SESSION 3:

FOOD SAFETY, TOXICOLOGY AND UTILIZATION OF MYCOTOXIN-CONTAMINATED GRAIN

Co-Chairpersons: David Kendra and David Schmale

A USER-FRIENDLY LAB-ON-A-CHIP CARTRIDGE FOR QUANTITATIVE DETERMINATION OF MULTIPLE MYCOTOXINS.

James Bloomberg[1*], Randy Myers[1], Jens Burmeister[2], Ingmar Dorn[2], Karin Wieczorek[3], Jerry Outram[3] and Friedrich Kerz-Möhlendick[3]

[1]Bayer CropScience, Research Triangle Park, NC 27709, USA; [2]Bayer Technology Services GmbH, Leverkusen, Germany; and [3]Bayer CropScience AG, Monheim, Germany
[*]Corresponding Author: PH: (919) 549-2948; E-mail: jim.bloomberg@bayercropscience.com

ABSTRACT

Since mycotoxins entering the food chain bear a significant risk to human health, legislators in Europe have set maximum levels for a number of mycotoxins in grain and grain products, which in turn impacts global trade flows. However, to date no suitable test is available that allows quantification of multiple mycotoxins directly at the point-of-interest, e.g. at grain collection points. Bayer Technology Services and Bayer CropScience have jointly developed a mycotoxin test kit based on the proprietary biochip platform planar waveguide technology (PWG) with plans for its market introduction from 2009. The system is based on fluorescently labelled antibodies and consists of a reader and an easy-to-use lab-on-a-chip cartridge that allows quantification of multiple mycotoxins in one analysis, within 20-30 min. including grinding and extraction. The kit can work in outside the laboratory environment in humid and dusty conditions associated with harvest time. First field tests in three sites in Germany have shown the suitability of the test to fit into the process at grain collection points. Analytical performance characterization demonstrated agreement of the method with EU guideline 401/2006. Performance comparison demonstrated superior accuracy of the newly developed test kit with commercially available ELISA-kits and performance closer to chromatographic analysis. The unique features of the PWG are its multiplexing capability and the ease of use in combination with quantitative results in a wide range of individual mycotoxin concentrations.

REDUCING THE COST OF DEOXYNIVALENOL TESTING SERVICES IN WHEAT AND BARLEY: MOVING TOWARD A SMALLER GRAIN SAMPLE.

T.L. Fetters[1], C.G. Griffey[2] and D.G. Schmale III[1*]

[1]Departments of Plant Pathology, Physiology, and Weed Science, and [2]Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA
[*]Corresponding Author: PH: (540) 231-6943; E-mail: dschmale@vt.edu

ABSTRACT

The trichothecene mycotoxin deoxynivalenol (DON) is a common contaminant of small grains in the U.S. Current DON testing labs supported by the United States Wheat and Barley Scab Initiative (USWBSI) operate sensitive and specific Gas Chromatography/ Mass Spectrometry (GC/MS) machines to detect and quantify DON with detection thresholds $\leq$ 0.05 ppm. Though DON assessments via GC/MS are extremely accurate, the machines and associated maintenance contracts are expensive. Standard extraction protocols for DON require large samples of homogenized grain (5.0g) and a substantial volume of extraction solvent (40mL) for each sample. Large grain samples require bulky extraction vessels, sizeable quantities of expensive solvents, and a considerable amount of space for processing. We hypothesized that wheat and barley samples of varying weights (0.5g, 1.0g, 2.5g, and 5.0g) taken from single 100g ground grain lots would yield similar concentrations of DON. The specific objective of this study was to evaluate the efficiency, accuracy, and repeatability of small grain samples (< 5.0 g) for DON testing. Grain was ground from 30 unique 100g grain lots (10 winter wheat, 10 hulled barley, and 10 hulless barley lots) from Virginia FHB field trials in 2007. Each ground 100g sample was divided into four sample weights (0.5g, 1.0g, 2.5g, and 5.0g), with each sample weight replicated at least twice. DON was extracted from grain samples with acetonitrile/water; 4 mL of the solvent was added for every 0.5g of sample. Extraction, clean-up, and quantification of DON were conducted following standard protocols. An analysis of variance showed that there was no difference in mean DON concentrations across sample weights ($P = 0.255$). Mean DON concentrations were significantly correlated across all of the sample weights ($P < 0.001$), with r ranging from 0.88 (0.5g and 5.0g) to 0.97 (1.0g and 2.5g). Our results indicate that small samples from 100g lots of wheat and barley (particularly those $\geq$ 1.0g) provide a reliable measure of DON contamination from FHB field trials. New front-end methods (before the sample is processed on the GC/MS) during sample preparation and DON extraction may help offset high operating costs associated with the GC/MS and improve the efficiency and timeliness of DON testing services. USWBSI DON-testing labs use an estimated 3,000L of acetonitrile every year (~75,000 samples, 40mL per sample), with an estimated cost of more than $100K (based on 19L containers purchased at $662 each). A 10-fold reduction in acetonitrile use (based on 0.5g samples) could save USWBSI DON-testing labs nearly $90K annually.

