DETECTION OF SCAB-DAMAGED WHEAT KERNELS BY NEAR-INFRARED REFLECTANCE

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ABSTRACT

Both wheat breeder and wheat inspector must currently deal with the assessment of scab in harvested wheat by manual human inspection. We are currently developing and examining the accuracy of a semi-automated wheat scab inspection system that is based on near-infrared (NIR) reflectance (1000 to 1700 nm) of individual kernels. Our initial work revealed that, for scanning, the kernels could be oriented in just a semi-random basis, in which the rotational angle about a kernel's long axis was arbitrary. Classification analysis has involved the application of various statistical classification techniques, including linear discriminant analysis, soft independent modeling of class analogy (SIMCA), partial least squares regression, and non-parametric (*k*-nearest-neighbor) classification. For the most recent year evaluated (2002), average cross-validation accuracy ranged from 82.1% (a wavelength difference, without kernel mass, model) to 89.6% (a *k*-nearest-neighbor, with kernel mass, model). Although the lower value in this range was indeed lower than that for a model using mass alone (83.8%), the corresponding accuracies of these models on a separate (fully independent) test set indicated that the spectrally based models, with accuracies in excess of 92% were clearly better than the mass alone model. Based on test set accuracy, there were only slight differences between models that were based on principal component scores and those that were based on a simple wavelength difference. Typically, test set accuracies were between 94 and 97 percent. For the *k*-nearest-neighbor model, the number of neighbors needed to achieve stable optimal accuracies was approximately 20. An exhaustive search of the most suitable wavelength pairs for the absorbance [$A = \log(1/R)$] difference, [$A_{\lambda 1} - A_{\lambda 2}$], revealed that the low-wavelength side of a broad carbohydrate absorption band (centered around 1200 nm) was very effective at discriminating between healthy and scab-damaged kernels, with accuracies at about 95%. The best wavelength difference was [$A_{1248\ nm} - A_{1140\ nm}$]. Although the average cross-validation accuracy was lower for this model (82.1%) compared to the *k*-nearest-neighbor model (83.3%), the test set accuracies were nearly identical (94.9% and 95.0%). Many other wavelength differences, [$A_x - A_y$], produced cross-validation accuracies that were within 0.5 percentage units of the optimal difference, with values for *x* favoring the 1150-1300 nm region, and values for *y* favoring the 1000-1150 region. Combined, these regions define the broad absorption band centered near 1200 nm, which is attributed to the second overtone of a carbohydrate CH stretch. The achieved accuracy levels demonstrate the potential for the use of NIR in inspection operations for wheat scab. Therefore, development of an automated, high-speed device utilizing as few as two wavelengths for wheat scab detection appears to be feasible.

UPDATE ON USWBSI DON DIAGNOSTIC LABORATORIES

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OBJECTIVES

To provide DON testing services for collaborators investigating Fusarium head blight (FHB) in barley and wheat.

INTRODUCTION

Deoxynivalenol (DON) is a mycotoxin produced by Fusarium molds. DON analysis is used as an indicator of FHB contamination. Researchers are studying ways to reduce/eliminate the effects of FHB contamination in grain and grain products. In 2003, the U.S. Wheat and Barley Scab Initiative again have provided funding to four regional labs for free DON analysis to researchers. The four regional DON testing labs are located in North Dakota, Minnesota, and Michigan. Contacts for these regional labs are as follows:

Patrick Hart, Ph.D., and Benjamin Munn, Department of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824;
Ph: 517-353-9428, Fax: 517-353-5598, E-mail: hartl@pilot.msu.edu
Method: Neogen Veratox test (ELISA)
Sample type(s): wheat

Paul Schwarz, Ph.D., and James Gillespie, Department of Plant Sciences, North Dakota State University, Fargo, ND 58105;
Ph: 701-231-7732, 701-231-1040, Fax: 701-231-8474, E-mail: James.Gillespie@ndsu.nodak.edu, Paul.Schwarz@ndsu.nodak.edu
Method: Tacke and Casper (1996) by GC/ECD
Sample type(s): barley, malt, and single kernel

Michelle Mostrom, Ph.D., and Beth Tacke, Dept. of Veterinary Diagnostic Services, North Dakota State University, Fargo, ND 58105;
Ph: 701-231-7529, Fax 701-231-7514, E-mail: Michelle.Mostrom@ndsu.nodak.edu
Method: Tacke and Casper (1996) by GC/ECD
Sample type(s): wheat and barley

Yanhong Dong, Ph.D., Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108;
Ph: 612-625-2751, Fax: 612-625-9728; E-mail: dongx001@umn.edu
Method: Tacke and Casper (1996) by GC/MS
Sample type(s): wheat and barley (bulk, single head, single spikelet, single kernel, and small fragment)

MATERIALS AND METHODS

Labs in North Dakota (Mostrom and Schwarz) use the method of Tacke and Casper (1). The lab in Minnesota (Dong) using the extraction procedure from Tacke and Casper but with quantification using GC/MS. The lab in Michigan (Hart) uses the Neogen Veratox system (ELISA).

All labs maintain there own intralab quality control with analysis of check samples. An interlab wheat check program was also maintained by Michigan State University. Each lab received monthly a wheat sample that was divided and analyzed on separate days for repeatability. Also North Dakota State University (Schwarz) sent out monthly check samples of barley and malt for analysis to the collaborative labs as part of a larger collaborative with industry.

RESULTS AND DISCUSSION

Table 1 shows the number of collaborators and number of state sending samples to be analyzed by the four regional labs. The numbers have increased in the past two years and are also shown in table 2 where the number of samples has increased the past four years with 30,000 samples expected to be analyzed in 2003/2004.

Intralab data shown in table 3 shows the coefficient of variances between 6-18% which is the same as previous years. Tables 4 and 5 show the interlab data from North Dakota State University and Michigan State University respectively. Data from both collaboratives with barley, malt, and wheat samples show that there are no significant differences between labs.

REFERENCES

Tacke, B.K., and Casper, H.H. 1996. Determination of deoxynivalenol in wheat, barley, and malt by column and gas chromatography with electron capture detection. J. AOAC Intl. 79:472-475.

Table 1. Number of collaborators and states.

DON Lab	Number of Collaborators				Number of States			
	00/01	01/02	02/03	03/04	00/01	01/02	02/03	03/04
MI-P. Hart	9	17	20	18	8	11	9	12
MN-Y. Dong	11	12	9	11	2	1	3	3
ND-P. Schwarz	4	3	4	8	2	2	3	4
ND- M. Mostrom	23	10	25	21	6	4	7	8
Total	47	42	58	58	18	18	22	27

Table 2. Samples analyzed for DON by labs.

DON Lab	Number of Samples Analyzed			2003/2004	
				Analyzed	Expected
	00/01	01/02	02/03	03/04	03/04
MI-P. Hart	2481	3371	3000	2420	4500
MN-Y. Dong	7533	8500	10000	3764	10500
ND-P. Schwarz	5222	4612	7500	2862	10000
ND- M. Mostrom	4436	4600	4000	3113	5000
Total	19672	21083	24500	12159	30000

Table 3. Intralab quality control date for DON analysis 2003/2004.

DON Lab	2000-2001				2001/2002			
	Sample	Number	Mean (ppm)	CV %	Sample	Number	Mean (ppm)	CV %
MI-P. Hart	Wheat	56	1.6	7	Wheat	94	2.3	5
MN-Y. Dong	Wheat	34	12.8	12	Wheat	38	9.0	14
ND-P. Schwarz	Barley	120	6.3	14	Barley	108	6.3	11
	Barley	112	1.6	16	Barley	104	1.5	16
	Barley	124	5.3	15	Barley	104	5.1	10
ND- M. Mostrom	Wheat	83	1.8	9	Wheat	31	1.7	5
	Barley	83	3.1	9	Barley	31	2.9	5
	Corn	83	5.0	9	Corn	31	4.6	7

DON Lab	2002/2003				2003/2004			
	Sample	Number	Mean (ppm)	CV %	Sample	Number	Mean (ppm)	CV %
MI-P. Hart	Wheat	122	0.9	12	Wheat	84	2.4	8
MN-Y. Dong	Wheat	30	7.2	13	Wheat	102	7.1	12
ND-P. Schwarz	Barley	31	13.8	15	Barley	75	1.9	18
	Barley	18	39.7	12	Barley	54	14.5	13
	Barley	9	2.1	13	Barley	18	40.3	12
ND- M. Mostrom	Wheat	104	1.8	7	Wheat	100	1.8	6
	Barley	104	3.1	6	New Wheat	100	1.1	10
	Corn	104	4.7	11	Barley	100	3.0	8
					Corn	100	4.7	7

Table 4. Interlab quality control data 2003 NDSU.

LAB	Malt Check Samples (ppm DON)									
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct
ND-P. Schwarz	1.70	1.10	0.70	0.40	0.50	0.50	0.07	0.00	0.40	0.43
ND- M. Mostrom	1.90	1.20	0.50	0.40	0.40	0.40	<0.20	<0.20	0.30	0.40
MN-Y. Dong	1.21	0.44	0.33	0.33	0.41	0.34	0.00	0.00	0.30	0.39
MI-P. Hart	3.20	1.20	0.50	<0.50	0.60	0.60	0.00	0.00		
Mean	2.00	0.99	0.51	0.38	0.48	0.46	0.02	0.00	0.33	0.41
SD	0.85	0.37	0.15	0.04	0.09	0.11	0.04	0.00	0.06	0.02
CV	42.41	37.20	29.81	10.73	19.52	24.85	173.21		17.32	5.12

LAB	Barley Check Samples (ppm DON)									
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct
ND-P. Schwarz	1.20	0.00	1.60	0.00	10.20	0.60	6.90	1.60	1.55	0.63
ND- M. Mostrom	1.30	<0.20	1.90	<0.20	10.40	0.80	5.70	2.30	1.60	0.80
MN-Y. Dong	0.77	0.00	1.67	0.00	10.90	0.66	4.96	2.08	1.38	0.72
MI-P. Hart	1.40	0.00	2.10	0.00	6.00	0.60	4.30	2.00		
Mean	1.17	0.00	1.82	0.00	9.38	0.67	5.47	2.00	1.51	0.72
SD	0.28	0.00	0.23	0.00	2.27	0.09	1.11	0.29	0.12	0.09
CV	23.75		12.53		24.20	14.19	20.39	14.65	7.64	11.87

Table 5. Interlab quality control data 2003 Michigan State University.

