainfall. Peanut and cotton crops only required one irrigation. Furrow diked corn was irrigated 3 times and non-diked corn required 5 irrigations. Despite abundant rainfall, higher levels of soil moisture were maintained in plots with furrow dikes compared to conventional plots. Similar yields were attained in all crops regardless of furrow diking. No detrimental effects such as water-logging, disease incidence, or digging losses in peanut were observed with furrow dikes. Levels of aflatoxin in corn ranged from 7 to 191 ppb and none was found in peanuts in 2005. In 2006, the season was much drier requiring more frequent irrigation. Corn was irrigated 12 times, peanut was irrigated 7 times, and cotton required 10 irrigations with dikes and 11 irrigations without dikes. Non-irrigated corn yield averaged 28 bu/A and irrigated corn averaged 180 bu/A. Aflatoxin levels in corn were inverse of yields, where non-irrigated averaged 115 ppb and irrigated averaged 25 ppb. There was no aflatoxin or yield response to diking in corn. At the time of this meeting, peanut and cotton had not been harvested.

Advances in Development of Kairomone-Augmented Control Tactics for Codling Moth and *Aspergillus* in Walnuts

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The boring of moth larvae into tree nuts provides the primarily invasion pathway for *Aspergillus*. Our goal is to diminish insect-caused nut damage through the use of novel, species-specific control systems based on host-plant kairomones. A single compound was identified from pears, ethyl (2*E*, 4*Z*)-2,4-decadienoate (pear ester, PE), that is a powerful kairomone, attracting both male, female and larval codling moths (CM). Through a CRADA, patents and license between USDA/ARS and Trécé, Inc. a seven year research program has now demonstrated four novel control tactics using the kairomone compound. Trécé, Inc. is currently petitioning the EPA for registration of the CM kairomone as an adjuvant for both mating disruption and larval insecticide sprays.

Various control tactics using the PE kairomone have been experimentally investigated including: mass-trapping and "attract – kill" (AK) of male and female moths, mating disruption (MD) of male moths, and AK "bait-sprays" targeting larvae. The most effective - inexpensive insecticides for CM control, the organophosphate insecticides, will within two – three years be banned or be highly restricted due to the Food Quality Protection Act 1996. Unfortunately, current alternative control materials, both insecticides and pheromone mating disruptants, and their required application rates are much higher or prohibitive in cost. Thus, both insecticidal and pheromone MD alternative strategies must be made more effective, affordable, and acceptable for control use. Our goal and hypothesis was that the pear-ester kairomone might improve the control efficacy and diminish the amount of insecticide and pheromone disruptant required to control CM in walnut orchards. Based on our research that male CM are more attracted to combination lures of kairomone + pheromone than to pheromone-alone baited traps, then perhaps MD would be more effective using the combination of pheromone with kairomone over the current pheromone-alone tactic. Also, newly-hatched neonate CM larvae are highly attracted to the kairomone, thus low-volume bait-sprays of kairomone + insecticide might AK target larvae more effectively.

Trécé, Inc. developed a micro-encapsulated (MEC) sprayable formulation of PE kairomone (PE-MEC) that has good residual field-release activity for *ca*. a month. To test its efficacy as a larval bait-spray adjuvant, PE-MEC was tank-mixed with reduced rates of four insecticides (choropyrifos, phosmet, methoxyfenozide, and granulosis virus) and applied to single replicate walnut trees by hand-gun sprayer. PE-MEC adjuvant reduced CM and navel orangeworm damage rates from 49% to 88% below rates incurred with insecticides alone. Effects of PE adjuvant on pheromone-based MD was tested using both sprayable PE-MEC in walnut orchards and hand-applied dispensers in apple orchards. PE-MEC was tanked mixed (2 grams/acre) with reduced-rates (10 gram/acre) sprayable pheromone and applied by fan-sprayer. Hand-applied dispensers were polymeric matrix dispensers formulated as PE combined with pheromone in 'Combo-dispensers' compared to pheromone-alone dispensers. Activity was evaluated by the "shut-down" of pheromone-baited traps and harvest damage. PE adjuvant combined with the pheromone disruptant reduced fruit/nut damage rates from 30% to 90+% below the low damage rates incurred with pheromone–alone dispensers. Key contribution of the kairomone adjuvant to MD was the reduction in multiple-matings by females.

These studies show that the kairomone can attract and eliminate CM and disrupt male mating orientation, and thereby improve damage and *Aspergillus* control. With expected EPA registration of PE adjuvant products next year, efficacy of kairomone-augmented tactics will be dramatically expanded through grower use in California walnuts and pome fruit orchards throughout USA.

PANEL DISCUSSION: CROP MANAGEMENT AND HANDLING, INSECT CONTROL, AND FUNGAL RELATIONSHIPS
Panel Leader – Russell Nuti

Question to Corley Holbrook: From your data it appears that nematodes can increase aflatoxin contamination in peanut. What can a grower do to avoid nematodes?

Answer: A grower can plant the nematode resistant cultivar that we plan to release this winter. This cultivar has major gene for resistance that was introgressed from wild peanut species. This gene results in near immunity to the nematode. Our cultivar also has excellent resistance to tomato spotted wilt virus and high yield and grade. It seems to me that use of this cultivar would be good insurance for any dryland farmer who has root-knot nematode.

Question to Corley Holbrook: Since this is a high level of resistance based on a single gene, how long do you think the resistance will last once the cultivar is widely grown?

Answer: This is always a concern when using a single major gene for resistance. The nematode resistance is a hypersensitive response conditioned by a single major gene. It is certainly possible that, over time, the nematodes may overcome this resistance. Dr. Patty Timper is already looking for additional genes for resistance, in case they are needed in future cultivars.

Question to Russell Nuti: How does soil type affect furrow diking?

Answer: We have done experiments in a range of heavy and light soils. Even in sandy soils, the dikes are still present at the end of the season. In all of the soils we have tested, the dikes are able to complete their main purpose of disturbing runoff of water from irrigation or rainfall.

Question to Corley Holbrook: Are there molecular markers for the resistance gene?

Answer: RFLP markers for the resistance trait were developed by groups in Texas and North Carolina. These markers were difficult to use, and in our program they were not adequately associated with phenotype. Juilette Chu, a scientist in Peggy Ozias-Akins lab, has developed a high throughput AFLP marker system that we are beginning to use in our breeding program. A manuscript on the development of these markers is in press in Crop Science.

Question to Russell Nuti: Have we tried to use the furrow diking system without including the ripper shank?

