METABOLISM OF TRICHOTHECENES BY WHEAT

L.-F. Chen[1,2]*, H.-Y. Yao[2], G. Yu[2], W.-P. Xie[1], and H.C. Kistler[1,3]

[1]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108;
[2]Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China; and
[3]USDA ARS Cereal Disease Laboratory, St. Paul, MN 55108
*Corresponding Author: PH: (612) 625 7790; E-mail: lfchen_nau@yahoo.com

ABSTRACT

Feeding experiments were conducted to determine whether wheat could metabolize exogenously added trichothecenes. Middle spikelets of a moderately resistant wheat cultivar Su8060 were fed in triplicate, on ten sets of plants, with a fixed amount of deoxynivalenol (DON, 4000 ng). One day after adding DON, the treated spike on one set of plants and adjacent tissue was removed for toxin analysis using GC-MS. The remaining nine sets of plants were fed with DON again. This daily process of toxin addition and sampling was continued until all spikes were harvested for toxin analysis. The same procedure was carried out with 15-acetyldeoxynivalenol (15ADON, 1000ng), 3-acetyldeoxynivalenol (3ADON, 500ng) or a combination of the three toxins (4000ng of DON, 1000ng of 15ADON and 500ng of 3ADON). The results showed that, when fed DON alone, DON was found in both the fed spikelets and adjacent fragments of rachis. Additionally in DON treatments, both 15ADON and 3ADON were recovered but only from the fed spikelets. When fed with 15ADON, both DON and 15ADON were recovered from both the fed spikelets and adjacent fragment of rachis. When fed with 3ADON, both DON and 3ADON were detected only in the fed spikelets. In all cases, change in the amount of toxins followed the same pattern: reaching the highest cumulative level on the fifth or sixth day of the experiment, and then decreasing to a lower level on the seventh or eighth day despite continued toxin addition. Toxin levels increased from the ninth day until end of this experiment. When fed with the combination of DON, 15ADON and 3ADON in the ratio of 8:2:1, the relative amounts of the three toxins recovered from the fed spikelets varied significantly, from 24:3:1 to 80:7:1. Nivalenol was not detected in any treatment. We hypothesize that the resistant wheat plant metabolizes trichothecenes into different forms as well as other uncharacterized metabolic products. (This study is supported by National Natural Science Foundation of China, 30170601and 39870471)

YEAST STRAINS ALLOWING PHENOTYPIC DETECTION OF ESTROGENIC ACTIVITY: DEVELOPMENT OF A SENSITIVE AND INEXPENSIVE YEAST BIOASSAY FOR ZEARALENONE

R. Mitterbauer[1], H. Weindorfer[1], N. Safaie[2], H. Bachmann[1] and G. Adam[1]*

[1]Center of Applied Genetics, University of Agricultural Sciences, Vienna, Austria; and
[2]Tarbiat Modarres University, Tehran, Islamic Republic of Iran
*Corresponding Author: PH: 43-1-36006-6380; E-mail: adam@edv2.boku.ac.at

ABSTRACT

Zearalenone (ZON) is a non-steroidal estrogenic mycotoxin produced by plant pathogenic species of *Fusarium*. As a consequence of infection with *F. culmorum* and *F. graminearum*, ZON can be found in cereals and derived food products. Since ZON is suspected to cause human disease such as premature puberty syndrome as well as numerous cases of hyper-estrogenism in farm animals, several countries have established monitoring programs and guidelines for ZON levels in grain intended for human consumption and animal feed. Austria, for instance, has set guideline levels of 60 µg/kg for wheat intended for human consumption and 50 µg/kg for whole feed for breeding pigs. In epidemic situations much higher levels have been found, for instance average levels of more than 500 µg/kg wheat have been measured in Northern Germany in 1998. In Northern Iran highly contaminated wheat has been reported for 1996 (35/35 samples positive, average ZON level of 3,4 mg/kg).

We have developed a low-cost method for monitoring of ZON contamination in grain based on a sensitive yeast growth bioassay. The indicator *Saccharomyces cerevisiae* strain YZRM7 is unable to grow, unless an engineered pyrimidine biosynthetic gene is activated by the ex-pressed human estrogen receptor in the presence of exogenous estrogenic substances. The deletion of the genes encoding ATP-binding cassette (ABC) transporters Pdr5p and Snq2p increases net ZON uptake synergistically. Less than 1 µg ZON per liter medium is sufficient to allow growth of the indicator strain. To prevent interference with pyrimidines potentially present in biological samples, we have also disrupted the genes *FUR1* and *URK1*, blocking the pyrimidine salvage pathway. The bioassay strain YZRM7 allows qualitative detection and quantification of total estrogenic activity in cereal extracts without requiring further clean up steps. The high sensitivity makes this assay suitable for low cost monitoring of contamination of maize and small grain cereals with estrogenic *Fusarium* mycotoxins.