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FY08 DEOXYNIVALENOL (DON) TESTING SERVICES AT VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY.

P.G. Gundrum[1, 2], D.M. Reaver[1], D. Cuadra[1], S. Grosse[1], W. Russell[1], T. Fetters[1], C.G. Griffey[2], C. Cowger[3], G.C. Bergstrom[4], A. Grybauskas[5] and D.G. Schmale III[1*]

[1]Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA; [2]Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA; [3]USDA-ARS, Department of Plant Pathology, North Carolina State University, Raleigh, NC; [4]Department of Plant Pathology, Cornell University, Ithaca, NY; and [5]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD
[*]Corresponding Author: PH: (540) 231-6943; E-mail: dschmale@vt.edu

ABSTRACT

Concerns about the mycotoxin deoxynivalenol (DON) continue to mount, and there is a growing need to develop and expand USWBSI diagnostic laboratories for mycotoxins throughout the United States. DON testing services are vital to the development of new varieties of wheat and barley with reduced mycotoxin potential and are necessary to identify and/or exclude appropriate strategies for managing FHB. In FY08, the Schmale Laboratory at Virginia Polytechnic Institute and State University launched a new regional diagnostic laboratory for mycotoxins in the eastern United States. Approximately 6,000 samples of wheat and barley are slated to be tested from USWBSI investigators (Bergstrom, Cowger, Griffey, and Grybauskas) in four states (New York, North Carolina, Virginia and Maryland). Most of the samples received for testing in FY08 were 100g kernel lots from FHB field trials, but some were ground 5-25g samples from greenhouse experiments. Extraction, clean-up, and quantification of DON were conducted following standard protocols. DON testing services are currently managed by two talented scientists (Patricia Gundrum and Diane Reaver) and four dedicated undergraduates (D'Lourdes Cuadra, Shannon Grosse, Tamara Fetters, and Will Russell). The ultimate goals of this work are to provide analytical services necessary to develop new cultivars of wheat and barley with reduced potential for DON contamination and to facilitate DON testing that will improve chemical and cultural practices necessary to reduce DON contamination in wheat and barley. The availability of these new testing services will continue to expedite the acquisition and delivery of data from DON analyses and will ensure increased uniformity, quality, and sample capacity for stakeholders in the eastern United States.

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DEALING WITH DON CONTAMINATED WHEAT – A MILLER'S PERSPECTIVE.

C.J. Lin*, Don Mennel and Rick Longbrake

The Mennel Milling Co., 1702 South Jefferson St., Roanoke, VA, 24016
*Corresponding Author: PH: (410) 435-8151 ext. 143; E-mail: cjlin@mennel.com

ABSTRACT

The Mennel Milling Company is a regional flour miller dating back to 1886. In 1996, there was a Fusarium epidemic in the Ohio soft wheat crop. This resulted in major financial losses to farmers and millers, raised the awareness of the dangers of FHB and DON, and changed the way we do business from purchasing to operations to sales. Each crop year we diligently monitor the development of the wheat. We scout fields and obtain samples prior to harvest. We no longer sell wheat ahead of harvest when the crop is at risk for FHB. We test each inbound load of wheat at harvest and segregate as necessary. We have improved our cleaning houses and thus, have taken defensive measures which have raised our costs of doing business, while also inconveniencing our suppliers and restricting the ability of our customers to buy forward flour when they may want to do so. FHB and DON in wheat continue to be major problems for the wheat flour milling industry.

RAPID DON TESTING AND METHOD PERFORMANCE EVALUATION AT THE USDA.

Tim D. Norden*

United States Department of Agriculture, Grain Inspection, Packers and Stockyards Administration, Federal Grain Inspection Service, Technical Services Division, Kansas City, MO
*Corresponding Author: PH: (816) 891-0470; E-mail: Tim.D.Norden@usda.gov

ABSTRACT

The USDA Grain Inspection, Packers and Stockyards Administration (GIPSA) provides official testing services throughout the U.S. for domestic and export grain lots for aflatoxins, deoxynivalenol (DON), fumonisins, zearalenone, and ochratoxin A. Rapid, simple, inexpensive, and accurate test methods are required for the effective facilitation of grain marketing. GIPSA evaluates and certifies the performance of both qualitative and quantitative rapid mycotoxin test methods according to specific criteria. Only GIPSA-certified rapid test methods can be used for official mycotoxin testing. Reference methods are developed and / or validated as needed to provide the benchmark criteria for evaluating the accuracy of potential rapid test methods. Current rapid DON testing technology and GIPSA method performance criteria for evaluation of this technology will be presented.

EVALUATION OF VISUAL AND OPTICAL SORTING OF *FUSARIUM*-DAMAGED KERNELS IN WINTER WHEAT.