Answer: Yes, we did a non-irrigated study in 2006 to compare the effectiveness of the furrow diking system with and without the ripper shank.

Question to Doug Light: Will a UV high voltage light compete (like an electrocution grid system) with kairomone type traps?

Answer: Other research has shown that the "black-light" electrocution grid system is effective in attracting and killing various insects including the codling moth. We found that adding the attractant pear ester did not improve the moth killing efficacy. And the problems with electrocution black-light traps are their expense, need for electrical power and the UV attracts various insect, so they are not selective or species-specific.

Question to Doug Light: What is the timing of control applications?

Answer: Timing is critical. We used kairomone lure-baited traps for monitoring of female emergence and egg-laying to time applications. This method reduced applications from 10 to 5 in Argentina.

Question to Doug Light: Will the kairomone adjuvant work in other crops?

Answer: This kairomone adjuvant is limited in crop scope, it works against just codling moths, but is effective in all three host crops, apple, pear, and walnut. This kairomone does not attract other pest species in other crops. New kairomones must be isolated and identified for other key pests, such as our group's current research efforts against the key nut pest of almonds and pistachios, the navel orangeworm.

Correlations Between Biotic Stresses and Aflatoxin Contamination in Maize

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Aflatoxin, a toxin produced by the fungus *Aspergillus flavus*, is the most potent carcinogen found in nature. Aflatoxin contamination of maize is a chronic problem in the southern US, where high temperatures, water stress, and insect damage produce conditions conducive to infection of maize by *A. flavus*. The purpose of this research was to determine the relationship between two biotic stresses, leaf feeding by the fall armyworm (FAW), *Spodoptera frugiperda*, and root feeding by the root-knot nematode (RKN), *Meloidogyne incognita*, and contamination of grain with aflatoxin. In the first experiment, twelve replications each of four hybrids (three commercial and one aflatoxin resistant) were planted, and half the replications were infested with FAW. Insect damage was evaluated at fourteen days after infestation. In the second experiment, three commercial hybrids were grown in a randomized complete-block design in a field with high population densities of RKN. A fumigant nematicide was used to create plots with minimal nematode damage to compare to non-fumigated plots with a high level of nematode damage. Early (pre-plant), mid, and late (at harvest) season nematode population levels were estimated based on soil samples. Both experiments have been conducted for two years in Tifton, GA. No correlations between FAW damage or levels of nematodes in the soil and aflatoxin contamination have been observed, though levels of nematodes were negatively correlated with yield.

Spatial Correlation between Aflatoxin Level and Ear-Feeding Insect Damage in Pre-Harvest Corn

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The correlation between insect damage and aflatoxin contamination has been examined previously; however, the spatial correlation between ear-feeding insect damage and aflatoxin accumulation in pre-harvest corn has not been well understood. In 2005, spatial correlation between ear-feeding insect damage and aflatoxin levels was assessed in a pre-harvest corn field (1/6 hectare) by taking samples according to an 8x8 m grid of the corn field. The top ears from five plants at each grid point were collected in the field when the kernel moisture was at 12%. The insect damage assessment included the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), as well as the percentage of stink bug-damaged kernels, which represented the kernel damage caused by a combination of the brown stink bug, *Euschistus servus* (Say) (Heteroptera: Pentatomidae) and the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) feeding. The aflatoxin levels from the corn samples were quantified using the VICAM® mycotoxin testing system. The aflatoxin level was correlated to the number of the maize weevils ($r = 0.3$, $P = 0.002$, $n = 92$), but not to either corn earworm damage ($r = 0.15$, $P = 0.17$, $n = 92$) or the stink bug damage ($r = 0.09$, $P = 0.37$, $n = 92$). In addition, the correlation between aflatoxin level and husk coverage was not significant ($r = -0.15$, $P = 0.1544$, $n = 92$), nor was the correlation between husk coverage and corn earworm damage ($r = 0.05$, $P = 0.66$, $n = 92$). The roles of both chewing and piercing-sucking insect feeding damage on aflatoxin and other mycotoxin (e.g., fumonisin) accumulations in pre-harvest corn are also discussed.

Effect of Glufosinate on Aflatoxin in Corn

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Aflatoxin is a secondary product metabolized by the fungus *Aspergillus flavus*, particularly when hot and dry environmental conditions prevail. Breeders have now developed lines with improved resistance, but other technologies to further reduce aflatoxin are still being investigated. One technology under consideration to reduce aflatoxin is the use of glufosinate (Liberty). Ammonia breaks down aflatoxin and has been used to treat contaminated grain. Glufosinate is believed to trigger ammonia production in the corn plant that in turn breaks down aflatoxin. Field tests were conducted in central Louisiana in 2005 and 2006 to determine the effects of glufosinate application on aflatoxin contamination in corn. *Aspergillus flavus* was both spread on the ground in one row of each plot and applied to ears in another row using a pin-bar cushion to enhance aflatoxin biosynthesis. Glufosinate herbicide was applied to 'N83-Z8' (Liberty Link) and 'N83-N5' (Non-Liberty Link) corn hybrids at 0, 4.25, 8.5, 17, and 34 ounces per acre at two different times after silking (targeted 40 and 60 days after mid-silk). Ears were allowed to reach harvest maturity in the field, then picked, shelled, ground to a fine meal and analyzed for aflatoxin concentration. The results of this study verified that the effect of glufosinate on corn remains elusive. It is apparent that glufosinate effects corn, however; the effect varies greatly from one environment to another. Glufosinate had little or no effect on aflatoxin in Liberty Link corn in 2005, but reduced aflatoxin in non-Liberty Link corn. The average aflatoxin contamination in corn sprayed with 4.25 and 8.5 ounces of glufosinate was significantly lower than the control and reduced aflatoxin by about 45% in ear-inoculated treatments and by about 86% in ground-inoculated treatments in non-Liberty Link corn in 2005. Rate of glufosinate had little or no effect on aflatoxin in non-Liberty Link corn in 2006. Early application of glufosinate resulted in lower aflatoxin in non-Liberty Link corn in 2005 compared to late application. The results were opposite in 2006. A major difference between the two years was that aflatoxin contamination was much higher in 2005. Glufosinate application to corn threatened by aflatoxin contamination has potential to be an economic tool for producers if the pathway of effect can be determined and beneficial reduction in aflatoxin stabilized. Use of glufosinate in this manner would probably require expanding the label. More field-plot and field-scale research is needed to confirm benefits and determine best practices.