LAB	Wheat Check Samples (ppm DON)								
	5	6	7	8	9	10	11	12	13
ND-P. Schwarz	0.06	2.30	1.50	0.90	1.20	1.30	1.00	1.22	0.38
ND- M. Mostrom	<0.20	2.20		1.20	1.25	1.65	1.10	1.40	0.50
MN-Y. Dong	0.12	1.24	1.46	1.11	1.17	1.02	1.01	1.36	0.51
MI-P. Hart	0.00	2.30	2.60	1.00	1.60	1.60	1.20	1.64	0.60
Mean	0.06	2.01	1.85	1.05	1.31	1.39	1.08	1.41	0.50
SD	0.06	0.52	0.65	0.13	0.20	0.29	0.09	0.17	0.09
CV	100.00	25.65	34.91	12.40	15.28	21.01	8.65	12.43	18.16

APPLICATION OF REAL TIME POLYMERASE CHAIN REACTION TO THE DETECTION AND QUANTIFICATION OF *FUSARIUM* IN WHEAT

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OBJECTIVES

To examine the relationship between Fusarium head blight symptoms, levels of deoxynivalenol, and the amount of *Fusarium* DNA in individual kernels of wheat.

INTRODUCTION

Fusarium head blight (FHB) of small grains has been problematic for commercial buyers and processors of small grains. To processors, the contamination of the grain with deoxynivalenol (DON) is a food safety issue, and the FDA has specific guidelines for the amounts of DON that can be in food and feed intended for animal and human consumption (1). Generally, small grains are screened for DON at the buying point using one of the several tests commercially available. The amount of testing is dependent on how much disease is observed in fields prior to harvest, which is usually based on visual symptoms on the heads. Jones, et al (2) reported a significant correlation of DON levels with FHB symptoms in spring wheat, but there was wide variation in DON for any one category of symptoms. Since the year 2000 there have been several reports of DON levels in winter wheat that don't always correlate with the amount of FHB symptoms in the field, i.e. low levels of DON when more than ten percent of the heads had FHB symptoms, and high levels of DON when FHB head symptoms were below ten percent. Our objectives in these preliminary experiments were to divide a single sample of grain into categories of kernel symptoms, and then examine individual kernels for DON and *Fusarium* DNA.

METHODS AND MATERIALS

A sample of *Fusarium* infected wheat seed was obtained from Gene Milus, University of Arkansas, with a DON level of >30 ppm. The sample was divided into seven categories based on visual symptoms. The categories were;

1) Normal appearing seed.
2) Seed slightly smaller than sample 1 seed.
3) Seed similar in size to sample 1 but with some whitish discoloration.
4) Shriveled seed but no discoloration.
5) Shriveled seed with some white discoloration.
6) Slightly shriveled seed with a definite white discoloration of the entire seed, and
7) Similar to 6 but seeds also had some red discoloration of the seed coat. An additional 8) Michigan seed from 2003 crop with kernels normal in appearance. Used as a negative control.

Ten individual seeds from each sample were weighed and ground in 500 µl water to extract DON. The homogenates were centrifuged for 5 min and the water transferred to new 1.5 ml eppendorf tubes. The remaining pellet was mixed with 600 µl of Qiagen extraction buffer and total genomic DNA was extracted

with Qiagen DNeasy Plant Mini Kit according to manufacturer instructions. The resulting DNA was diluted 10^2 times (980 µl PCR water + 20 µl DNA eluate) to approximately 1 ng/µl (1µg/ml).

Real time PCR was carried in 25 µl volumes and consisted of 12.5 µl SYBR Green PCR Master mix, 300 nM of each primer, PCR water, and 10 µl of diluted DNA. Forward primer Tri5F (5'-CGACTACAGGCTTCCCTCCAA-3') and reverse primer Tri5R (5'-ATCCGCCATGCACTCTTTG-3') were designed using Primer express software and used to amplify an 85 bp sequence of Tri-5 DNA. Wheat ribosomal DNA 18 S Forward primer (5'-GCCTTCGTGCAAGTGATCCT-3') and reverse primer (5'-CAAGCGGTCAAACCAACCA-3') were used to amplify 18 S ribosomal DNA to normalize the quantities of Tri5 DNA. DNA standard were prepared for both Tri5 gene and 18S gene from the known amounts of DNA. Real-time PCR was performed in Microamp optical 96-well reaction plates using the automated ABI Prism 7000 sequence detector. The Real-time PCR reactions were run as follows: initial denaturation at 95°C for 10 min followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. PCR results were analyzed using ABI Prism 7000 SDS software. The amounts of DNA in each reaction well were calculated by the software according to standard DNA amounts (Standard Curve Method). Relative amounts of fungal DNA was obtained by normalizing the quantities of fungal DNA to the quantities of Wheat 18S DNA and by calibrating with a calibrator (wheat sample number 8).

RESULTS AND DISCUSSION

The standard curves for estimating concentrations of *Fusarium* and wheat DNA are shown in figures 3 and 4, respectively. There was a good correlation between DON and DNA in individual kernels (r^2=74.2%), and between sample means of DON and DNA (r^2=99.0%) (Table 1, and Figures 1 and 2). Using DON as a comparison with symptom categories, the categories in increasing order of DON were samples 8, 3, 1, 4, 2, 5, 6, and 7 (Table 1). Using increasing levels of DNA to compare with visual symptoms, the categories were samples 8, 3, 1, 4, 5, 2, 6, 7. Samples 5, 6 and 7 had high levels of *Fusarium* DNA and DON. These results suggest that DON and DNA are not necessarily correlated well with kernel symptoms of FHB infection. Schnerr, et al (3) also reported a good correlation between DNA and DON in infected wheat kernels, but they did not correlate either with FHB symptoms of individual kernels. Although these results are from a single sample of wheat divided into subjective categories based on appearance, they do suggest that individual kernels can be variable in relation to levels of infection and DON. More research is needed to support these data indicating that high levels of DON can occur in wheat with minimal symptoms of infection.

Table 1. Sample means and standard errors for DNA concentration (pg), DON (ppm), and kernel weight (mg).

SAMPLE	DNA (pg)		DON (ppm)		SEED WEIGHT (mg)	
	Mean	Std.Dev	Mean	Std.Dev	Mean	Std.Dev
Sample8	0.5	0.3	0.1	0.4	40.0	7.4
Sample3	3.0	2.8	0.9	1.3	23.7	6.1
Sample1	3.9	5.3	1.8	2.8	35.9	3.3
Sample4	5.2	5.7	3.9	8.6	16.0	3.2
Sample2	20.6	26.0	7.7	5.3	35.4	3.6
Sample5	15.9	10.8	31.6	56.4	15.5	4.1
Sample6	94.1	89.5	106.9	77.6	27.8	2.3
Sample7	207.8	132.6	245.8	126.5	15.1	1.9

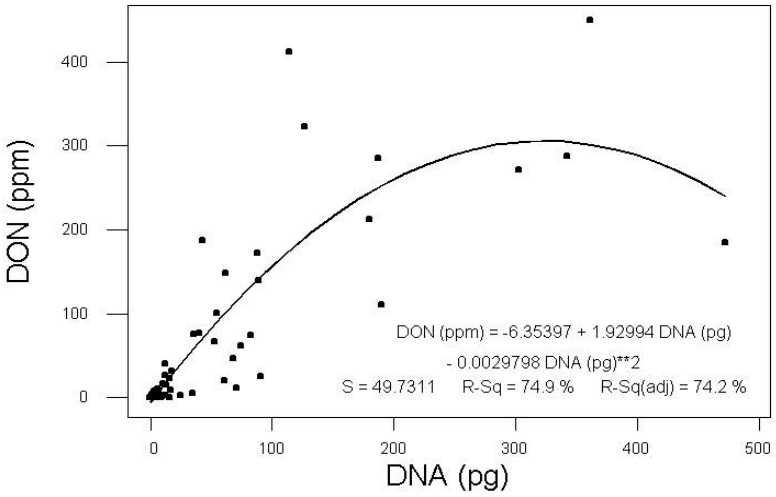

Figure 1. Regression plot for DON (ppm) and DNA (pg) for each of the eighty samples used in the study.

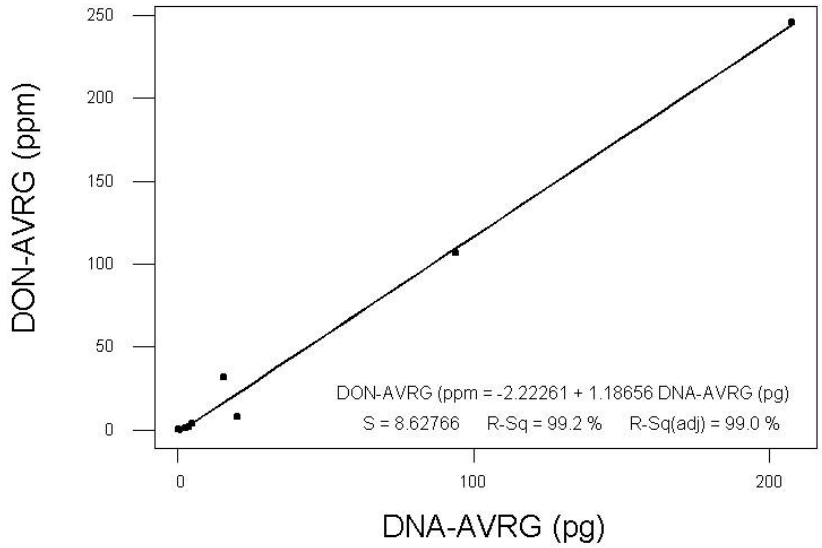

Figure 2. Regression plot of sample means for DON (ppm) versus DNA pg).