Stephen N. Wegulo[1*] and Floyd E. Dowell[2]

[1]Department of Plant Pathology, University of Nebraska, Lincoln, NE, 68583-0722;
and [2]USDA ARS, Grain Marketing and Production Research Center,
Engineering Research Unit, Manhattan, KS 66502
[*]Corresponding Author: PH (402) 472-8735; E-mail: swegulo2@unl.edu

ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a destructive disease of wheat. FHB causes premature whitening or bleaching of infected spikelets. Bleached spikelets are either sterile or contain shriveled and/or discolored kernels, which are referred to as *Fusarium*-damaged kernels (FDK). *Fusarium*-damaged kernels lower yield, test weight, and quality of grain, and often contain the toxin deoxynivalenol (DON). Therefore, FDK is a major grain grading factor and is routinely measured for purposes of quality assurance. Measurement of FDK usually is done visually. Visual sorting of grain for FDK can be laborious especially if many samples need to be sorted. Furthermore, if several raters sort grain for FDK, as is often necessary, inconsistency can result from variability in intra-rater repeatability and/or inter-rater reliability. The objective of this study was to assess the ability of a single-kernel near-infrared system to detect FDK. Twenty one wheat grain samples were obtained from fields, a grain inspection facility, and elevators in south central and eastern Nebraska, USA, an area where severe epidemics of FHB occurred in 2007. Four 100-kernel subsamples from each of the 21 samples were sorted by an automated single-kernel near-infrared (SKNIR, Perten Instruments, Stockholm, Sweden) system. The system feeds single kernels into a near-infrared spectrometer, and then sorts each kernel into either a healthy or FDK portion using a partial least squares regression calibration model. The same subsamples sorted by the SKNIR system were visually sorted by an experienced rater and a recently trained rater. Agreement in FDK sorting between the SKNIR system and the two raters (inter-rater reliability) was strong. Correlation coefficients between the SKNIR system and the raters were $r = 0.91$ (rater #1) and $r = 0.89$ (rater #2). Agreement between the two raters (inter-rater reliability) also was strong ($r = 0.91$). Agreement between replicate runs in sorting FDK (intra-rater repeatability) was strongest for the SKNIR system ($0.91 \leq r \leq 0.96$, $P < 0.0001$) followed by rater #1 ($0.68 \leq r \leq 0.80$, $P \leq 0.0007$) and rater #2 ($0.49 \leq r \leq 0.66$, $P \leq 0.0236$). The mean FDK in each of the 21 samples ranged from 1 to 71% for the SKNIR system, 7 to 51% for rater #1, and 4 to 44% for rater #2. Compared to the SKNIR system, the raters generally overestimated low FDK and underestimated high FDK. Plots of standard deviations of FDK means showed that the SKNIR system was more consistent in sorting FDK than the two raters. In conclusion, visual sorting was strongly correlated with sorting by the SKNIR system; the SKNIR system had a wider range of FDK detection than visual raters; visual raters overestimated low FDK and underestimated high FDK; and the SKNIR system was more consistent than visual raters.

DEOXYNIVALENOL ALTERED CIRCULATING AND SPLENIC LEUKOCYTES AND CELL MIGRATION MARKERS: TIME COURSE AND DOSE RESPONSE IN YOUNG AND OLD BALB/C MICE.

Xianai Wu[1], Joan Cunnick[2], Marian Kohut[3], Ted Bailey[4] and Suzanne Hendrich[1*]

Departments of [1]Food Science and Human Nutrition, [2]Animal Science, [3]Kinesiology and[4]Statistics, Iowa State University, Ames, IA 50011
[*]Corresponding Author: PH: (515) 294-4272; E-mail: shendric@iastate.edu

ABSTRACT

It was hypothesized that deoxynivalenol (DON) changed leukocyte subset numbers and their migration potential in peripheral blood with interaction of age and sex. These leukocyte markers could be functional biomarkers of DON exposure in humans. In BALB/c mice fed DON at 0, 1.0 and 2.0 ppm, age, sex and feeding interval altered immune response of peripheral blood (PB) and splenic leukocytes, as measured by flow cytometry using cell surface markers. In 2-3 month old female mice, after feeding DON for 14 d, PB granulocytes were increased at 1 and 2 ppm DON, but not after 28 d. Also, a decreased percentage of PB $CD4^+$ cells and a decreased percentage of $CD11b^+$ (macrophage) splenic leukocytes at 2.0 ppm DON were seen after 14 d only. An increased percentage of $CD19^+$ cells occurred at 2.0 ppm DON after 14 and 28 d. In old females, decreased PB $CXCR5^+$ B cells were noted after feeding 2.0 ppm DON for 14 d, and 1.0 ppm DON decreased splenic $CD11b^+$ cell %, but no dose-response in these cells was observed. In old male mice, 1.0 and 2.0 ppm DON increased granulocytes and $CD29^+CD11a^+$ neutrophils after 14 d and 2.0 ppm DON increased $CCR9^+$ T cytotoxic cells after 28 d suggested that DON stimulated digestive system inflammation in old male mice. DON caused no changes to immune cell markers in young male mice. As a conclusion, low-dose DON changed leukocyte balance in peripheral blood and spleen, and interrupted B cell, neutrophil, and T cell migration in interaction with sex and age.