Effect of Exogenous Jasmonic Acid Application on *Aspergillus flavus* Kernel Infection and Aflatoxin Production in Two Maize Hybrids (*Zea mays* L.)

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Jasmonic acid (JA), produced by the octadecanoid pathway, is a phytohormone that triggers induced resistance against certain pathogens and arthropod herbivores. The octadecanoid pathway has been implicated in playing a role in the *Aspergillus flavus*-maize seed interaction. In field studies, the effect of exogenous applications of nonphytotoxic levels of JA at 8 and 13 days after midsilk were evaluated in two maize hybrids differing in resistance to *A. flavus* infection (Mo18W x Mp313E and GA209 x Mp339) for any effect on kernel infection by *A. flavus*, subsequent aflatoxin accumulation and a natural infection of *Fusarium verticillioides*. As a parent in single crosses, Mp313E has been effective in reducing aflatoxin contamination levels in other studies. An application of 71.5 µg JA·plant-1, whether applied topically to silks or injected directly into the side of the ear, did not have a significant effect on reducing aflatoxin production. There were significant differences in aflatoxin accumulation and kernel infection by *A. flavus* and *F. verticillioides* between the two hybrids. GA209 x Mp339 tended to have higher levels of *A. flavus* kernel infection (7%) and aflatoxin (477 ng·g-1); yet, lower levels of *F. verticillioides* (14%) than Mo18W x Mp313E with 2% *A. flavus* kernel infection , 145 ng·g-1 aflatoxin, and 26% *F. verticillioides* infection. This concentration of jasmonic acid is ineffective in reducing *A. flavus* kernel infection and aflatoxin accumulation in an agronomical setting.

Biological Control of *Aspergillus flavus* by *Pichia anomala*: Efficacy and Practical Application

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The fungus, *Aspergillus flavus,* produces aflatoxin which is the most potent carcinogen known. This mycotoxin is very hazardous to the health of both human and animal. Aflatoxin contamination is associated with wounding in corn, peant, cotton seed and tree nut. There is no conventional fungicide to control *A. flavus*. Economic losses are in the billions of dollars per year due to aflatoxin contamination of agricultural commodities. Growers and processors are looking for effective means to control *Aspergillus* infestations and subsequent contamination of food crops.

Numerous literatures indicate that wounds in plant tissues including insect provide the entry to *A. flavus*. By mechanically wounding pistachio nut-fruits, sufficient number of nut-fruits conducive to *A. flavus* and fungal infection were generated. The wounded nut-fruits are easily recognized for sampling. Two experiments were conducted in a commercial pistachio orchard in the summer of 2005 in collaboration with Dan Parfitt and Brent Holtz (University of California, Davis, CA). The biopesticide, *P. anomala* WRL-076 reduced the frequency of *A. flavus* colonization by 4 to 10 times and decreased the total propagules of *A. flavus* by 80 to 99% in comparison to control nut-fruits sprayed with water. Similar result was observed in almond orchard.

Furthermore in laboratory experiments, PEG (polyethylene glycol) 8000 was used to adjust medium a_w to 0.96, which mimicked a water stress condition of –5.62 MPa.. *P. anomala* WRL-076 can grow at this low water activity (a_w). The yeast cells formed a film and inhibited the growth of *A. flavus* inoculated to the medium. Analysis of cellular trehalose accumulation and genes responsible for trehalose biosynthesis are in progress.

P. anomala WRL-076 does not produce allergenic spores and killer toxin. This species has been demonstrated to control a variey of fungi such as *Aspergillus flavus*, *A. parasitics, A. ochraceus, Penicillium roqueforti, Penicillium spp, Botrytis spp, etc.*. It has been tested in corn to reduce aflatoxin by Tom Isakeit of Texas A&M University. Researchers in California are testing this biopesticide to control Alternaria disease in almond and pistachio.

Aflatoxin Control in Figs: Biocontrol using Atoxigenic Strains

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For several years, we have investigated the use of atoxigenic strains (strains not able to produce aflatoxins) of *Aspergillus flavus* as biocontrol agents to reduce aflatoxin contamination of figs in California. This approach has been very successful in commercial cotton fields in Arizona where the atoxigenic strain AF36 has substantially reduced aflatoxin contamination of cottonseed. In 2003 and 2004, we applied the atoxigenic strain AF36 (as infected wheat at the rate of 41.2 g wheat/tree (equivalent to 10 lbs/acre)) once each year during early summer in a drip-irrigated Calimyrna fig orchard. No AF36 was applied in this orchard in 2005 or 2006. On 7 September, 2005, soil, leaf, and fruit samples were collected. The soil had a higher density (508.7 cfu/g soil) of *A. flavus*/*A. parasiticus* in the areas under the drip lines where infected wheat had been placed (in early summer of the previous year) than in the middles (17.4 cfu/g) or under the drip lines in the untreated areas (6.8 cfu/g). During the harvest period in the late summer of 2005, almost all of the *A. flavus* isolates (98.9%) obtained from the soil under the drip lines in the areas treated in 2003 and 2004 belonged to the strain AF36 compared to 90.2% in the middles and to 71.6% of the isolates from the untreated areas, suggesting that AF36 survived for at least one year at high levels and that there was movement of AF36 from the applied areas under the drip lines to untreated areas. The density of *A. flavus*/*A. parasiticus* and the incidence of AF36 on leaves did not differ significantly between treatments. No decay by *A. flavus* was found in 900 dried figs that were examined, suggesting that applying AF36 does not significantly increase decay of the figs. Our results suggest that the use of AF36 in fig orchards should result in the atoxigenic strain becoming the dominant *A. flavus* strain where applied without significantly increasing fig decay.

In 2006 we did not apply any atoxigenic strains in this orchard. However, we did collect samples in order to determine the survival and spread of the previously applied atoxigenic strains. On 4 October (normal harvest period), soil and fruit samples were collected and are currently being evaluated.

The incidence of atoxigenic strains among *A. flavus* isolates occurring naturally in commercial fig orchards in California was determined. A total of 348 isolates of *A. flavus* from commercial fig orchards were evaluated, and all three atoxigenic strains AF36 (6.9%), A815 (0.6%), and A564 (0.3%) were detected. The greater occurrence of AF36 among the strains examined suggests that using AF36 for the biocontrol of aflatoxin contamination in figs was a better choice than using any other strain. In another study, we determined the natural occurrence in commercial fig orchards of 15 additional atoxigenic *A. flavus* strains (obtained from California orchards). Only three of the 15 atoxigenic strains were detected: CAP (2.5% of the *A. flavus* isolates), CAD (1.3%), and CAM (1.3%). Based on these findings, it seems likely that AF36 is the most common atoxigenic strain occurring naturally in commercial fig orchards in California.