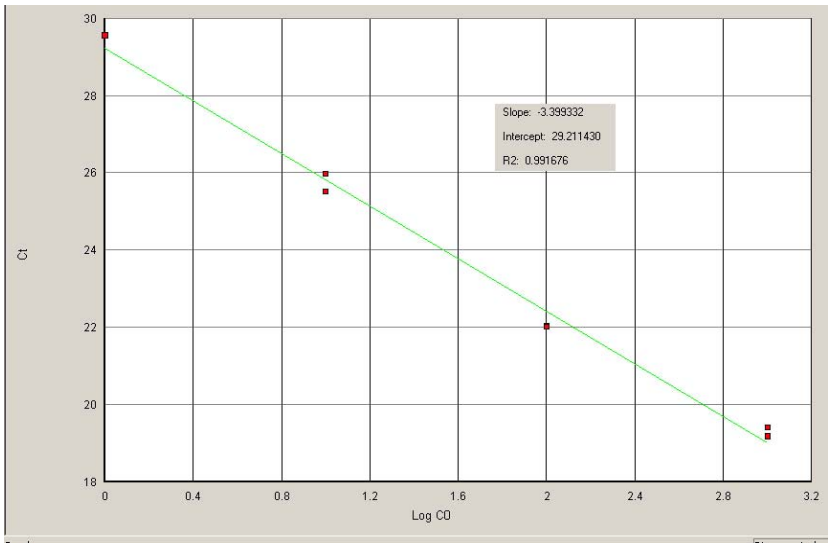

Figure 3. Standard Curve for Tri-5 gene primers-Tri5F-Tri5R.

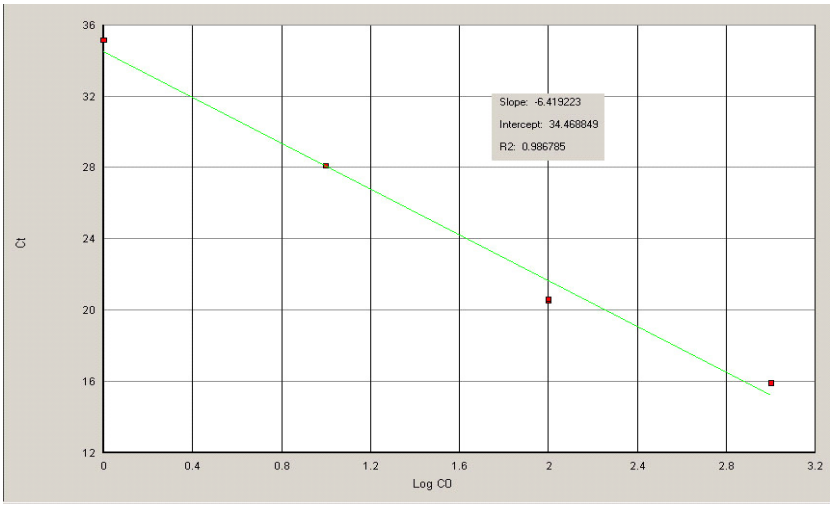

Figure 4. Standard Curve for Wheat rDNA 18S gene primers- W18SF-W18SR.

REFERENCES

Jones, R. K., and Mirocha, C. J. 1999. Quality parameters in small grains from Minnesota affected by Fusarium head blight. Plant Dis. 83:506-511.

McMullen, M., R. Jones, D. Gallenburg. 1997. Scab of wheat and barley. A re-emerging disease of devastating impact. *Plant Disease* 81:1340-1348.

Schnerr, H., L. Niessen, and R. F. Vogel. 2001. Real time detection of the *tri5* gene in *Fusarium* species by LightCycler™-PCR using SYBR Green I for continuous fluorescence monitoring. Intl. J. Food Microbiol. 71:53-61.

FUSARIUM HEADBLIGHT QUALITY ASSURANCE VIA IMMUNOCHEMISTRY

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OBJECTIVE

Determine the relationship between quantitative values of *F. graminearum* and DON in barley

INTRODUCTION

Quality assurance and quality control of any product is predicated upon a **direct, accurate, simple, and cost-effective** method of quantifying the component to quality. Currently FHB quantification is limited to a visual scoring system of seeds or seed heads. Visual scores have limitations to disease quantification and have led to the following conclusion. "… disease ratings in the field with other agronomic characteristics (yield) may reveal the limitations of relying on visual ratings customarily used to quantify disease severity" (Smith et al., 2003). This could also be the reason why no genetic source with complete resistance to FHB is yet known (Miedaner et al., 1995; 2003). Secondary fungal metabolites (DON) have also been used as an indicator of *Fusarium* presence. In the case of barley, however, we can not currently predict malt DON levels from barley DON levels with a high degree of accuracy when DON is <2.0 ppm (Schwarz et al 1995; Beattie et al., 1998). This is because DON production is dependent on environmental conditions (temperature and moisture), physiological development of the fungus (Hooker et al., 2002; Martins and Martins, 2002;) and storage conditions (Beattie et al., 1998). Thus, a need exists to develop a more direct and quantitative analysis for *Fusarium* to replace, or be used in conjunction with, FHB and DON analyses. Immunochemistry is a proven analytical tool that capitalizes on the specificity of antigen/antibody interactions capable of detecting minute quantities of the target antigen. Previously, we capitalized on our immunochemical experiences assessing infection status and toxin production of fungal endophytes in grasses to develop monoclonal antibodies specific to *Fusarium graminearum* during the winter of 2001/2002 (Hill et al., 2002a, 2002b, 2002c). Accordingly, our objective was to assess quantification of *Fusarium graminearum* with species-specific monoclonal antibodies in barley.

MATERIALS AND METHODS

Characterization of Antibody Utility for Barley – Two hundred and fifty grams of three barley samples were used to develop immunochemical methods. The three samples were a non-contaminated sample of 'Drummond' grown in Arizona, a low-contaminated sample of Drummond grown in Langdon, ND, and a highly contaminated sample of ND9712 grown in Langdon, ND. 1) Antigen extraction protocols were tested to determine the optimum conditions for immunoblot analysis by testing: a) temperature and time requirements for antigen extraction, and b) seed aspect when placed on nitrocellulose membranes for antigen extraction. 2) Antigen extraction protocols were tested to determine the optimum conditions for ELISA assays by testing: a) extraction of whole vs. ground seed, b) time necessary to extract the antigens, and c) single vs. a sequential extraction of antigens (antigens extracted after DON extraction performed).

Analysis of Barley Lines - Eighty-five doubled haploid (DH) lines from the cross Zhedar 2/ND9712/ Foster were planted into single row plots at Osnabrok and Langdon, ND in spring 2003. Corn was previously grown on each field, the stubble was chiseled in the fall, and plots planted directly into the corn stubble. The lines had two replicates at each location. The plots were rated for FHB at the soft dough stage of kernel development. After harvest, each line was tested for DON using HPLC-mass spectroscopy at North Dakota State University. Seed from each line was shipped to the University of Georgia, antigens extracted with the best protocol from antibody characterization studies in 2a, 2b, and 2c, and indirect ELISA used to quantify *F. graminearum* in each. Fifty seed from each sample were placed on nitrocellulose membranes and immunoblot analysis conducted using the best protocol from 1a and 1b to determine percent infection.

Analysis of variance was conducted to examine means and coefficients of variability for FHB, DON, and ELISA data within each location. FHB, DON, and ELISA data were correlated with each other within locations. To compare consistency of analysis among locations, FHB from Osnabrock was regressed with FHB from Langdon, DON from Osnabrock was regressed with that from Langdon, and ELISA quantification of *F. graminearum* from Osnabrock was regressed with that from Langdon.

RESULTS AND DISCUSSION

Characterization of Antibody Utility for Barley – We found the best method to extracting antigens for immunoblot was to place seed on top of nitrocellulose membranes saturated with extraction buffer. Seed had to be placed with the **crease side up** and extracted overnight at room temperature (Figure 1). Placing seed with the crease side down resulted in a meniscus of water in the crease that, upon removal of seed, led to spreading of the antigens over the entire nitrocellulose membrane. We found that ground seed decreased the detectable F. graminearum in our ELISA test. Antigen detection was possible with as little as 15 minutes seed incubation on the nitrocellulose membrane but we found that incubating overnight gave greater intensity of color development in seed contaminated with *F. graminearum* while having no effect on the seed that were not.

Extraction of *F. graminearum* antigen from ground seed suppressed the ELISA analysis. Mean of whole seed and ground seed extracts in our experiment was 148 and 92 ug *F. graminearum* per g seed, respectively. Antigen presence in whole seed were greatest when extracted for 1 hour (Figure 2) and ELISA quantification of *F. graminearum* in the three samples gave results consistent with DON even though the two North Dakota samples were 100% infected (Table 1).

Analysis of Barley Lines - The range of ELISA data for *F. graminearum* was greater than either FHB or DON. CV's were greatest for FHB followed by DON and ELISA data (Table 2). FHB scores did not correlate with DON or ELISA in the Langdon samples but were correlated to both in the Osnabrock samples. DON and ELISA data correlated with one another regardless of where the samples originated (Table 3). FHB scores for barley lines grown at Osnabrock did not correlate with those at Langdon, but DON and ELISA values for the lines among locations had low correlations.

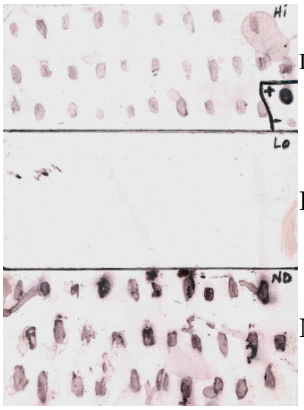

Figure 1. Immunoblot of *F. graminearum* in Drummond from ND, Drummond from AZ, and ND18172 from ND.

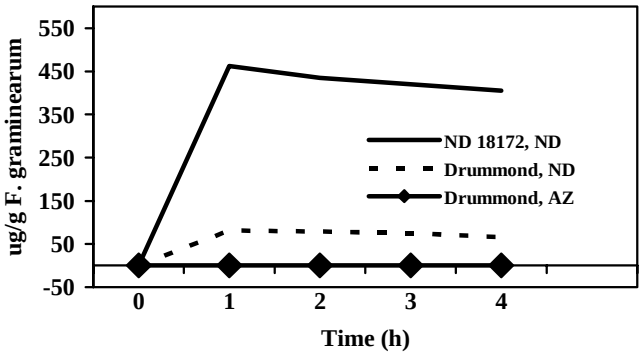

Figure 2. ELISA quantification of *F. graminearum* as affected by extraction time in barley.

