SESSION 4:

FOOD SAFETY AND TOXICOLOGY

MYCOTOXIN CONTAMINATION OF CORN DISTILLERS' DRIED GRAINS WITH SOLUBLES FROM FORTY-SEVEN ETHANOL PLANTS IN THE U.S. IN 2011

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ABSTRACT

Several known fungal plant pathogens in the genus *Fusarium* produce dangerous mycotoxins that may contaminate corn (*Zea mays* L.) and small grains destined for fuel ethanol production and the resulting ethanol co-products known as distillers' dried grains with solubles (DDGS). Corn DDGS are a significant source of animal feed. Fuel ethanol production may concentrate mycotoxins in DDGS, posing a significant threat to the health of domestic animals. A recent survey of mycotoxins in corn DDGS reported that 12% of the samples (67 DDGS samples from 8 ethanol plants in the U.S.) contained mycotoxin levels that exceeded FDA advisory levels. In the present study, we used GC-MS to screen 141 DDGS samples collected in 2011 from 47 ethanol plants located in 12 states for the mycotoxins deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), and zearalenone (ZON). Mean DON levels ranged from <0.5 ppm (LOQ) to 15 ppm, mean 15-ADON levels ranged from <0.25 ppm (LOQ) to 3.9 ppm, and mean ZON levels ranged from <0.1 ppm (LOQ) to 1.0 ppm. None of the DDGS samples contained 3-ADON. DON levels were significantly correlated with levels of 15-ADON (R^2= 0.96, $P < 0.0001$) and ZON (R^2=0.93, $P < 0.0001$). Twenty five percent (35/141) of the samples contained a mean of 1 to 5 ppm DON, 3% (4/141) of the samples contained a mean greater than 5 ppm but less than 10 ppm DON, and 3% (4/141) of samples contained a mean of 10 or more ppm DON. DDGS lots contaminated with unacceptable levels of DON evaded detection prior to their commercial distribution and were consequently sold as feed products. These observations underscore the need for new and improved detection and mitigation strategies for mycotoxins in DDGS.

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HOW THE *FUSARIUM* TOXIN DON IS MADE AND DELIVERED TO PLANTS

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ABSTRACT

Several species of the fungus *Fusarium* colonize wheat and barley and produce toxic small molecules that contaminate grain, rendering it unsuitable for consumption. Among the most destructive of these species is *F. graminearum*, which causes Fusarium head blight disease and often infests the grain with harmful trichothecene mycotoxins such as deoxynivalenol (DON). Previous studies have shown that the fungus is remarkably adapted for producing DON by precisely regulating the genes for its synthesis in order to promote toxin accumulation in the host plants. We have now found, by labeling proteins for toxin synthesis with fluorescent proteins, that these proteins are directed to subcellular toxin factories; small vesicles called toxisomes that appear to serve as the staging area for the toxin biosynthetic assembly line. When cell culture conditions are changed in order to promote toxin biosynthesis, another pathway supplying precursor molecules for toxin synthesis may be shifted within the cell to toxisomes, streamlining the path to toxin synthesis. By making toxin in a confined vesicle within the cell, the fungus may protect itself from the inhibitory effects of its own toxin and may allow for an efficient way to deliver it to the plant. This work establishes that toxin synthesis requires a complex developmental event in the fungus which ultimately determines the outcome of plant infection and plant health.

TRICHOTHECENE CHEMOTYPES AND ZEARALENONE OF *FUSARIUM* ISOLATES FROM NATURAL CONTAMINATED WHEAT GRAINS FROM BRAZIL

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ABSTRACT

Wheat production in Brazil has developed and grown over the years. Aiming for safety and crop quality, we investigate the mycotoxins: deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), nivalenol (NIV) and zearalenone (ZEA) profiles of 60 *Fusarium* isolates from wheat grains harvested from three states of Brazil: Rio Grande do Sul, Sao Paulo and Parana in 2011. Prior to chemical analyses, *Fusarium* spp. were identified through EF-1 partial gene sequence and all isolates studied are within Species Complex *Fusarium graminearum*. For toxin quantification, isolates were incubated for 10 days in YES medium (Himedia) at 25ºC. Toxins were extracted from mycelium plus medium, according to Zachariasova et al. 2010. HPLC system consisted of a Shimazdu model with UV (trichothecenes) and fluorescence (ZEA) detectors. Chromatographic separations were performed on a Phenomenex C-18 reversed-phase column (250x 4.6mm, 5 µm particle size). Mobile phase consisted of acetonitrile: water (70:30 v/v) at a flow rate of 0.5 mL min^{-1}. With an average recovery rate of 88%, detection limits of DON, 3-ADON, 15-ADON, NIV and ZEA were 83.3; 167.0; 167.0; 83.3 and 54.0 ug/L, respectively. We identified four different phylogenetic species: *F. graminearum senso stricto* (33%), *F. meridionale* (48%), *F. cortaderiae* (17.4%) and *F. astroamericanum* (1.6%). Our results showed a prevalence of DON (58%) and NIV (48%) followed by 15-ADON (29%), ZEA (26%) and 3-ADON (1,4%). Although DON was more frequent, mean NIV production was much higher (24,306 µg/kg) than DON (3,839 µg/kg). DON derivative 15-ADON was predominant over 3-ADON. We were surprised with the frequency found of ZEA and its mean production of 10,378 µg/kg. In addition, specie-toxin relations showed that 78% of *F. meridionale* produced NIV, 28% produced NIV and DON both, as to *F. graminearum*, production varied between DON, 15-ADON and NIV. *F. cortaderiae* produced just NIV and *F. astroamericanum* was the only isolate which produced 3-ADON. These results give us a bigger understanding of mycotoxins profiles of *Fusarium* isolated from wheat grain in Brazil. In the future, we intend to add isolates and analyze the wheat grains harvested for the same mycotoxins.

EFFECTS OF *LACTOBACILLUS RHAMNOSUS* VT1 CULTURE SUPERNATANT ON *FUSARIUM GRAMINEARUM* GROWTH AND MYCOTOXIN PRODUCTION IN CULTURE AND BARLEY MALT

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ABSTRACT

Cell free *Lactobacillus* culture supernatant (CFLCS) was demonstrated to inhibit *Fusarium graminearum* growth in glucose-yeast extract-peptone (GYEP) broth and homemade potato dextrose agar (HPDA).

The CFLCS at concentrations of 30%, 40% applied in rice culture inhibited the growth of *F. graminearum,* but increased mycotoxin concentrations in rice culture. At a concentration at 50% CFLCS, *F. graminearum* growth was inhibited completely and no mycotoxins were detected.

As replacements of steeping water during the malting process, the CFLCS concentrations of 30% and 50% significantly reduced the *F. graminearum* growth and mycotoxin accumulations in naturally infected barley. However, the germinative abilities of the barley samples were inhibited.