Aflatoxin Control in Pistachios: Biocontrol using Atoxigenic Strains

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For the past several years, we have investigated the use of atoxigenic strains (strains not able to produce aflatoxins) of *Aspergillus flavus* as biocontrol agents to reduce aflatoxin contamination of pistachios in California. This approach has been very successful in commercial cotton fields in Arizona where the atoxigenic strain AF36 has substantially reduced aflatoxin contamination of cottonseed. In 2001 and 2002, the three promising atoxigenic strains AF36, A564, and A815 were applied (as infected wheat seeds at the rate equivalent to 10 lbs/acre) in a flood-irrigated research pistachio orchard. In order to determine the survival and spread of the atoxigenic strains, soil samples were collected on 29 August, 2005. The density of *A. flavus*/*A. parasiticus* in the soil did not significantly differ among treatments. The percentages of *A. flavus* isolates from the soil were 57.7, 46.0, and 17.7% for AF36, A815, and A564, respectively, in the areas that were treated with each of those specific strains, which demonstrated that AF36 persisted better than the other strains applied. The applied strains were detected in the untreated areas at low levels (0.6 to 9.1% of the isolates, depending on strain), suggesting only slight movement of the applied atoxigenic strains to untreated areas. In 2006 additional soil samples were collected and are currently being evaluated.

Starting in 2003, the atoxigenic strain AF36 was applied in a different research pistachio orchard, which was irrigated by microsprinklers. In 2005 wheat seeds infected with AF36 were applied after collecting soil samples on 29 June. On 12 September (during the normal harvest period), samples of leaves, early split nuts, and soil were collected. On the following day, the nuts were harvested, and samples collected from the harvested nuts. For the soil samples collected in September, the density of *A. flavus*/*A. parasiticus* was not significantly different between treated areas and untreated areas. The incidence of AF36 among *A. flavus* isolates increased from before applying the wheat to late summer in the areas treated in 2004 and 2005 from 56.9% to 82.0%, but only increased slightly in the areas treated in 2003 and 2004 (83.9 and 84.8%) and in the untreated areas (5.3 and 18.3%). The incidence of AF36 on leaves did not significantly differ between areas treated with AF36 and untreated areas. No kernel decay by *A. flavus* was found in the 500 early split nuts examined, suggesting that applying AF36 does not significantly increase decay of the nuts. In addition, the treatments did not differ significantly in the density of *A. flavus*/*A. parasiticus* on the surface of the hulls of freshly harvested nuts. In 2006 AF36 was not applied in this orchard. Soil and nut samples were collected during the harvest period in 2006 in order to determine the survival and spread of the AF36 previously applied. And these samples are currently being evaluated.

The incidence of atoxigenic strains among *A flavus* isolates occurring naturally in commercial pistachio orchards in California was determined. All three atoxigenic strains, AF36 (4.6% of the isolates), A564 (1.0%), and A815 (0.9%) were detected among 824 isolates of *A. flavus* from commercial pistachio orchards. In another study we determined the natural incidence of 15 additional atoxigenic *A. flavus* strains (obtained from California orchards) among 200 *A. flavus* isolates (20 isolates from each of 10 commercial pistachio orchards). Only four of the 15 atoxigenic strains were detected: CAV (1.5% of the isolates), CAD (0.5%), CAG (0.5%), and CAM (0.5%). The strain AF36 seems to be the most common atoxigenic strain occurring in commercial pistachio orchards in California.

Preparations are being made for applying the atoxigenic strain AF36 in commercial pistachio orchards in 2007. A request for an Experimental Use Permit has been submitted in early October 2006 to the Environmental Protection Agency to get permission to apply AF36 in commercial orchards in California. In addition, we are determining which orchards have consistently high levels of aflatoxin contamination by using the results of aflatoxin analyses of 300 to 500 library samples (20-pound samples of fresh nuts taken at the processing plant as nuts are being unloaded) yearly from the 2001 to 2005 harvests. We plan on applying AF36 in those orchards that have the most severe aflatoxin contamination.

Potential for Reducing Aflatoxin in Corn with Field Application of a Nontoxigenic Strain of *Aspergillus flavus*

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Research conducted over several years has resulted in the development of a product for biological control of aflatoxin contamination. The biopesticide is now produced commercially under the trade name, afla-guard®, and it is approved for use in commercial peanut production. Afla-guard® is used to establish a dominant population of a nontoxigenic strain of *Aspergillus flavus* (NRRL 21882) in soil where the applied strain competitively excludes toxigenic strains in the colonization of peanuts during periods of late-season drought. Because corn is also commonly contaminated with aflatoxins, the purpose of this study was to determine the efficacy of afla-guard® in controlling aflatoxin in corn. The two-year study design was a randomized complete block with two plantings of corn, four biocontrol treatments, and eight replications. The 64 individual plots consisted of four rows of 60 feet and were separated by 12 feet laterally and 40 feet end to end. The area around and among plots was not planted. The two plantings were separated by three weeks. Treatments included: 1) untreated control; 2) afla-guard® applied to soil at 20 lbs/ac; 3) afla-guard® applied to plant whorls at the same rate; and 4) a liquid spray suspension of spores applied four times during silking. The soil application was made when corn plants were about two feet high and whorl application was made just prior to tasseling. The spray suspension contained 1×10^6 spores/mL applied at a rate of 13 gal/ac; the first application was made at the beginning of silking, and three other applications were spaced 2-3 days apart. In 2005, corn was harvested with a combine and half the corn from each plot was collected for analysis. In 2006, all corn from each plot was hand-picked because severe drought conditions resulted in very poor yields. Corn from each plot was ground in a Romer subsampling mill set to provide an analytical subsample of approximately 2-3 kg. That subsample was then ground with an equal volume of water in a vertical cutter mixer to homogenize fungal propagules and aflatoxins, and a subsample was taken for dilution plating to determine *A. flavus* density in corn (CFU/g) and for HPLC analysis to determine aflatoxin concentrations. The incidence of toxigenic/nontoxigenic *A. flavus* in corn was then determined by culturing 10 *A. flavus* isolates per sample on a liquid growth medium and analyzing cultures for aflatoxins and cyclopiazonic acid. Data were log transformed when necessary to normalize distributions and were subjected to two-way ANOVAs to determine significant differences. In 2005 there was a significant ($P < 0.05$) difference in aflatoxin for the two planting dates but not for biocontrol treatments, and there was no interaction between planting date and treatment. Mean aflatoxin for the first date was 30.9 ppb compared with 80.5 ppb for the second. Total *A. flavus* in corn was also significantly higher for the second date (3.9×10^5 CFU/g) than the first (2.1×10^5 CFU/g), but differences among treatments were not significant. There were no differences for date or treatment in the incidence of the nontoxigenic strain of *A. flavus* in corn, which ranged from an incidence of 83.8% for the soil inoculation treatment to 93.1% for whorl application. A prolonged and severe drought in 2006 produced a greatly reduced yield of corn. Colonization of corn by *A. flavus* and contamination with aflatoxins were higher than in 2005. Significant differences for aflatoxin were found for both planting date and treatments. Mean concentrations for planting dates 1 and 2 were 93.9 and 167.6 ppb, respectively. The whorl application produced a significantly ($p < 0.05$) lower mean aflatoxin of 49.5 ppb compared with all other treatments (control = 191.6; soil application = 108.3; spray application = 173.7). Both the soil and spray application treatments were significantly reduced compared with control. There was significantly more total *A. flavus* in corn from the second planting (5.2×10^6 CFU/g) than from the first (2.6×10^6 CFU/g), and the whorl treatment had significantly less than the other treatments. The incidence of the nontoxigenic strain was highest in the whorl treatment at 86.5%, but the untreated control still had a quite high incidence of 69.1%. Although plots in this study were separated from each other, it was apparent that a major infiltration of the applied nontoxigenic strain infected and colonized corn in the untreated control plots. Nevertheless, the application of afla-guard® to the whorls of corn plants resulted in the greatest reduction of aflatoxin, averaging 74% in 2006. These results warrant large scale field studies to determine the true potential for biological control of aflatoxin in corn.