Table 1. Infection frequency and mycelial mass of *F. graminearum* in three barley samples. These values correspond to the samples from the immunoblot in Figure 1, to the left of this table.

Line ID	DON	% infected	*F. graminearum*
	ppm	(immunoblot)	ug/g seed
Drummond-AZ	0	0	0.00
Drummond-ND	1.3	100	81
ND18172-ND	24.7	100	462

Table 2. Range, means, and coefficients of variation (CV) for head blight scores, DON, and ELISA quantification of *F. graminearum* in North Dakota field-grown barley.

	Osnabrock			Langdon		
	FHB	DON	ELISA	FHB	DON	ELISA
	%	ppm	ug/g seed	%	ppm	ug/g seed
Range	.06-.43	13.0-71.6	58.9-133.0	0.0-8.7	19.8-106.1	85.9-252.4
Mean	0.18	34.3	97.3	1.36	54.0	147.4
CV (%)	58.1	39.9	14.1	84.5	25.7	28.8

Table 3. Correlation matrix among FHB, DON, and ELISA data for barley grown at Osnabrock and Langdon, ND.

	Osnabrock			Langdon		
	FHB	DON	ELISA	FHB	DON	ELISA
FHB	-	0.42	0.49	-	-0.04	-0.14
DON			0.57	-	-	0.44
Osn vs. Langdon	0.07	0.21	0.23			

REFERENCES

Beattie, S. P.B. Schwarz, R. Horsley, J. Barr, and H.H. Casper. 1998. The effect of grain storage conditions on the viability of *Fusarium* and deoxynivalenol production in infested malting barley. J. Food Protection 61:103-106.

Hooker, D.C., A.W. Schaafsma and L. Tamburic-Ilincic. 2002. Using weather variables pre- and post-heading to predict deoxynivalenol content in winter wheat. Plant Dis. 86:611-619.

Hill, N.S., R.W. Stack, and A.E. Glenn. 2002a. Monoclonal antibodies to *Fusarium graminearum*. Agronomy Abstracts.

Hill, N.S., E.E. Hiatt, J.P. DeBatista, J.P. Costa, M.C. Costa, C.H. Griffiths, J. Klap, D. Thorogood, and J.H. Reeves. 2002b. Seed testing for endophytes by microscopic and immunoblot procedures. Seed Sci. and Technol. 30:347-355.

Hill, N.S., J.H. Bouton, F.N. Thompson, L. Hawkins, C.S. Hoveland, and M.A. McCann. 2002c. Performance of tall fescue germplasms bred for high- and low-ergot alkaloids. Crop Sci. 42:518-523.

Martins, M.L. and H.M. Martins. 2002. Influence of water activity, temperature and incubation time on the simultaneous production of deoxynivalenol and zearalenone in corn (*Zea mays*) by *Fusarium graminearum*. Food Chemistry 79:315-318.

Miedaner, T., B. Schneider, and H.H. Geiger. 2003. Deoxynivalenol (DON) content and Fusarium head blight resistance in segregating populations of winter rye and winter wheat. Crop Science 43:519-526.

Miedaner, T., D.E. Ziegler, and H.H. Geiger. 1995. Variation and covariation for quantitative resistance to head blight (*Fusarium culmorum*) in two testcross series of S_2 lines in winter rye. Plant Breed. 114:155-159.

Schwarz, P.B., Casper, H.H., and Beattie, S. 1995 The fate and development of naturally occurring *Fusarium* mycotoxins during malting and brewing. J. Am. Soc. Brew. Chem. 53(3(:121-127.

Smith, J.D., K.K. Kidwell, M.A. Evans, R.J. Cook, and R.W. Smiley. 2003. Assessment of spring wheat genotypes for disease reaction to Rhizoctonia solani AG-8 in controlled environment and direct-seeded field evaluations. Crop Sci. 43:694-700.

EVALUATION OF ELECTRON-BEAM IRRADIATION FOR REDUCING *FUSARIUM* INFECTION AND MICROBIAL LOADS IN BARLEY MALT

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ABSTRACT

Utilization of *Fusarium* infected barley for malting may lead to mycotoxin production and decreased malt quality. Electron-beam irradiation of *Fusarium* infected barley may prevent these safety and quality defects and allow use of otherwise good quality barley. We evaluated electron-beam irradiation for effectiveness in reducing *Fusarium* infection while maintaining germinative energy in barley samples. Four barley samples with varying deoxynivalenol concentrations (0.10, 0.40, 1.20, and 1.27 µg/g) were evaluated for this study. 200g samples were placed in sterile plastic bags and irradiated at doses of 0, 2, 4, 6, 8, and 10 kGy. Treatments were done at Surebeam Corporation, Chicago and repeated three times. Treated samples were malted in a pilot-scale malting unit at North Dakota State University. The barley samples were analyzed for *Fusarium* infection (FI), germinative energy (GE), aerobic plate counts (APC), and mold and yeast counts (MYC). Malted barley samples were analyzed for FI, APC, and MYC. FI decreased with increase in radiation dosage in both the barley and malted samples. In barley samples exposed to 10 kGy, FI was reduced by 50-98%. APC significantly decreased (1-5 logs) in barley with increase in irradiation dosage. A 5-log reduction in APC was observed at 10 kGy for all barley samples. MYC significantly decreased in barley with increase in irradiation dosage. A 1-2.5 log reduction in MYC was observed for all barley samples exposed to 10 kGy. A 20-70% increase in FI in malt, as compared to 8-10 kGy irradiated barley, was observed. The APC's for malts from barley exposed to 8-10 kGy were significantly higher than in other malted samples. A 5-6 log increase in APC, as compared to 8-10 kGy irradiated barley, was observed. MYC exhibited similar trends as observed with APC in malted barley. A 1-3 log increase in MYC, as compared to 8-10 kGy irradiated barley, was observed. GE in barley samples was significantly decreased (3-15%) at higher irradiation dosages. The largest decrease (14%) in GE was observed for the 1.27 ppm sample treated at 10 kGy. The results suggest that dosages between 6-8 kGy may be effective in reducing the FI significantly while maintaining the GE in barley. Dosages over 8 kGy reduce GE and appear to lead to higher microbial loads in malt. Whether the significant increase in APC and MYC counts after malting of irradiated barley was due to cross-contamination or due to the growth of resistant flora requires further investigation.

INHIBITION OF MOUSE SPLEEN LYMPHOCYTE PROLIFERATION BY DEOXYNIVALENOL

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ABSTRACT

We hypothesized that acute exercise stress combined with feeding deoxynivalenol (DON, 2 ppm) for 14 d would exascerbate immunosuppressive effects of DON. Twenty-four 8-wk-old male BALB/c mice were fed DON for 14 d after 1 wk acclimation to AIN-93G diet. On d 14, mice were placed on a treadmill (10-20 m/min) and exercised to exhaustion (2.5-4.25 h). Mice were then killed by decapitation. Trunk blood and spleens were collected. Single cell suspensions of splenocytes were made by stomacher. Splenocytes were cultured at 5 x 10^5 cells/well for 72 h in the presence of the mitogen concanavalin A (10 µg). Cell proliferation was measured by Cell-Titer™dye uptake. Proliferation of splenocytes from DON-fed mice was 40% of non-exercised controls. Exercised controls and exercised animals fed DON showed proliferation of 68-70% of non-exercised controls. Only the non-exercised DON-fed mice showed significant suppression of lymphocyte proliferation. Thus, our hypothesis was not confirmed, suggesting a protective effect of exercise stress against DON toxicity.

MODERN METHODS FOR DETECTION AND QUANTIFICATION OF TRICHOTHECENE PRODUCERS AND DON LEVELS IN CEREALS AND MALT

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OBJECTIVES

To develop diagnostic tools for detection and quantification of trichothecene producing *Fusarium spp.* and for DON as the major mycotoxin produced in cereals and cereal products, to compare the results with established methods and to apply the newly developed tools in the analysis of contaminated sample materials.

INTRODUCTION

Several *Fusarium spp.* are widespread pathogens on cereals in both temperate and semitropical areas, including the cereal growing regions of Northern America and Europe (Bottalico 1998). Warm and rainy weather conditions during the flowering stage promote infection of cereals with *Fusarium* species, resulting in a disease known as Fusarium Head Blight. Low temperatures following infection may promote production of trichothecene mycotoxins in some species (Jacobsen *et al.* 1993). Contamination of grains with *Fusarium ssp*. and their toxins is of great economic concern to cereal producers and to the grain-processing industry (Demcey Johnson *et al.* 1998; Windels 1999), including maltsters and brewers. Deoxynivalenol (DON) and nivalenol (NIV) have been found to be the most frequently occurring trichothecene contaminants in agricultural crops throughout the world. Fusaria which are capable of producing these key toxins may grow in cereals before harvest but also during storage and food processing under unfavourable conditions (Müller *et al.*, 1998; Homdork *et al.*, 2000). As a result of their high stability during storage and processing (Widestrand and Pettersson 2001) and their occurrence in a wide range of agricultural commodities, trichothecenes are regularly detected in animal feed and in human food (WHO 1990). The U.S. Food and Drug Administration advisory level for DON in cereals and cereal products for human consumption is 1 mg kg^{-1}. With respect to health hazard and consumer protection, uniform regulations of trichothecene limits in several countries and in the European Community are also to be expected within the near future.

Standard methods for the analysis of mould contamination in grain comprise micro-biological investigation of samples on suitable agar media. However, this procedure is time-consuming and only viable mycelia can be detected. Therefore, qualitative and quantitative estimation of fungal biomass is often inaccurate.

The polymerase chain reaction (PCR) offers an alternative to micro-biological procedures. Quantitative PCR methods have previously been developed for fungal pathogens including *Fusarium spp.* (Nicholson *et al.* 1998 ; Niessen *et al.* 1998b ; Doohan *et al.* 1999; Edwards *et al.* 2001). Recently, a group specific quantitative PCR assay for trichothecene producing *Fusarium spp.* was established, which is based on

real-time quantification of the *tri5* gene sequence using the LightCycler™system (Schnerr *et al.* 2001). This assay provides the basis for a rapid quantification of toxigenic *Fusarium* species in cereal samples.

