The 1998 National Fusarium Head Blight Forum

CHAPTER 2

Food Safety, Toxicology, and Utilization

Click here for Chapter Index

U.S. Wheat & Barley
Scab Initiative

Michigan State University • East Lansing, Michigan USA
October 26-27, 1998

Compiled by:
Patrick Hart, Richard Ward, Rhonda Bafus, and Kathleen Bedford

We would like to gratefully acknowledge contributions of the following companies and organizations for partial sponsorship:

USDA ARS
Michigan Agricultural Experiment Station, Michigan State University
American Malting Barley Association, Inc.
Monsanto — Food • Health • Hope
Neogen Corporation
Novartis

Michigan State University

Copies of this publication can be viewed at HYPERLINK http://www.scabusa.org
Printed in the United States by University Printing, East Lansing, MI Production and Layout: O'Connor Creative

The 1998 National Fusarium Head Blight Forum

CHAPTER 2

Food Safety, Toxicology, and Utilization

CLICK ON AUTHOR'S NAME TO GO TO ARTICLE

(Includes Biotechnology, Variety Development, and Germplasm Introduction)

P

Potential Utilization Of Highly DON-Contaminated Wheat Via Extrusion Processing

M. Accerbi, V.E.A. Rinaldi and P.K.W. Ng. Department of Food Science and Human Nutrition, Michigan State University.

OBJECTIVES

The objectives of the present study were (1) to evaluate the effect of extrusion on lowering DON content of (a) naturally highly DON-contaminated wheat soaked in sodium bisulfite solutions (SBs) and of (b) flours and whole meals obtained from the DON-contaminated wheat tempered with SBs; and (2) to examine changes in the rheological properties of the flour samples.

INTRODUCTION

Deoxynivalenol (DON, vomitoxin) is a mycotoxin usually found in *Fusarium*-contaminated grains that causes, in farm animals, vomiting, feed refusal, reduced weight gain, emesis and altered immune function (Pestka and Bondy, 1990; Rotter et al., 1996), and in humans, abdominal aches, sore throats, vomiting and diarrhea (Israel 1989). At low contamination levels, DON is typically located near the exterior surface of the kernel, but when it is present in higher concentrations it is distributed almost uniformly throughout the kernel (for review, Pomeranz et al., 1990). Furthermore, the degree of *Fusarium* infection, and consequently the DON content, varies considerably among kernels regardless of the grain sample's total DON level (Seitz and Bechtel, 1985). For wheat, the processing steps have been widely investigated to determine the possibility of reduction in DON concentration (see Patey and Gilbert, 1989, Charmley and Prelusky, 1994, for reviews). So far the most promising decontamination procedures seem to be chemical treatment of the grain. Among the chemicals tested, sodium bisulfite (SB) was responsible for the greatest reduction in DON levels, depending on the amount of reagent employed or on the treatment time (Young et al., 1986). Although flour obtained from SB-tempered wheat contained only very low levels of DON, baking of that flour resulted in an increase in DON amount (Young et al., 1986). Those results can be explained by the fact that SB reacts with DON to form a DON-sulfonate adduct (DON-S), unstable at high temperature and high pH (Young, 1986). When DON-contaminated corn was treated with SB and fed to pigs, no short-term deleterious effects were observed (Young et al., 1987). Furthermore, DON-S administered orally to pigs did not induce any acute toxic effect at levels equivalent to those of DON that had caused emesis (Young et al., 1987).

MATERIALS AND METHODS

Wheat. Michigan soft white wheat naturally contaminated with DON at 7.3 ppm was obtained from a local supplier.

DON determination. DON contents were determined by Gas Chromatography with Electron Capture Detection (Tacke and Casper, 1996).

Sodium bisulfite solutions. Solutions of SB were prepared based on the amount of sulfur dioxide (SO_2; 67% SO_2 in SB, weight per weight).

Soaking of wheat. Samples of one kg each were soaked in replicates for one hour in one liter of water or SBs containing the equivalent of 0.5, 1.0, 1.5, 2.0, 2.5 or 5.0% SO_2. From each sample, 250 g were dried and ground with a Udy Cyclone Mill (0.5mm screen), and the remaining 750 g were set aside for extrusion.