We have furthermore constructed yeast strains allowing phenotypic detection of the estrogenic activity of ZON by engineered *ADE2* and *MEL1* genes. Together with a positive selection marker (*URA3* with estrogen responsive elements in the promoter), such easily screenable markers are valuable tools for cloning ZON degradation genes by expression of cDNA libraries in yeast.

DIAGNOSTIC VOMITOXIN (DON) SERVICES IN 2002/2003 SAMPLES

M.S. Mostrom[1]*, P. Schwarz[2], Y. Dong[3] and P. Hart[4]

[1]Dept. of Veterinary Diagnostic Services, [2]Dept. of Cereal Science, North Dakota State University, Fargo, ND 58105; [3]Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108; and [4]Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824
*Corresponding Author: PH: (701) 231-7529; E-mail: Michelle.Mostrom@ndsu.nodak.edu

OBJECTIVES

To provide analytical services for deoxynivalenol testing for researchers investigating mitigation of Fusarium head blight in wheat and barley.

INTRODUCTION

Deoxynivalenol (DON or vomitoxin) concentrations in cereal grains are an indicator of Fusarium head blight (FHB). Researchers evaluating techniques to reduce the adverse effects of in grains have used DON to determine the resistance in cultivars. Mycotoxin testing, specifically DON testing, is an important part of the cooperative efforts to reduce FHB. In 2002, the U.S. Wheat and Barley Scab Initiative provided grants to four regional DON testing laboratories in Michigan, Minnesota, and North Dakota to analyze for DON in wheat and barley cultivars.

MATERIALS AND METHODS

The four laboratories involved in DON testing are listed below. The analytical methods used by the laboratories include ELISA, gas chromatography with electron capture detection, and gas chromatography with mass spectrometry analysis. The sample preparation method used for the gas chromatography analysis was developed by Tacke and Casper (1996).

The individual laboratories conduct their own intralab quality control on appropriate control pools throughout the analysis period for DON testing to ensure quality of the analysis. The intralab quality control data spans the time from the beginning of DON testing in the labs through the end of October in 2002. Additionally, a collaborative quality assurance program, using wheat, was conducted among the laboratories (P. Hart, coordinator). Each laboratory was requested to perform analyses from a divided sample on two successive days within a 30 day period. The collected data were summarized and sent back to the laboratories. These check samples allowed each laboratory to evaluate the accuracy and precision of their system. Data from three check sample tests are included (May through October 2002).

Data (May through October 2002) are also included from a larger collaborative quality assurance program conducted by North Dakota State University, Department of Cereal Sciences. All four laboratories participate in this program, which uses malt and barley as check samples. These data are included for additional matrix quality assurance information.

Laboratories :

Patrick Hart, Ph.D., Department of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824; Phone: 517-353-9428, FAX: 517-353-5598, e-mail: hart@msu.edu
Method: water extraction and DON quantitation with the Neogen Veratox test (ELISA)
Sample types: wheat

Yanhong Dong, Ph.D., Department of Plant Pathology, University of Minnesota, St. Paul, MS 55108; Phone: 612-625-2751, FAX: 612-625-9728; e-mail: dongx001@umn.edu
Method: acetonitrile and water extraction, silylation and quantitation by gas chromatography/ mass spectrometry (GC/MS)
Sample types: wheat, barley, (bulk, single head, single spikelet, single kernel, and small fragment)

Paul Schwarz, Ph.D., Department of Cereal Science, North Dakota State University, Fargo, ND, 58105; Phone: 701-231-7732, FAX: 701-231-7723, e-mail: Paul.Schwarz@ndsu.nodak.edu
Method: acetonitrile and water extraction, silylation and quantitation by gas chromatography/ electron capture detection (GC/ECD)
Sample types: barley, malt, single kernel

Beth Tacke, B.A., Department of Veterinary Diagnostic Services, North Dakota State University, Fargo, ND, 58105; Phone: 701-231-8309, FAX: 701-231-7514, e-mail: Beth.Tacke@ndsu.nodak.edu
Method: acetonitrile and water extraction, silylation and quantitation by GC/ECD
Sample types: wheat, barley

RESULTS AND DISCUSSION

Table 1 summarizes the participating number of collaborators (principal investigators), number of states, and estimated number of DON samples to be analyzed in the grant year of May 2002 through April 2003. Approximately 24,500 cereal grain samples submitted by about 57 principal investigators in 14 different states investigating FHB will be analyzed for DON by the four laboratories in 2002/2003.