Analytical procedures for trichothecene mycotoxins usually differ in extraction, clean-up, and final analytical steps, depending on the kind of substance is actually analysed. A detailed review of analytical standard methods such as GC, GC/MS, HPLC and immunochemical techniques has been published by Krska et *al.* (2001).

Real-time Biomolecular Interaction Analysis (BIA) from Biacore AB (Uppsala, Sweden) uses the optical phenomenon of surface plasmon resonance (SPR) to monitor biomolecular interactions, without labelling any of the interactants. It detects changes in the concentration of molecules in a surface layer of the solution in contact with the sensor surface. Bound antibody can be removed using chaotropic reagents, which allow the sensor surface to be reused repeatedly. BIAcore has been used in many applications such as kinetic and affinity analysis and investigations of specificity and concentration of ligands. The system has been previously used in the detection of various mycotoxins (van der Gaag *et al.*, 1998), aflatoxin B_1 (Daly *et al.*, 2000), and fumonisin B_1 (Mullett *et al.*, 1998).

The current study describes the use of a real-time PCR based system to detect and quantify the contamination of cereal samples with trichothecene producing *Fusarium spp.* as well as a dip stick based system for specific detection of *F. graminearum* PCR products. Also quantification of DON in an inhibitive indirect immunoassay on a BIAcore SPR device is described. Results obtained with the new analytical tools are compared with results obtained using established analytical protocols.

MATERIALS AND METHODS

Prepartion of DNA - DNA from pure fungal cultures was prepared using the method previously described (Niessen & Vogel, 1997). The method described by Knoll et al. (2002a) was used for rapid preparation of *Fusarium*-DNA from cereal and malt samples.

Real-time PCR analysis - The LightCycler™system was applied for amplification and online quantification of extracted DNA. The PCR primers Tox5-1 (forward) and Tox5-2 (reverse) were used to amplify a 658 bp fragment from the trichodiene synthase gene (*tri5*) of trichothecene producing *Fusarium spp.* (Niessen & Vogel, 1998a). The PCR master mix used was composed as previously described (Schnerr et al., 2001). One microliter of DNA template either extracted from a pure fungal culture of from sample material was used per reaction. Thirty two samples were run in parallel by performing 35 cycles of amplification in LightCycler™capillaries (Roche Diagnostics, Mannheim, Germany) under the following thermal cycling protocol. Samples were preheated at 95°C for 2 min. Subsequently 35 cycles of 0 sec 95°C, 5 sec 63°C, 30 sec 72°C (heating and cooling at 20°C/sec) were run. Detection of fluorescent product was carried out after the last step of each cycle at 83°C. Following the final amplification cycle, a melting curve was acquired by one cycle of heating to 95°C, cooling to 75°C at 20°C/sec and slowly heating to 95°C at 0.1°C/sec with continuous measurement of fluorescence at 520 nm. For quantitative analysis, a serial dilution of purified *F. graminearum* DSM 4527 DNA was used to set up a calibration curve.

PCR Detection Test Strips (Roche) for F. graminearum – PCR using digoxygenin labelled *F. graminearum* specific primers was carried out as described by Niessen and Vogel (1997). Following PCR, 9 µl of the reaction were mixed with 1 µl of product specific oligonucleotide probe biotinylated at both ends (5 µmol l^{-1} in 4 x SSC buffer) (Niessen et al. 1998b). The mix was boiled for 5 minutes and immedi-

ately transferred on ice for hybridisation. Five microliters of the mixture were applied to a DNA Detection Test Strips ™ and the reaction developed for 10 min as recommended by the manufacturer.

BIAcore immunoassay for DON –Purified antibodies from a polyclonal anti-DON antiserum (Niessen et al., 1993) were used to detect and quantify free DON in sample extracts. Unreacted antibody was detected using a BIAcore X device (Biacore AB, Uppsala, Sweden) and a sensor chip SA (research grade) with pre-immobilised streptavidin to which biotinylated DON (produced according to Casale et al., 1988) was coupled. The sensor surface was regenerated with 3 µl 6 M guanidinium-chloride in 10 mM glycine, pH 2.9 after each measuring cycle. DON was extracted from 4 g of ground wheat or barley with 12 ml of 10 % (v/v) methanol in water with 6 % (w/v) polyvinylpyrrolidone by shaking on an orbital shaker at 100 rpm for 30 min at ambient temperature. Extracts were clearyfied by centrifugation (1 min, 15,000 x g) and cleaned up with MycoSep™columns (Romer Labs Inc., Union, USA). For analysis 10 µl of the extract or of a serial dilution of purified DON (Sigma-Alsdirch, Deisenhofen, Germany) were incubated with 30 µl of a 0.5 µg μl^{-1} anti-DON antibody solution for 1 min at ambient temperature and subsequently passed over the surface of the sensor chip. Evaluation of results was done using the BIAevaluation® Software 3.0 softtware package (Biacore AB (Uppsala, Sweden).

RESULTS AND DISCUSSION

Real-time PCR analysis of DON contaminated wheat - Figure 1A shows real-time PCR kinetics obtained with a serial dilution of purified DNA from *F. graminearum* DSM 4527 and the calibration curve calculated from the data (see figure 1B). Real-time PCR on the LightCycler™ (Wittwer et al., 1997) was applied to analyse 300 samples of wheat which had previously been field inoculated with *F. culmorum*. All samples were analysed for DON using a GC/MS method (data kindly provided by BASF, Limburgerhof, Germany). Concentrations of the toxin ranged from not detectable to 34.3 mg/kg. Concentrations of template DNA in the samples were calculated from the calibration curve given in figure 1B and expressed as ppb DNA per gram sample.

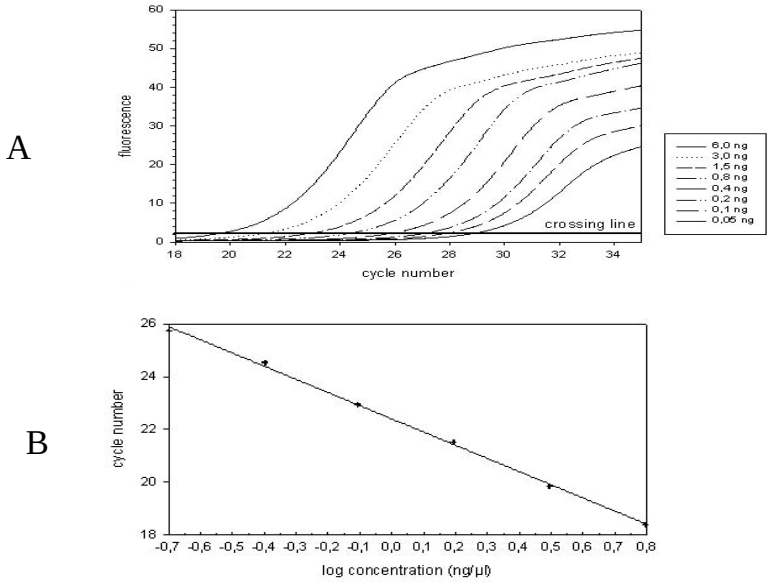

Figure 1: Calibration of the LightCycler™ PCR assay. **A**. Real-time PCR kinetics of a dilution series of DNA isolated from a pure culture of *F. graminearum* DSM 4527 used as template. B. Calibration curve calculated from the data obtained in A, cycle number at crossing line, r = 1.0. Redrawn from Schnerr et al., 2001.

DNA concentrations of trichothecene producing *Fusarium spp.* ranged between not detectable and 16.3 mg/kg in the 300 samples studied. For correlation analysis, DNA concentrations were plotted against DON concentrations and analysed by linear regression (SigmaPlot®4.0 for Windows®, SPSS Inc. Chicago, USA). A coefficient of correlation between both parameters of r=0,9557 was calculated on the basis of all samples. Calculation based only on samples which had DON concentrations between not detectable and 1,5 mg/kg (n = 234) revealed a coefficient of correlation of r = 0,7476 (see figure 2). The correlation was statistically highly significant. Only 12 of the 234 samples had data points which were outside the calculated interval of confidence (95 %). A similarly high correlation was found, when DNA contents and DON concentrations (HPLC) analysed in 100 naturally contaminated wheat samples were plotted against each other (data not shown).

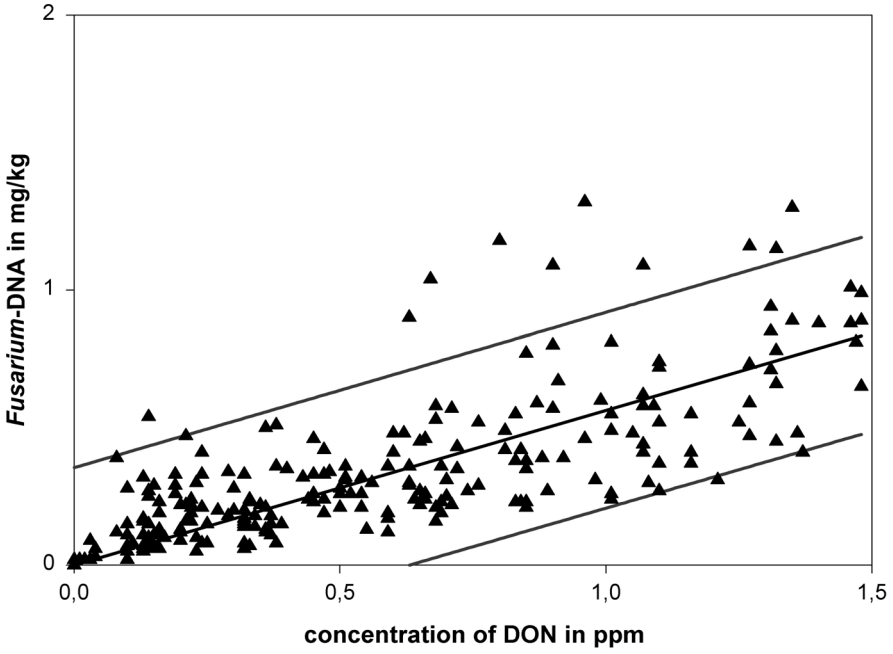

Figure 2: Correlation between concentrations of trichothecene producer DNA and DON concentration in samples of wheat infected with *F. culmorum.* (n = 234; r = 0.7476). Brighter lines indicate 95 % interval of confidence. Redrawn from Schnerr et al., 2002a.