Milling of wheat. Three samples of 18 kg each were tempered to 15% moisture with water or SBs containing the equivalent of 5.0 or 10% SO_2. Subsamples of 16 kg of each tempered sample were milled with a laboratory mill (Buhler MLU-202) to obtain bran, shorts and flour. Whole meal was obtained by blending all mill fractions, and flour by blending the 3 break and 3 reduction flours.

Extrusion. A laboratory co-rotating and intermeshing twin screw extruder (APV Baker MP 19TC-25) was used. The variables involved were two screw configurations (low and high shear) and two temperature profiles (low, 40˚C-145˚C, and high, 40˚C-170˚C). Replicates of 750 g of soaked wheat from each treatment were drained and extruded. Replicates of whole meals and flour samples were extruded. After extrusion, all extrudates were dried and ground with a Udy Cyclone Mill.

Rheological properties of flours. The Farinograph was performed according to AACC Method 54-21 and analysis with the Rapid Visco Analyser was performed according to the manufacturer's suggested profile.

RESULTS AND DISCUSSION

Wheat soaked in sodium bisulfite solutions. The reduction in DON in SB-soaked wheat is shown in Figure 1. At a SO_2 concentration as low as 0.5%, DON was lowered from 7.3 to 2.3 ppm (a reduction of 68.5%), and at 5% SO_2, a reduction of about 89% (from 7.3 to 0.8 ppm) was obtained.

Extrusion of soaked wheat. The two screw configurations tested had no effect on DON contents of the extruded products (data not shown). On the contrary, an effect of the temperature profiles used was observed. Low temperature extrusion of wheat soaked in water reduced slightly the DON level (Table 1), and the high temperature profile was even more effective. Extrusion of wheat soaked in SB reduced the DON level slightly and the maximum effect was observed for the sample from wheat soaked in the 5% SO_2 solution (Table 1).

Extrusion of whole meal and flour. As for the soaked wheat, the screw configurations tested did not affect the DON content of the extruded whole meal and flour samples, but the temperature profiles did influence the presence of detectable DON. For both whole meal and flour from water-tempered wheat, extrusion with the low temperature profile was slightly more effective in lowering DON (Table 2) than high temperature extrusion. In general, extrusion of whole meals and flours from SB-tempered wheat caused an increase in DON amount (Table 2), which was more obvious when the high temperature profile was used. This observation could be due to the formation of the DON-sulfonate adduct (by reaction between DON and SB) which is unstable at higher temperatures (Young, 1986).

Physical properties of flours. In comparison with the flour from the water-tempered wheat, the flour obtained from 10% SO_2-tempered wheat showed consistent deviation in both Farinograph and RVA parameters (Table 3). These data clearly indicate that the presence of high amounts of SB has deleterious effects on the flour properties.

Summary

Soaking of highly DON-contaminated wheat with a SB solution for only one hour can lower DON level as much as 89%. In addition, extrusion can be utilized to process soaked wheat to remove moisture and most of the SB odor. Furthermore, potential extruded products can be made from flour, even though rheological properties of the flour have been altered by tempering the wheat with SB solutions.

REFERENCES

CHARMLEY L.L. and PRELUSKY D.B., 1994. In: Mycotoxins in grain. Components other than aflatoxin. Pg. 421-433. Miller J.D. and Trenholm H.L. Eds. Eagan Press, St. Paul, MN.

ISRAEL E., 1989. IDRC Rep. 18:18-19.

PATEY A.L. and GILBERT J., 1989. In: Fusarium. Mycotoxins, Taxonomy and pathogenicity. Pg. 399-420. Chelkowski J. Eds. Elsevier.

POMERANZ Y., BECHTEL D.B., SAUER D.B., SEITZ L.M., 1990. In: Advances in cereal science and technology. Vol. X, pg. 373-433. Y. Pomeranz Ed. Am. Assoc. Cereal Chem., St. Paul, MN.

PESTKA J.J. and BONDY G.S., 1990. Physiol.Pharmcol. 68:1009-1016.

ROTTER B.A., PRELUSKY D.B., PESTKA J.J., 1996. J. Toxicology Environmental Health 48: 1-34.