Isolation of Bacterial Antagonists of *Aspergillus flavus* and *Fusarium verticillioides* from Mississippi Corn Soils

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Bacterial populations from Mississippi corn field soil and corn rhizosphere samples were evaluated for their potential as biological control agents against *Aspergillus flavus* and *Fusarium verticillioides*. Bacteria were isolated by direct plating of washes of soil subsamples and intact corn roots. Isolated strains were initially screened for antagonistic activities in liquid coculture against *A. flavus* strain Papa827, a *nor* mutant strain that accumulates norsolorinic acid under aflatoxigenic conditions. Bacteria that showed inhibition of *A. flavus* growth were further screened for antifungal phenotypes on agar media against *A. flavus* strain Papa 827, and against *F. verticillioides* strain 935, a fumonisin-producing isolate from Mississippi. After screening 600 soil isolates, we identified 208 isolates that inhibited *A. flavus* growth, and of those, 79 isolates that also inhibited *F. verticillioides* growth. Likewise, of 418 bacteria isolated from corn rhizosphere samples, 235 strains inhibited *A. flavus* growth, and 142 of those also inhibited *F. verticillioides* growth. These bacteria were further differentiated by their production of extracellular chitin- and yeast cell wall-hydrolyzing enzyme activities (visualized as clearing zones on chitin and yeast cell wall agar), as well as production of antifungal metabolites (visualized as zones of fungal growth inhibition on potato dextrose agar). Bacterial strains were identified by 16S rDNA sequence analysis and by nutritional analysis using the Biolog microbial identification system. The most prevalent genera isolated from rhizosphere samples were *Burkholderia* and *Pseudomonas*, while the most prevalent genera isolated from non-rhizosphere soil were *Pseudomonas* and *Bacillus*. Less prevalent genera included *Stenotrophomonas*, *Agrobacterium*, *Variovorax*, *Wautersia* (formerly *Ralstonia*), and several genera of coryneform and enteric bacteria. Quantitative coculture assays against mycotoxigenic *A. flavus* and *F. verticillioides* in different media demonstrated differences in the inhibitory activities among bacterial isolates, suggesting that multiple laboratory assays should be used in determining candidates for potential field applications in the development of bacterial strains for biological control.

Studies on Aflatoxin Inhibition by Intra-specific "Competition"?

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Atoxigenic *Aspergillus flavus* is considered a promising biocontrol approach to minimize pre-harvest aflatoxin contamination. Intra-specific competition for nutrients and competitive exclusion in the soil are considered possible mechanisms. Fifty *A. flavus* cultures were isolated from kernels from Louisiana growers' corn fields and divided into two groups: 9/50 toxigenic and 41/50 atoxigenic. All 41 atoxigenic isolates were screened for ability to inhibit aflatoxin B1 production by a single toxigenic isolate 53 in a suspended disc assay. Eight isolates completely inhibited aflatoxin production and 4 were highly inhibitory. The inhibition was independent of vegetative compatibility group as the 12 isolates were from 5 VCG's and two isolates are in the same VCG as isolate 53. A filter insert-plate well system was adopted to continue the work. There was no inhibition when isolate 53 and previously effective atoxigenic isolates were separated by a 0.4 μm or 3.0 μm filter. Approximately 50% inhibition occurred when separated by a 12.0 μm filter, and complete inhibition occurred with a 74 μm membrane. If atoxigenic/toxigenic conidia were mixed together on both sides of the filter, nearly complete inhibition occurred. The diameter of the *A. flavus* conidia and hyphae used was between 3.5-7.0 μm. These results showed that physical touching or growth in intimate contact is required for toxin inhibition and that it is independent of nutrients. *A. flavus* toxigenic isolate *Af*70s-GFP was acquired to microscopically visualize the inhibitory effect. However, none of the atoxigenic completely inhibitory isolates from our collection nor NRRL 21882, a biocontrol isolate for peanut, inhibited toxin production by *Af*70s-GFP. Subsequently isolate K49 and two Australian cryptic species II isolates were shown to suppress *Af* 70s-GFP's toxin production. This showed there is specificity in the touch inhibition interaction. It suggests that multiple atoxigenic isolates each specific to a subset of the toxigenic population may be required for effective atoxigenic biocontrol. The intra-specific competition appears due to touch or close physical contact which initiates an unknown signaling pathway to prevent toxin synthesis and may be the mechanism of biological control.