The intralab coefficient of variation for the four laboratories varies from 6 to 16 % on the grain control pools that were analyzed with the samples during 2002 (Table 2). The interlab proficiency check samples for FHB testing show that the four laboratories are determining similar results, in a wheat matrix, using different analytical methods (Table 3).

Additional interlab check samples were analyzed in malt and barley matrices by the same four laboratories using the same methodology (Table 4). The data in Table 4 represent the time frame from May through October 2002, and are part of a two-year check sample program involving a number of participants. The *z*-value is given for each laboratory by month. [Note that the *z*-values were calculated as the lab's individual value minus the sample mean divided by the sample standard deviation. A smaller *z*-value represents less spread of actual results and higher accuracy and precision.] The data in Table 4 show that the *z*-values for the different

laboratories are fairly small (close to zero) and no major differences were observed in analytical values at lower DON concentrations.

The repeatability of results on successive days reflects the precision of the analysis and was good for the interlab FHB check samples for those cooperating laboratories. Also, the intralab coefficients of variations on the control pools were fairly low for the participating diagnostic lab. These results indicated that the variation in analyses over several days is low and no major differences in analytical values of check samples occurred between the four DON diagnostic centers.

Table 1. Estimated DON analyses by laboratories in 2002 through 2003

DON Laboratory	Number of Collaborators	Number of States	Estimated Number of Samples Tested in 2002
MI: P. Hart	20	9	3,000
MN: Y. Dong	9	3	10,000
ND: P. Schwarz	4	3	7,500
ND: B. Tacke	25	7	4,000

Table 2. Intralab quality control data for DON testing through October 2002

DON Laboratory	Grain	Number	Mean (ppm)	Standard Deviation	Coefficient of Variation (%)
MI: P. Hart	Wheat	122	0.9	0.1	12
MN: Y. Dong	Wheat	30	7.2	0.9	13
ND: P. Schwarz	Barley	31	13.8	2.0	15
	Barley	18	39.7	4.9	12
	Barley	9	2.1	0.3	13
ND: B. Tacke	Wheat	104	1.8	0.1	7
	Barley	104	3.1	0.2	6
	Corn	104	4.7	0.5	11

Table 3. Interlab proficiency check samples for DON testing for FHB

DON Laboratory	Grain	DON (ppm)					
		Test 1		Test 2		Test 3	
MI: P. Hart	Wheat	1.0	1.0	4.0	4.4	<0.5	<0.5
MN: Y. Dong	Wheat	0.77		3.8		0.3	
ND: P. Schwarz	Wheat	0.6	0.4	3.0	3.2	0.3	
ND: B. Tacke	Wheat	0.9	1.0	2.8	2.6	0.4	0.4
Mean ± std.dev.		0.8 ± 0.2		3.4 ±0.7		0.4 ± 0.1	

Table 4. Interlab check samples for DON in barley (bar) and malt from May through October 2002 by four laboratories (part of a larger check sample program, North Dakota State University, Department of Cereal Sciences)

Lab	DON (ppm)											
	May		June		July		August		September		October	
	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt
MI: P. Hart	5.00	0.60	2.40	0.60	1.70	<0.5	3.20	0.60	4.40	<0.5	4.30	0.70
MN: Y. Dong	5.35	0.26	2.57	0.31	1.43	0.13	4.02	0.40	4.58	0.12	18.47	0.39
NDSU: P.Schwarz	4.60	0.20	2.20	0.30	1.50	0.15	2.80	0.20	4.00	0.10	20.30	0.50
NDSU: B. Tacke	5.50	0.40	2.40	0.40	1.60	<0.2	3.70	0.40	5.00	0.20	17.30	0.40
Sample MEAN	5.11	0.37	2.39	0.40	1.56	0.14	3.43	0.40	4.5	0.14	15.09	0.50
Sample Std. Dev.	0.40	0.18	0.15	0.14	0.12	0.01	0.54	0.16	0.41	0.05	7.30	0.14
Z-values by Lab	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt
MI: P. Hart	-0.28	1.32	0.05	1.42	1.21		-0.43	1.22	-0.23		-1.48	1.41
MN: Y. Dong	0.59	-0.59	1.17	-0.66	-1.08	-0.71	1.10	0.0	0.20	-0.38	0.46	-0.75
NDSU: P.Schwarz	-1.28	-0.93	-1.27	-0.74	-0.49	0.71	-1.17	-1.22	-1.19	-0.76	0.71	0.02
NDSU: B. Tacke	0.97	0.20	0.05	0.0	0.36		0.50	0.0	1.22	1.13	0.30	-0.68