Using the PCR assay described, the minimum detectable quantity of template DNA in sample material was 16 µg/kg corresponding to 290 haploid genomes (Xu *et al.* 1995). The quantitative PCR application analyses the potential for production of trichothecenes in *Fusarium* contaminated cereals. In the current study it was used to analyse relations between this potential, measured as ppm of trichothecene-producer DNA, and the concentrations of DON actually found in a corresponding sample. Data obtained demonstrate that a positive, linear correlation exists between both parameters in the grain samples analysed. This correlation was shown to be statistically highly significant. It was clearly demonstrated that specific diagnosis and quantification of quality relevant *Fusarium spp.* are possible in only a small portion of time and expenditure of labour compared to both microbiological methods and to chemical analysis of mycotoxins. The correlation between DNA and DON concentrations was shown in field inoculated sample material. According to Edwards *et al.* (2001), relationship found here might be weaker in naturally infected samples, because various strains of the different *Fusarium spp.* may be present with different capabilities for production of trichothecenes. This statement was contrasted by results obtained from the analysis of 100 samples of

naturally contaminated wheat in the authors lab, where also a coefficient of correlation exceeding 0.9 was found between DNA concentrations and DON as analysed by GC/MS.

Results reported here clearly show that analysis of samples for the presence of contaminants potentially producing trichothecenes by quantitative PCR may provide a powerful tool for future quality control in cereals. Furthermore, correlation of biomass with other parameters like fungicide treatment, irrigation, regional and climatic differences or even *Fusarium* resistance of cereals might be worthwhile to study with the system developed.

SPR based biosensor for the determination of DON in cereals **-** As an alternative to established analytical procedures for the key mycotoxin DON, a BIAcore-based indirect inhibition immunoassay was developed for the rapid quantification of this mycotoxin in sample material. A calibration curve ranging between 0.07 and 50 ng/µl injection volume (i.v.) of pure DON was used to quantify the toxin in wheat samples (figure 3). The detection limit of the assay for DON was 2.5 pg μl^{-1} i.v., corresponding to 7.5 ppb DON in a contaminated wheat sample. The working range of detection (lower limit + 3x standard deviation) lay between 0.13 and 10.0 ng μl^{-1} i.v. corresponding to 390 and 3000 µg kg^{-1} DON in sample material. For this range of contaminations a R^2 = 0.992 was calculated for the correlation between DON concentration and response signal. The 50 % inhibitive concentration of this assay was calculated to be IC_{50} = 0.72 ng μl^{-1} i.v.

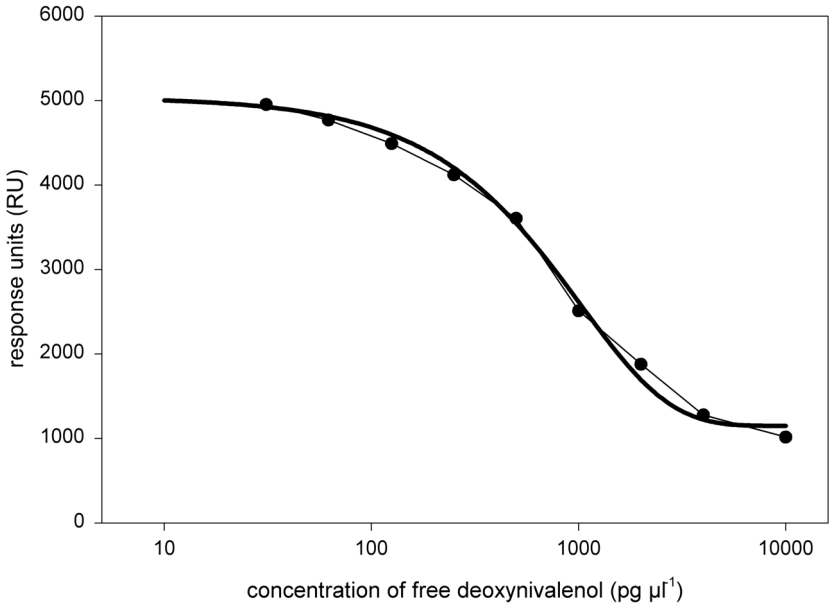

Figure 3: Calibration curve of the inhibition immunoassay for DON. A constant concentration of anti-DON antibodies was mixed with a dilution series of the toxin in binding buffer. The concentration of unreacted antibody was quantified using the BIAcore X SPR device. Redrawn from Schnerr et al., 2002b

Average recovery of DON in wheat samples spiked at levels of 50, 100, 500 µg/kg was 104 ± 15 % with the extraction and cleanup procedure described in materials and methods. Analysis time was 15 min per sample including extraction and quantification. Correlation of DON concentrations analysed with the biochip and with GC/MS (n = 15) or HPLC (n = 50) data showed coefficients of correlation of R^2 = 0.9464 (GC/MS) and R^2 = 0.9066 (HPLC), respectively.

Only few immunoassays have been described using a surface plasmon resonance biosensor for the detection of mycotoxins, such as aflatoxin, ochratoxin A, fumonisin B_1 and zearalenone (van der Gaag *et al.*, 1998, Mullett *et al.*, 1998, Daly *et al.*, 2000). In the present study, simple and rapid sample preparation was combined with real-time measurement applying the optical phenomenon of surface plasmone resonance in a BIA application. For the grain-processing industry the BIAcore assay developed may provide high-speed measurement of DON concentrations for screening of large sample numbers. The significant advantage of this technique is that a biosensor can be used as a rapid, on-line detection system without any further reactions to detect the binding event. In addition, a sensor surface may withstand up to 100 cycles, leading to considerable decrease of costs per analysis. This permits analysis of high sample numbers in the food and feed industry for improved quality control and as a tool for realisation of HACCP concepts. Assays of the BIAcore type are rapid and easy to use even by low trained personnel. It can therefore be anticipated that assays like the one described here might become increasingly important in the cereal industry in the future.

***Test strip for detection of* F. graminearum** – In order to compare sensitivity of the Test Strip™ method with conventional methods of product detection, e.g. ethidium bromide staining, PCR product analyses was performed by gel electrophoresis and the new method in parallel experiments. Several dilutions of purified *F. graminearum* DSM 4527 DNA were amplified by PCR. One aliquot of the reaction mixture was applied to agarose gel electrophoresis following PCR (figure 4B). A second aliquot of the same reaction was used for product detection with the DNA Detection Test Strips™ (figure 4B). The limit of detection was found to be $2.6*10^{-4}$ µg template DNA/reaction with the Test Strip™ system. Comparing results in the agarose gel, a clearly visible signal was still present at a concentration of $6{,}50*10^{-5}$ µg template DNA/ reaction showing a slightly higher sensitivity of product detection in agarose gels.

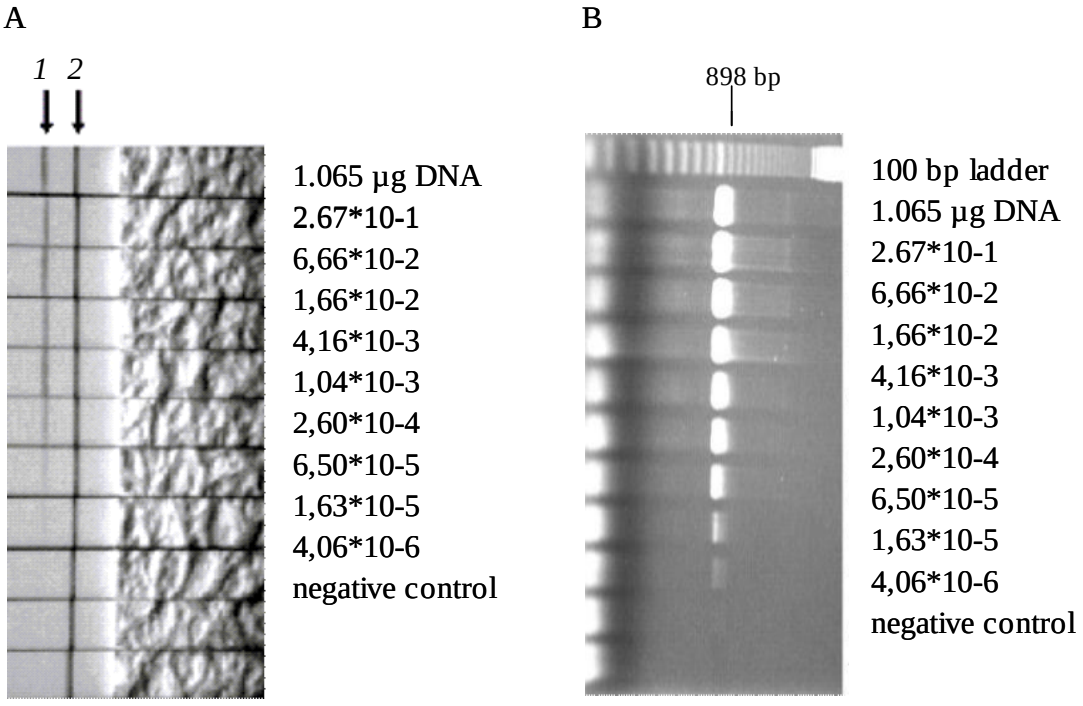

Figure 4: Comparison of two detection methods for PCR products. A serial dilution of DNA from *F. graminearum* DSM 4527 was used as template. Sterile distilled water was used as negative control. **A**: DNA Detection Test Strips™ 1: Signal from PCR product (streptavidin line) 2: Signal from internal control (immuno-gold labelled anti-mouse antibody). **B**: Agarose gel with PCR products stained with ethidium bromide. Redrawn from Knoll et al., 2002.