Colonization and Aflatoxin Accumulation of Corn Following Pin Bar Inoculation with Various *Aspergillus flavus* Strains

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This study was conducted to assess the colonization potential of *Aspergillus flavus* strains as biological control agents to reduce aflatoxin contamination. Under field conditions developing corn ears were inoculated with various *A. flavus* strains using a pin-bar inoculation technique in 2004, 2005 and 2006. Non-aflatoxigenic strains K49 and CT3 and toxigenic strain F3W4 were compared against the carrier control (aqueous tween 20). Following inoculation 5 to10 corn ears were sampled every other day and visually assessed for infected kernels and modeled using the Gompertz growth model or the best fitting regression. Aflatoxin concentration was determined by HPLC at each sample date in 2005 and 2006. In all three years, the non-aflatoxigenic strain K49 demonstrated superior colonization to strain CT3. The toxigenic strain F3W4 exhibited differential colonization in all three years. The Gompertz model characterized kernel colonization for all inoculated treatments in all years of study, except for F3W4 in 2006 ($r^2 > 0.954$). Aflatoxin accumulation in corn inoculated with F3W4 was 35,000 and 22,000 ppb in 2005 and 2006 respectively and patterns of accumulation was well described by the Gompertz equation, while less than 200 ppb was observed in corn inoculated with non-aflatoxigenic strains CT3 and K49 and accumulation described as either linear or logistic. In 2006 a competition study was included with mixed inoculation of equal rates of F3W4 and either CT3 or K49. Total aflatoxin accumulated after 20 days in corn inoculated with F3W4 and CT3 (40,000 ppb) was greater than F3W4 (16,000 ppb) alone, while corn inoculated with F3W4 and K49 were less than 2000 ppb, indicating the superiority of strain K49 as a biological control agent. This data indicates that this technique can be used to elucidate colonization potential of non-toxigenic *A. flavus* in corn in relation to biological control of aflatoxin production.

PANEL DISCUSSION: MICROBIAL ECOLOGY
Panel Chair: Jeffrey D. Palumbo

Kenneth Damann was asked to distinguish physical interactions between fungi in terms of touching versus large non-diffusible molecules. He discussed the distinction, adding that in live versus dead competitors, dead cells do not function the same. This indicates some metabolic or physiological activity of the competitor is involved for the effect to occur. Further discussion suggested that there may be a slowly diffusible compound, containing a lipid moiety, for example, involved in this interaction, and that spore washes or other fractionation might give clues as to the nature of this interaction. A comment was made suggesting that the conditions necessary to demonstrate the inhibition effect of touching, while it may be a laboratory phenomenon, might explain why AF36 has not been effective in northern Arizona, especially if there is specificity in the touching effect leading to variability in the response.

Jeffrey Palumbo was asked to comment on the target of delivery for bacterial biocontrol agents on corn. He replied that the primary target was soil, with the goal of initially reducing the potential inoculum population of *A. flavus* and *F. verticillioides*, and secondary applications on corn plants will also be tested. Other comments suggested another strategy for identifying bacterial biocontrol agents would be to begin with several crops and screen for widely distributed antagonists, then narrow the candidates down by crop. He agreed, but mentioned the prohibitive scale of such an approach.

Hamed Abbas addressed a question to the panel as to whether the variability in the efficacy of non-aflatoxigenic *A. flavus* agents was a result of the method of application of these strains in corn. His response was that this variability is why his strategy is to target the kernel. Other comments were made regarding silk channel delivery and its relationship to stress, and the combination of insect damage, stress conditions, and limited delivery of biocontrol strains as relating to aflatoxin contamination.

Mark Doster was asked about the distribution of non-aflatoxigenic *A. flavus* strains in terms of the drip diameter of the irrigation lines. He responded that the drip zones stay relatively wet even between irrigation events, and the population of *A. flavus* presumably varies up and down seasonally, as well as in response to cyclic wet and dry conditions.

Themis Michailides and Mark Doster were asked whether they compared the effect of suspended versus on-ground drip lines in their studies. They responded that this comparison was not made, since the growers at the site they study installed only the suspended drip lines. In response to a question regarding the effect of non-aflatoxigenic strains on the aflatoxin content of pistachios or figs, they emphasized that their study was aimed at demonstrating displacement of toxigenic *A. flavus* in soil, rather than targeting the aflatoxin content in the crops.

A final comment was made suggesting that a unifying goal would be to target soil treatments to reduce or eliminate S-strain *A. flavus* to eliminate aflatoxin contamination potential.

Antimicrobial Activity of Pyrrocidines from *Acremonium zeae* against Endophytes and Pathogens of Maize

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Acremonium zeae produces pyrrocidines A and B, polyketide-amino acid-derived antibiotics, and is recognized as a seed-borne protective endophyte of maize which augments host defenses against microbial pathogens causing seedling blights and stalk rots. Pyrrocidine A displayed significant *in vitro* activity (MIC) against *Aspergillus flavus* and *Fusarium verticillioides* in assays performed using conidia as inoculum, with pyrrocidine A being more active than B. In equivalent assays performed with conidia and/or hyphal cells as inoculum, pyrrocidine A revealed potent activity against major stalk and ear rot pathogens of maize *Fusarium graminearum, Nigrospora oryzae, Stenocarpella* (*Diplodia*) *maydis*, *Rhizoctonia zeae* and kernel rotting fungal pathogens *A. flavus*, *Alternaria alternata* and *Cladosporium cladosporioides*. Protective endophytes, including mycoparasites which grow asymptomatically within healthy maize tissues, show little sensitivity to pyrrocidines. Pyrrocidine A also exhibited potent activity against *Clavibacter michiganense* subsp. *Nebraskense*, causal agent of Goss's bacterial wilt of maize, *Bacillus mojaviense* and *Pseudomonas fluorescens*, maize endophytes applied as biocontrol agents, but were ineffective against the wilt- producing bacterium, *Pantoea stewartii*. *A. zeae* is recognized as a potential confounding variable in maize variety trials for resistance to pathogenic microbes and their mycotoxins.