REFERENCES

Tacke, BK and Casper, HH. 1996. Determination of deoxynivalenol in wheat, barley, and malt by column and gas chromatography with electron capture detection. J A O A C International 79:472-475.

HUMAN SUSCEPTIBILITY TO TRICHOTHECENE MYCOTOXINS

James J. Pestka[1,2] *, Kristen Penner[1] and Jennifer Gray[2]

[1]Dept. of Food Science and Human Nutrition and [2]Dept. of Microbiology and Public Health,
Michigan State University, East Lansing, MI 48824
*Corresponding Author, PH: 517-353-1709; E-mail: pestka@msu.edu

ABSTRACT

The trichothecene deoxynivalenol (DON), also given the colloquial name vomitoxin, has occurred with alarming frequency in wheat, corn and barley produced Michigan and Midwest. A major concern is that, because of the paucity of information on human toxicity, action levels might be set artificially low, thereby reducing the marketability of Michigan wheat containing trace levels of DON but posing no risk. Based on studies in the mouse model, we believe that the most critical step for toxicity induction by DON and other trichothecenes are their action on leukocytes (white blood cells) either by activation of cellular hormones known as cytokines or by the induction of programmed cell death (apoptosis). If human leukocyte cytokine dysregulation and/or apoptosis induction are indeed targeted by the same levels of DON and other 8-ketotrichothecenes in mice as in the mouse, then the risk of low ppm levels of DON to humans will be extremely small when one considers the diversity of the human diet and the actual potential level of DON exposure in human tissues. Two types of models are being used to test this hypothesis- cloned and primary cells.

DON and other 8-ketotrichothecenes induce, in the U937 human macrophage clonal model the production of three critical proinflammatory mediators, namely, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and the chemokine, interleukin-8 (IL-8). Interestingly, the higher trichothecene concentrations markedly reduced proliferation and were cytotoxic. The key signals for cytokine upregulation are likely to involve ribosomal binding DON ribosome binding ÿ protein kinase R/Hck kinase ÿ MAPKinases ÿ cytokine upregulation. DON also affected a cloned human T lymphocyte model (Jurkat cells). Although DON stimulated IL-2 production, the four other 8-ketotrichothecenes did not stimulate production of this cytokine. DON and 15-acetyl DON at 60 to 500 ng/ml and 3-acetyl DON at 600 to 5000 ng/ml could induce IL-8 production, whereas NIV and FX were not stimulatory. Again, the higher trichothecene concentrations markedly impaired proliferation and were cytotoxic. conditions optimized for the primary culture of human leukocytes and conducted preliminary experiments on the effects of DON.

Two primary leukocyte culture approaches have been evaluated. The first involved direct culturing of human blood obtained from volunteers. Using the first approach, we have observed that DON will directly induce IL-6. Of particular importance was the finding that some donors were much more sensitive to DON-induced IL-6 than others in terms of minimum effective DON concentration and magnitude of response. Furthermore, the doses required for these effects in primary cells appear to be lower than for cloned cell models suggesting that human primary cells are slightly more sensitive to DON and potentially other trichothecenes. Analogous results were found for IL-8 but sensitive donors did not correspond to IL-6 responders. Rather, the low IL-6 responders were high IL-8 responders. The second type of primary culture

involved leukocytes obtained from processed Red Cross blood. Similar variability and sensitivity was observed in these cells. However, since we did not have control of these samples from initial blood draw and know nothing about the donors, it will be difficult to reproduce or interpret findings from with Red Cross materials. Thus, we will focus all future efforts on human blood culture.

It will be important to ascertain whether blood cells from specific individuals are more or less sensitive to the toxin and whether these effects are consistent over repeated blood collections. If so, it will suggest that toxin-susceptible and resistant individuals may exists among the human population, possibly because of genetic polymorphisms related to toxin metabolism or cellular target interaction. If responses are variable among the same individual, it will be possibly indicative that a hormonal or environmental factor (eg diet, medication) differentially affects an individual response to DON. Either type of information will be critical for conducting accurate risk assessments for DON and other 8-ketotrichothecenes.