DNA was extracted from cereal samples contaminated with *F .graminearum* and amplified in a PCR as described under materials and methods. Product was detected using DNA Detection Test Strips™ and results were compared to those obtained with detection in agarose gel electrophoresis with ethidium bromid staining (figure 5). Samples with no *F. graminearum* contamination showed no signal neither with the Test Strips™ nor on agarose gels. In the group of samples with 1-30 % infected grains (microbiological analysis of surface disinfected grains on SNA medium, Nirenberg 1981) the number of samples which were positive in PCR was 10 % higher with detection in agarose gels as compared to detection using the Test Strips™. In the group of samples with more than 40 % of contaminated grains the percentage of PCR positive results in agarose gels and with the Test Strips™ were found to be identical.

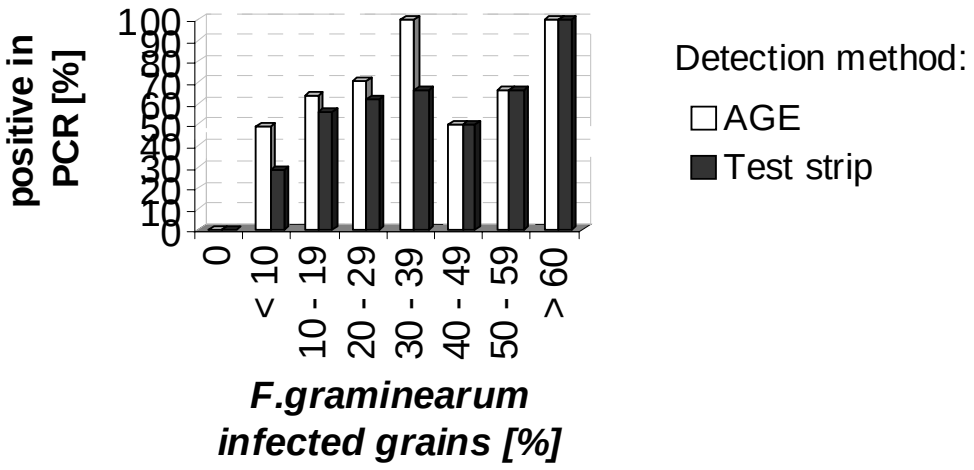

Figure 5: Comparison of detection of *F. graminearum* from cereal samples with agarose gel electrophoresis and DNA Detection Test Strips™. Samples were grouped according to their percentage of grains infected with *F. graminearum*. AGE = agarose gel electrophoresis. Redrawn from Knoll et al., 2002b.

Currently, detection of amplification products in conventional PCR is most frequently performed by staining agarose electrophoresis gels with fluorescent dyes such as ethidium bromide or SYBR Green I, which fluoresce upon intercalation into the DNA double strand. Besides the need for appropriate electrophoresis equipment, analyses are labour intensive and time consuming and mutagenicity of DNA dyes pose a hazard to human health. In contrast, DNA Detection Test Strips™ need no instrumentation, handling is easy and no hazardous reagents are used. Results are obtained within 20 minutes, compared to at least 70 minutes if agarose gel electrophoresis is applied. Detection and verification are achieved with one experiment because the specifically amplified PCR product is detected by a product specific probe. The method described here facilitates detection of *F. graminearum* in routine applications and in screening studies.

Conclusions - Results presented in the current study point to the fact that the modern analytical tools described can act as useful tools in the screening of large numbers of samples in the agro-food industry. Real-time PCR methods like the one described indicate the potential of a sample for the presence of trichothecene mycotoxins within short time omitting time consuming pre-incubation steps and mycological analysis. Antibody based bio-sensorial methods can quantify key mycotoxin like DON within 15 min or

even less in cereals but also in processed samples. Finally, use of DNA Detection Test Strips™ may contribute significantly to a broader acceptance of PCR as a powerful tool in the quality control of the food and feed industry.

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REFERENCES

Bottalico, A. 1998. *Fusarium* diseases of cereals: species complex and related mycotoxin profiles, in Europe. J. Plant Pathol. 80:85-103.

Casale, W.L., Pestka, J.J. and Hart, L.P. 1988. Enzyme-linked immunosorbent assay employing monoclonal antibody specific for deoxynivalenol (vomitoxin) and several analogues. J. Agric. Food Chem. 36:663-668.

Daly, S.J., Keating, G.J., Dillon, P.P., Manning, B.M., O'Kennedy, R., Lee, H.A. and Morgan, M.R. 2000. Development of a surface plasmon resonance-based immunoassay for aflatoxin B_1. J. Agric. Food Chem. 48:5097-5104.

Demcey Johnson, D., Flaskerud, G.K., Taylor, R.D. and Vidyashankara, S. 1998. Economic impacts of Fusarium head blight in wheat. In: Agricultural Economics Report No. 396, Department of Agricultural Economics, Agricultural Experiment Station, North Dakota State University, Fargo.

Doohan, F.M., Parry, D.W. and Nicholson, P. 1999. Fusarium ear blight of wheat: the use of quantitative PCR and visual disease assessment in studies of disease control. Plant Pathol. 48:209-217.

Edwards, S.G., Pirgozliev, S.R., Hare, M.C. and Jenkinson, P. 2001. Quantification of trichothecene-producing *Fusarium* species in harvested grain by competitive PCR to determine efficacies of fungicides against Fusarium head blight of winter wheat. Appl. Environ. Microbiol. 67 :1575-1580.

Homdork, S., Fehrmann, H. and Beck, R. 2000. Influence of different storage conditions on the mycotoxin production and quality of *Fusarium*-infected wheat grain. J. Phytopathol. 148:7-15.

Jacobsen, B.J., Bowen, K.L., Shelby, R.A., Diener, U.L., Kemppainen, B.W. and Floyd, J. 1993. Mycotoxins and Mycotoxicoses, http://www.aces.edu/dept/grain/ANR767.htm

Knoll, S., Mulfinger, S., Niessen, L. and Vogel, R.F. 2002a. Rapid preparation of *Fusarium* DNA from cereals for diagnostic PCR using sonification and an extraction kit. Plant Pathol. 51:728-734.

Knoll, S., Vogel, R.F. and Niessen, L. 2002b. Identification of *Fusarium graminearum* in cereal samples by DNA Detection Test Strips™. Lett. Appl. Microbiol 34:144-148.

Krska, R., Baumgartner, S. and Josephs, R. 2001. The state-of-the-art in the analysis of type-A and –B trichothecene mycotoxins in cereals. Fres. J. Anal. Chem. 371:285-299.

Müller, H.M., Reimann, J., Schumacher, U. and Schwadorf, K. 1998. Natural occurrence of *Fusarium* toxins in oats harvested during five years in an area of southwest Germany. Food Addit. Contam. 15:801-806.

Mullett, W., Lai, E.P. and Yeung, J.M. 1998. Immunoassay of fumonisins by a surface plasmon resonance biosensor. Anal. Biochem. 258:161-167.

Nicholson, P., Simpson, D.R., Weston, G., Rezanoor, H.N., Lees, A.K., Parry, D.W. and Joyce, D. 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Phys. Mol. Plant Pathol. 53:17-37.

Niessen, L., Böhm-Schraml, M., Vogel, H. and Donhauser, S. 1993.Deoxynivalenol in commercial beer - screening for the toxin with an indirect competitive ELISA. Mycotoxin Res. 9:99-109.

Niessen, L. and Vogel, R.F. 1997. Specific identification of *Fusarium graminearum* by PCR with *gao*A targeted primers. Syst. Appl. Microbiol. 20:111-123.

Niessen, L. and Vogel, R.F. 1998a. Group specific PCR-detection of potential trichothecene producing *Fusarium*-species in pure cultures and cereal samples. Syst. Appl. Microbiol. 21:618-631.

Niessen, L., Klusmann, J. and Vogel, R.F. 1998b. Quantitative estimation of *Fusarium graminearum* DNA using a solid phase PCR assay (DIAPOPS). J. Food Mycol. 1:73-84.

Nirenberg, H.I. 1981.A simplified method of identifying *Fusarium spp.* on wheat. Can. J. Bot. 59:1599-1609.

Schnerr, H., Niessen, L. and Vogel, R.F. 2001. Real time detection of the *tri5* gene in *Fusarium* species by LightCycler™PCR using SYBR®Green I for continuous fluorescence monitoring. Int. J. Food Microbiol. 71:53-61.

Schnerr, H., Niessen, L. and Vogel, R.F.2002a. Correlation between DNA of trichothecene-producing *Fusarium* species and deoxynivalenol concentrations in wheat-samples. Lett. Appl. Microbiol. 35:121-125.

Schnerr, H., Niessen, L. and Vogel, R.F. 2002b. A biosensor-based immunoassay for rapid screening of deoxynivalenol contaminations in wheat. Food Agric. Immunol. 14:313-321.

Van der Gaag, B., Stigter, E., van Duijn, G., Bleeker, H., Hofstra, H. and Wahlström, L. 1998. Application development on BIACORE for the detection of mycotoxins in food and feed, http://www.biacore.com/keyareas/pdf/Mycotoxin2.pdf

WHO 1990. Selected mycotoxins: ochratoxins, trichothecenes, ergot. Environmental Health Criteria 105:71 ff.

Widestrand, J. and Pettersson, H. 2001. Effect of time, temperature and solvent on the stability of T-2 toxin, HT-2 toxin, deoxynivalenol and nivalenol calibrants. Food Addit. Contam. 18:987-992.

Windels, C.E. 2000. Economic and social impacts of Fusarium Head Blight: changing farms and rural communities in the northern great plains. Phytopathology 90:17-21.

Wittwer, C., Ririe, K.M., Andrew, R.V., David, D.A., Gundry, R.A. and Balis, U.J. 1997. The LightCycler™: a microvolume multisample fluorimeter with rapid temperature control. BioTichniques 22:176-181.

Xu, J.R., Yan, K., Dickman, M.B. and Leslie, J.F. 1995. Electrophoretic karyotypes distinguish the biological species of *Gibberella fujicuroi* (*Fusarium* section *Liseola*). Mol. Plant Mic. Int. 8:74-84.