Migration of *Fusarium verticillioides* between Inoculated and Non-inoculated Ears in Field-grown Corn Plants

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Consequences of *Fusarium verticillioides* colonization of corn kernels may be plant disease and/or mycotoxin production. Plant disease may reduce crop production and mycotoxins may cause harmful, and often fatal, effects on humans and animals. Understanding migration patterns of *F. verticillioides* among corn plants is essential for developing strategies to prevent diseases of corn plants and eliminate mycotoxins from our food chain. The purpose of the current research was to analyze the field dissemination of *F. verticillioides* from corn ears inoculated with *F. verticillioides* PATg, a transformant with a selection gene, *hph*, for hygromycin resistance (hyg^r) and a reporter gene, *gusA*, coding for ß-glucuronidase (GUS). Corn ears were inoculated through either the shuck or the silk channel at two stages of development, green silks and brown silks. The average expression of hyg^r was 66% and GUS was 96% for mycelia emerging from kernels of ears inoculated with *F. verticillioides*. Mycelia resistant to hygromycin appeared in cultures of kernels from non-inoculated and water-inoculated ears, but only at ≤ 3%. However, none of the mycelia stained positive for GUS in kernels originating from non-inoculated ears and only 7% of those isolated from kernels of water-inoculated ears. Thus, dissemination of *F. verticillioides* PATg was minimal from plant to plant under field conditions existing in Georgia during the growing seasons of 2001, 2002, and 2003. The only slight evidence of dissemination occurred in a few ears inoculated with water through the silk during the green silk stage of development. Perhaps, the mechanical injury during inoculation provided an entry site for the spores of *F. verticillioides* PATg dispersed by abiotic factors, such as rain or wind.

Molecular and Microscopic Studies on the Interactions of *Pichia anomala* and *Aspergillus flavus*

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Several hundred strains of yeasts were screened for their antagonistic activities to prevent the growth of *A. flavus* by a visual bioassay. The yeast, *Pichia anomala* was shown to inhibit both the growth of *A. flavus* and aflatoxin production. It is important to understand the mechanisms by which antagonistic yeasts control the target fungus because the resulting information can facilitate the development of effective methods for formulation, application and commercial registration.

The objective of this study was to probe the antagonistic effect of the yeast, *P. anomala* antagonistic to *A flavus* by using RT-PCR technique and a vital fluorescent stain, FUN-1. Yeast and fungi were inoculated into a 250 ml-flask containing 50 ml potato dextrose broth (PDB) at yeast to fungus (Y : F) ratios of 1:1, 5:1, 10:1, 30:1, and 50:1. yeast cells and hyphae of *A. flavus* were harvested. Total RNAs from yeast cells were extracted and used for first-strand cDNAs synthesis followed by PCR. The resulting DNA fragments were analyzed by gel electrophoresis. Fugal hyphae were stained by a fluorescent compound, FUN-1 and then viewed through a Leica DMRB epifluorescence microscope.

The gene which is coding for the cell wall degrading enzyme, exo-β-1, 3 glucanase in *P. anomala* WRL-076 was chosen for analysis. Our preliminary experiments indicate the induction of *Paexg2* gene expression of *P. anomala* WRL-076 by *A. flavus* when co-culturing in the same medium. This enzyme probably caused damage on the hyphae of *A. flavus* resulting in significant reduction fungal growth.

The FUN-1 fluorescent stain is a membrane-permeant, halogenated cyanine compound that binds to nucleic acids. Biochemical processing of the dye by metabolically active fungal hyphae yielded cylindrical intra-vacuolar structures (CIVS) that were markedly red shifted in fluorescent emission and spectrally distinct from the nucleic acid bound form of the dye resulting in green. The formation of CIVS is mediated by ATP production. Metabolically active *A. flavus* hyphae accumulated red fluorescence in vacuoles, while hyphae that were inhibited by *P. anomala* stained green. The result indicates that the yeast might inhibit the ATP system of *A. flavus*, which causes a significant reduction of fungal biomass.

Intracellular Sugar Alcohol and Sugar Accumulation by the Biocontrol Yeast, *Pichia anomala* WRL-076

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The biocontrol yeast, *Pichia anomala WRL-076* is applied as a foliar spray to pistachio and almond trees for control of *Aspergillus flavus*, therefore it is naturally exposed to environmental stress such as temperatures fluctuation, desication, water availability and UV-irradiation. Numerous studies implicate that the dissacharide, trehalose acts as a protectant for cells under environmental stresses. Upon dessication and thermal inactivation, the presence of trehalose can prevent cell leakage and membrane damage to preserve cell viability. The objective of this research is to examine the effects of carbon sources in the media on intracellular concentration of trehalose and osmotic solutes such as glycerol, arabitol and sorbitol. A protocol for extracting intracellular sugars and polyols was described. High-performance liquid chromatographic (HPLC) method with evaporative light-scattering detection (ELSD) was applied to analyze intracellular sugars and polyols.

Nutrient broth (8g/l) and yeast extract(5g/l) were supplemented with either glucose (10g/l), glycerol (10g/l), sorbitol (10g/l), maltose (10g/l) or sucrose (10g/l) as carbon source. Yeast cells were harvested by centrifugation in a 15 ml conical tube with Sorvall RC-5C Plus centrifuge. The cell pellet was suspended in 1 ml dist. water. Yeast cells were disrupted in a Branson Sonifier® cell disruptor (Branson Ultrasonic Corp., Danbury, CT) for 2 min., then boiled for 5 min. and cooled down to room temperature. Acetonitrile was added to each tube to a final concentration of 75%. After votexing, the mixture in each tube was centrifuged in a Sorvall RC-5C Plus centrifuge for 10 min. The supernatant containing extracted sugars and polyols was filtered and transferred to a glass vial. A high-performance liquid chromatographic method (HP ChemStation, Agilent Technologies, Santa Clara, CA) with evaporative light-scattering detection (PL-ELSD, Amherst, MA) was used for analysis of intracellular sugars and polyols. Separation of these compounds was achieved on a Prevail™ Carbohydrate ES HPLC column (Alltech Associates, Inc., Deerfield, IL). The mobile phase was acetonitrile: water 75:25 (*v*/*v*), at a flow rate of 1 ml/min.
Yeast cells at early and late exponential growth phases, were harvested at 24 and 48 h after initial inoculation. Intracellular sugars and polyols concentration was very low in those samples. When the yeast cells reached to stationary phase (72 h after initial inoculation), intracellular sugars and polyols increased significantly.

The information should facilitate the production of effective yeast cells adapted to environmental stress for biocontrol of *A. flavus*.

Development of Non-Toxigenic Strains of *Aspergillus flavus* for Control of Aflatoxin in Maize

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Aflatoxin (AF), produced by *Aspergillus flavus*, can be a major problem in Mississippi Delta maize (*Zea mays* L.) causing economic losses if levels of contamination are high. Although research has been directed at reducing maize AF contamination, no consistent control methods are available. This presentation summarizes research approaches in development of non-aflatoxigenic *A. flavus* strains to control maize AF contamination. In a survey of *A. flavus* isolates from Mississippi Delta soil, two non-toxigenic strains (K49 and CT3) were identified, that when applied to maize as soil inoculants, suppress AF by competitive displacement. Four years of field testing showed these non-toxigenic isolates reduced AF contamination by 60-94%. The non-toxigenic strains displace the toxigenic *A. flavus* populations in soil. K49 may be a more suitable biocontrol agent than CT3 because K49 also does not produce cyclopiazonic acid. Both strains displace toxigenic isolates when applied to soil, however, K49 may have a greater soil colonization potential, based on faster growth than CT3 and the ability to form sclerotia. Using a pinbar inoculation technique, K49 displayed a more rapid maize colonization than CT3. These results suggest the basic method for reducing AF contamination is by competitive exclusion. Further work to improve biocontrol efficacy in reducing AF contamination is in progress.