USING NEAR INFRARED TRANSMITTANCE AS A SCREENING TOOL FOR DON IN BARLEY

H. Pettersson[1], L. Aberg[2], J.A. Persson[2], H. Andren[2], and M. Matteson[3]*

[1]Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden; [2]Foss Tecator AB, Hoganas, Sweden; and [3]Foss North America, Eden Prairie, MN, USA
*Corresponding Author: PH: (952) 974-9892; E-mail: mmatteson@fossnorthamerica.com

ABSTRACT

Near infrared transmittance (NIT) spectroscopy has been investigated for rapid estimation of deoxynivalenol (DON) in whole kernel barley (and wheat). Barley samples, spectra, and reference analysis data provided by study participants from the USA, Austria, and France. All spectra (570 – 1100 nm) collected using the FOSS Infratec 1241 Grain Analyzer with the added color module, and reference analysis using HPLC or GC methods. Calibrations tested included partial least squares (PLS) and artificial neural network (ANN) for DON (ppb, ppm) and log (DON ppb). Independent validation data showed best performance using the ANN calibration log (DON ppb) across locations (N 257, Slope 0.79, Correlation 0.88, SED 0.3024, and Bias –0.0013). Based on current findings, it appears NIT can be used as a screening tool to measure DON in barley. It is recommended to use a limit of 3.5 (3.2 ppm), values above 3.5 indicate the sample might be infected and should be set aside for further testing (HPLC, GC). DON calibration development work will continue, by expanding calibration databases to include additional growing seasons, locations, and by addressing issues associated with sampling and laboratory errors, to improve accuracy and precision of DON analysis using NIT.

STORAGE OF SCABBY WHEAT: *FUSARIUM* GOES AWAY, DON DOESN'T

Robert W. Stack[1]*, Howard H. Casper[2], and Dennis J. Tobias[1]

[1]Dept. of Plant Plathology and [2]deceased, formerly Dept. of Veterinary Science and Microbiology, North Dakota State University, Fargo, ND 58105
*Corresponding Author: PH: (701) 231-7077; E-mail: rstack@ndsuext.nodak.edu

ABSTRACT

Some 60 years ago, R. G. Shands at the University of Wisconsin reported that scabby barley stored for five years (1931-1936) retained its emetic activity when fed to pigs. He proposed that the effect was due to some "toxic principle", then unknown, and not to the presence of the live fungus since the barley remained toxic although *Fusarium* cultures could no longer be recovered from it (Phytopathology 27:749-762). Today we would recognize that his "toxic principle" affecting the pigs was likely DON. Shands did actual feeding experiments with grain extracts to demonstrate the toxicity of his five-year-old barley samples. The widespread outbreak of FHB in the northern spring grain region in 1993 was perhaps the worst since 1928, the one that had prompted Shands' interest. We had evaluated a large number of scabby grain samples from the 1993 crop in eastern North Dakota and northwestern Minnesota. Many of these grain samples had DON levels in excess of 10 ppm, some as high as 50 ppm. Some grain samples from this survey had been retained in storage since 1993. For the present study, we chose 50 of the 1993 wheat samples to re-analyze for DON in 2001. As originally analyzed, the grain samples had contained from <0.5 ppm to 18 ppm. The same procedure for extraction and analysis by GC-MS was used in 1993 and in 2001. The level of DON found in the 2001 analysis of these samples was about 73% of that found in 1993. That ratio of the two analyses was remarkably consistent for most of the samples ($R^2 = 0.90$). The correlation of DON to presence of tombstone kernels in grain was nearly the same for ine 1993 as the 2001 analyses ($R^2 = 0.60, 0.62$, respectively). When cultured in 1994, 65% of the kernels in 1993 grain samples had given colonies of *Fusarium graminearum*. Despite the substantial amount of DON remaining in this grain in 2001, not a single culture of *F. graminearum* could be recovered when the 660 representative scabby kernels from these samples were plated out on media suitable for recovery of *Fusarium*. Our results show that the commonly-held assumption that DON is long-lasting in grain is correct for wheat as well as barley. The wheat samples tested represented multiple locations in the region and several cultivars; neither site not cultivar showed any particular deviation from the overall relationship of 1993 to 2001 DON. (This poster was presented at the 2002 Annual Meeting of the Canadian Phytopathological Society, Waterton Lakes, Alberta, June 2002.)