HUMAN CYTOKINE MRNA RESPONSE TO DEOXYNIVALENOL (VOMITOXIN) USING WHOLE BLOOD CULTURES

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ABSTRACT

Deoxynivalenol (DON or vomitoxin),a tricothecene mycotoxin, is a naturally occurring contaminant frequently found in grain-based foods. This toxin has adverse effects on human and animal health, causing a multisystemic shock-like syndrome including dermal irritation, nausea, vomiting, diarrhea, hemorrhage, leukopenia and anemia. A critical step in DON toxicity is action on leukocytes by activation of cytokines. Previous studies have shown an upregulation of several cytokines, in the U937 cells, a cloned human macrophage model. To study the acute effects of DON on human cytokine production in peripheral mononuclear blood leukocytes, we have developed a culture approach using a 20% dilution of whole blood in RPMI-1640 media and a 6 hour exposure. Cultures were exposed to DON at concentrations of 0, 10, 50, 100, 250, and 500 ng/ml. RNA was then isolated and assayed using real-time PCR primer/probes, for both cytokines (IL-6, IL-8, and TNF-alpha) and 18S rRNA. Cytokine levels were normalized using 18S rRNA levels and relative expression levels determined. IL-6 was significantly induced by DON at 250 ng/ml (~9.5 fold) and 500 ng/ml (~14 fold). IL-8 was significantly induced by DON at 250 ng/ml (~8.5 fold) and 500 ng/ml (~3 fold). TNF-alpha was significantly induced by DON at 10 ng/ml (~1 fold), 250 ng/ml (~1 fold), and 500 ng/ml (~4 fold). Taken together, the capacity of DON to induce IL-8, IL-6, and TNF-alpha gene expression and the threshold doses to achieve these effects were consistent with previous findings in cloned human and mouse macrophage cultures. Interestingly, a high degree of variability was observed in blood cultures from different donors, thus raising the possibility that some individuals may have greater sensitivity to DON than others. Further research is being undertaken to clarify this possibility. (This abstract was presented at the Society of Toxicology Annual Meeting, Salt Lake City, UT, March 10-13, 2003)

DETECTION OF DEOXYNIVALENOL IN BLOOD AND TISSUE BY ELISA

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ABSTRACT

Deoxynivalenol (DON or vomitoxin) is a trichothecene mycotoxin commonly found in cereal grains that adversely affects the gastrointestinal and immune function. In assessing risk of this toxin to humans, it is important to be able to monitor its absorption, disposition and clearance in tissues of exposed animals and compare this to in vitro studies with human and animal. Here we developed approaches to analyze for DON in tissue by immunochemical assay and used these to follow the kinetics of distribution and clearance of this mycotoxin in the mouse. Spiking studies revealed that DON in plasma could be analyzed directly using a competitive direct enzyme-linked immunosorbent assay (ELISA) providing that standard curves were prepared in human plasma as diluent. For analysis of organs, tissues were homogenized in phosphate buffered saline (1:10 ratio). Resultant extracts were then heated in boiling water for 5 min, centrifuged, and supernatants analyzed by ELISA. Tissue disposition and clearance of DON were measured in B6C3F1 male mice (8 wk-old) that were orally administered 25 mg/kg BW of the toxin. Blood was collected from retro orbital plexus and organs removed after 0.08 (5 min), 0.25 (15 min), 0.5 (30 min), 1, 2, 4, 8 and 24 hr intervals. Maximal DON was detected at 5 and 15 min in all tissues tested with a rapid clearance over a 24 hr period. At 5 min, DON concentrations in ng/g of tissue were 19552 ± 1910 in liver, 12140 ± 461 in plasma, 7568 ± 515 in kidney, 7294 ± 839 in spleen, 6755 ± 854 in heart, 5486 ± 175 in thymus and 723 ± 79.4 in the brain. DON concentrations were significantly higher in all the organs tested from 5 min to 8 hr compared to untreated mice. At 24 hr, DON concentrations were also significantly higher except in heart and kidney. Taken together, the results showed that, in the mouse, DON rapidly distributed in all organs within a short time after exposure according to the rank order liver> plasma> kidney> spleen> heart> thymus> brain. The approach presented here will enable predictive studies on human toxicity by correlating in vivo animal studies to ongoing in vitro animal and human investigations.

FIRST REPORT OF TRICHOTECENES PRESENCE IN COMMERCIAL BARLEY GRAINS IN THE HIGHLANDS OF CENTRAL MEXICO

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OBJECTIVES

The present study was carried out to detect and quantify trichothecene levels in commercial barley grain in the Highlands of Central Mexico.

INTRODUCTION

In México, malting barley (*Hordeum vulgare* L.) is grown on approximately 301,000 hectares under both irrigated and non-irrigated conditions. The main malting barley producing area of México is the Central Highlands (encompassing the states of Hidalgo, Tlaxcala, México, and Puebla) (6). Within this region, the area between the valleys and hills of Hidalgo (Apan and Cd. Sahagún) and Tlaxcala (Calpulalpan, Tlaxco, Españita, and Benito Juárez) accounts for nearly 50% of México's total barley area.

Since 1998, Fusarium Head Blight of cereals (*Fusarium spp.*) has increased, affecting barley (*Hordeum vulgare* L.) in the Highlands of Mexico. This region includes the states of Mexico, Tlaxcala, Puebla and Hidalgo (2,3) where the disease causes losses in both yield and grain quality (3).

MATERIALS AND METHODS

At harvest in the 2001 and 2002 growing seasons, samples (1.5 kg) of grain produced by the growers in the Central Highlands of México were taken. Sampling was carried out in warehouses in the Calpulalpan and Apan areas. Samples were taken directly from grain shipments from each area; the objective was to obtain a significant number of samples representative of the Highlands.

Samples were analyzed in CIMMYT's Toxins Laboratory in El Batán, México. The grain samples were ground (Braun commercial mill) and processed using the technique of Romer Laboratories, Inc. (Don FluoroQuant™ method #FQD1NC, version 95.9).

RESULTS

For the 2001 growing season, 87.1% of samples tested positive for trichothecenes (0.05-2.10 ppm), whereas in the 2002 season, 67.7% of samples were positive (0.03-7.10 ppm). In general, toxins were found in 96.8% of the grain from all sampling sites (Table 1) in one of the two years, which indicates that pathogens are present throughout the sampled area.

Results of this study show that 67.7% of the samples from the 2001 growing season and 32.3% of those from the 2002 growing season have a toxin content above 0.5 ppm, the maximum tolerated level used in this study. This finding suggests that most commercial barley grain in México could be contaminated in the future a condition that might be dangerous for the known harmful effect of toxin in human health (4).

To control toxin contamination, the European Community recommends a maximum tolerance level of 0.5 ppm in cereal grains for direct human consumption and 0.75 ppm in flour used as a raw material in food products. Germany restricts the levels to 0.35 ppm in bread and pasta, and 0.10 ppm in edible cereals for children and babies (Notification 2002/138/D).

In countries such as Russia, the USA, and China, the maximum level tolerated in wheat for human consumption is 1.0 ppm (1). Furthermore, the FAO-Codex Alimentarius Commission has proposed a maximum of 0.5 ppm for all cereal-derived products for human consumption, except those for babies, which should contain no more than 0.1 ppm (1). In this study the tolerance level applied was 0.5 ppm, the same recommendation followed by Anheuser-Bush, Inc., the most important beer manufacturer in the USA (5).

CONCLUSIONS

Toxins were found in 96.8% of the sampling sites in one of the two test years. About 68% of the samples from the 2001 growing season and 32% of those from the 2002 growing season have a toxin content above 0.5 ppm.

Failure to take measures to control and manage the disease could result in economic losses due to grain yield reductions and the presence of toxins in the grains. This might also have consequences for barley producers and the brewing industry in the Central Highlands of México.

REFERENCES

FAO-Codex Alimentarius Commission. 2002. Codex Committee on food additives and contaminants. info@codexalimentarius.nl

Gilchrist, S.L. 2000. Problemas fitosanitarios de los cereales de grano pequeño en los Valles Altos de México. Revista Mexicana de Fitopatología 18:132-137.

Ireta, M.J. 2000. Fusariosis de los cereales. *In:* G. Fuentes D. (ed). Fitosanidad de cultivos básicos. Segunda edición. Sociedad Mexicana de Fitopatología. México.

Miller, J.D., Young, J.C., and Sampson, D.R. 1985. Deoxynivalenol and Fusarium head blight resistance in spring cereals. J. of Phytopathology 113:359-367.

Pederson, J.D., Horsley, R.D., and McMullen, M.P. 2001. Efficacy of fungicides in controlling Fusarium head blight on barley genotypes with partial resistence. *In*: S.M. Canty, J. Lewis, L. Siler, and R.W. Ward (eds). 2001 National Fusarium Head Blight Forum Proceedings. U.S. Wheat and Barley Scab Initiative. USA. 82-86.

SAGAR. 1999. Anuario estadístico de la producción agrícola. Secretaría de Agricultura, Ganadería y Desarrollo Rural - Centro de Estadística Agropecuaria.

Table 1. Detection and quantification of trichothecenes (deoxynivalenol + nivalenol) on the commercial barley variety Esmeralda in the Central Highlands of México (Tlaxcala-Hidalgo), 2001-2002.

	Trichothecene concentration (ppm)	
Sampling site	2001	2002
Almoloya	2.10	2.50
Apan	0.90	0.00
Calpulalpan	1.10	0.00
Chimalpa y Tlalayote	0.10	0.00
Coatlaco	0.83	0.00
Emiliano Zapata	0.05	0.00
La Estancia	2.00	1.00
La Laguna	0.52	0.44
La Soledad	1.70	0.52
La Unión	1.10	0.39
Lagunillas	0.40	0.31
Lázaro Cárdenas	1.70	0.11
Lomorriel	0.80	0.69
Matamoros	1.30	1.00
Ocotepec	0.00	0.03
Paredón	2.10	0.28
Rancho Nuevo	1.50	0.00
San Andrés Buenavista	2.00	1.80
San Diego	1.40	0.00
San Felipe Sultepec	0.88	0.00
San José Jiquilpan	0.00	0.46
San Juan Ixtilmaco	1.80	0.14
San Mateo Activan	0.17	0.18
Santa Bárbara	0.00	0.00
Santa Clara	0.48	0.82
Santiago Tletlapayac	0.00	0.13
Santiaguito	0.33	0.00
Teapan	1.40	0.68
Tepetlayuca	1.10	0.49
Tierra y Libertad	0.40	0.97
Zotoluca	2.10	7.10
Ranges	0.05-2.10	0.03-7.10
Sites where toxins found (%)	87.10	67.7