Population Ecology of *Aspergillus flavus* and other Fungi Associated with Mississippi Delta Soils

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Understanding the source of *Aspergillus flavus* is required to effectively manage within-field aflatoxin contamination of maize (*Zea mays* L.). Studies assessed the density of *A. flavus* propagules and other soil microflora (*Fusarium* spp., total fungi) associated with Mississippi Delta soils, and correlated soil factors with these populations. Soils from 12 and 15 sites were collected in 2000 and 2001, respectively. Propagule density of *A. flavus* ranged from log (10) 1.97 to 4.31 colony forming units (cfu) g^{-1} soil, while total fusaria ranged from log (10) 2.99 to 5.37 cfu g^{-1} soil. The highest populations of *A. flavus* were associated with soils containing higher organic matter, especially in sites under no-tillage management. The frequency of aflatoxin production in isolates ranged from 13 to 81% depending on soil. In 2001, there was a highly significant correlation between *Aspergillus* propagules and history of maize cultivation. However, in 2000, this was not observed. Overall soil fertility factors such as organic matter content, nitrate and extractable phosphorus correlated with the density of *Aspergillus* as well as *Fusarium* spp., and total fungi, but little relationship was observed with soil texture. The relationship between soil parameters and *Aspergillus* populations may be useful in predicting the contribution of soil microflora to aflatoxin contamination.

Systemic Movement of *Aspergillus parasiticus* in Maize Stalks and Ears

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Preharvest infection of corn (*Zea mays*) kernels by *Aspergillus flavus* and *A. parasiticus* is a chronic problem in the southern United States. It has been reported that these fungi infect developing kernels via the silk. This study was conducted to explore other avenues of infection of corn ears by *Aspergillus* spp. An *A. parasiticus* NOR mutant (NRRL 6111) was cultured in Petri dishes containing V-8 agar with sterilized toothpicks placed on the surface. Corn hybrids resistant and susceptible to aflatoxin contamination were grown in the field and inoculated at the VT stage by inserting the infested toothpicks into stalks between the 5th and 6th node below the lowest ear shoot. Beginning two weeks after inoculation, fungal growth and movement was determined weekly by collecting ear shank tissue and stalk tissue from the nodes between the infection sites and the developing ears. Cross sections of the stalks and ear shanks were dipped in ethanol, flame sterilized, and placed in Petri dishes containing Czapek solution agar amended with NaCl (7.5%). Ears were collected at the end of the growing season to determine the level of kernel infection by the NOR mutant. Kernels from individually shelled ears were surface sterilized with NaOCl and plated in Petri dishes containing Czapek solution agar amended with NaCl. The *A. parasiticus* NOR mutant was isolated from all stalk node tissues and ear shank tissue in the resistant and suceptible hybrids at the first collection date two weeks after inoculation. The NOR mutant was isolated from kernels of the susceptible hybrids in 2003 and 2004. Infection rates of kernels in infected ears were very low (< 1.0 %). In 2005, the fungus was found in only one kernel from an ear of the resistant hybrid. The NOR mutant was never isolated from stalks, ears, or kernels from control (uninoculated) plants grown in the plots with inoculated plants. Although infection levels of maize kernels was low, systemic movement of the *A. parasiticus* up the stalk appears to be another possible infection route to developing corn ears.

Investigating the Bright Greenish-Yellow Fluorescence (BGYF) Properties of Corn Kernels with an Imaging Spectrometer

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The objective of the present study was to analyze hyperspectral bright greenish-yellow fluorescence (BGYF) response of corn kernels under UVA excitation. Traditionally, corn kernels were examined for evidence of BGYF, an indicator of the presence of *Aspergillus flavus* when illuminated with a high-intensity ultra-violet light. Because under certain specific conditions the fungus can produce aflatoxin, BGYF is also an indirect link to the possible presence of aflatoxin. BGYF is produced by the reaction of kojic acid formed by the fungus and a peroxidase enzyme from living corn and other agricultural products. The BGY fluorescence is exhibited when light of longer wavelengths (visible) is released from living objects after absorption of the shorter wavelength from the black light. The BGYF test is typically the first step that leads to an in-depth chemical analysis for possible aflatoxin contamination of corn kernels. Hyperspectral imaging technology offers a novel non-invasive approach toward screening for toxigenic fungi and the presence of toxins associated with them by identifying a given specimen based on its spectral signature. Additional spectral information about the fungus and possibly the presence or absence of the toxin may be gained from combining hyperspectral imaging with UV radiation resulting in fluorescent response images. Because certain compounds emit fluorescence in the visible range when excited with UV, the fluorescent image of a target may be more revealing than the reflected image.

The target corn samples were collected from a commercial corn field in 2005 and showed abundant BGYF response. The BGYF positive kernels were individually hand picked and placed in a black, clay dish. The other half of the same dish was used to hold the control kernels randomly selected from the BGYF negative corn. As a comparison with the BGYF corn kernels, a group of discolored corns were also selected and imaged under the same settings. Under human eye inspection, the discolored corn had similar size and general appearance as the BGYF positive corn. All kernels were imaged with a visible near-infrared hyperspectral imaging system under UV radiation with excitation wavelength centered at 365 nm.

Initial results exhibited strong emission spectra with peaks centered from 500 nm to 515 nm wavelength range for BGYF positive kernels. Interestingly, a spectral shift separated the BGYF positive kernels from the normal controls and the discolored BGYF negative kernels. Aflatoxin concentrations in the BGYF positive and negative groups of corn kernels were measured subsequently with high performance liquid chromatography. The mean aflatoxin concentration level was 5114 ppb for the BGYF positive and undetectable for the normal kernels. Our results illustrate the potential of fluorescence hyperspectral imagery for mold and toxin detection and classification on corn.

Antibody Detection of *Aspergillus* in Peanuts

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Is There More Value in Quantifying Mycotoxins or Fungus for Plant Breeding?

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