SEITZ L.M. and BECHTEL D.B., 1985. J. Agric. Food Chem. 33:373-377.

TACKE B.K. and CASPER H.H., 1996. J. AOAC Int. 79:472-475.

YOUNG J.C., 1986. J. Agric. Food Chem. 34:919-923.

YOUNG J.C., SUBRYAN L.M., POTTS D., MCLAREN M.E., GOBRAN F.H., 1986. J. Agric. Food Chem. 34:461-465.

YOUNG J.C., TRENHOLM H.L., FRIEND D.W., PRELUSKY D.B., 1987. J. Agric. Food Chem. 35:256-261.

Figure 1. Change in DON levels (ppm) of wheat soaked in sodium bisulfite solutions.

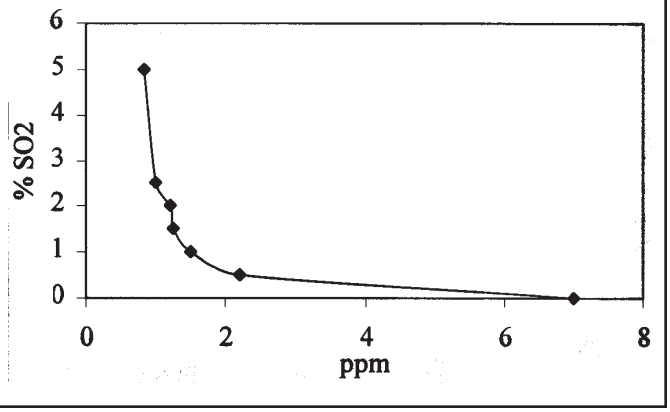

Table 1. DON levels (ppm) upon extrusion[1] of sodium bisulfite-soaked wheat

	Concentration of SO_2 solution				
	H_2O	0.5%	1.5%	2.5%	5.0%
Soaked	4.9	2.3	1.3	1.0	0.8
Extruded, LT	4.4	2.0	0.6	0.7	0.3
Extruded, HT	4.1	2.7	1.0	0.6	0.3

[1]LT: low temperature profile; HT: high temperature profile.

Table 2. Effect of extrusion[1] on DON levels (ppm) of whole meal and flour

	Concentration of SO_2 solution		
	H_2O	5%	10%
Whole meal, non-extruded	6.9	3.4	3.5
Whole meal, extruded LT	6.2	3.2	3.6
Whole meal, extruded HT	6.5	3.8	4.2
Flour, non-extruded	4.2	3.0	3.1
Flour, extruded LT	3.7	3.1	3.1
Flour, extruded HT	3.9	3.3	3.5

[1]LT: low temperature profile; HT: high temperature profile.

Table 3. Effect on rheological properties of flours milled from wheat tempered with various concentrations of SO_2

	H_2O	5%	10%
Farinograph			
Absorption, %	57.6	59.5	59
Arrival time, min.	1	1	1
Peak time, min.	1.5	1.5	1
Stability, min.	1.5	2	0.5
Breakdown time, min.	2.5	3	2
Mixing Tolerance Index, BU[1]	100	140	180
Rapid Visco Analyser			
Peak viscosity[2]	180.3	182	162
Hold[2]	87.3	88.3	73
Breakdown[2]	93	93.7	89
Final viscosity[2]	119.6	109.3	89.6
Setback[2]	32.3	21	16.6
Peak time, min.	5.7	5.7	5.6
Pasting temperature, °C	83.5	83.5	82.8

[1] Brabender Units.
[2] RVA Units.

P

Relationship between Visual Scab Ratings and Deoxynivalenol in Wheat Cultivars.

Bai, G-H, Plattner, R. and Desjardins, A., NCAUR/ARS/USDA, 1815 N. University St. Peoria, IL, 61604; Kolb, F., University of Illinois, 1102 S. Goodwin Ave, Urbana, IL. 61801

Introduction

Wheat scab, caused by *Fusarium graminearum*, is an important disease in the US. Both wheat grain yield and quality can be dramatically reduced by infection with this fungus. Infected grains are often contaminated with deoxynivalenol (DON) which is produced by most strains of *Fusarium graminearum*. DON contamination of grain is a major concern for animal production and human health. Breeding wheat cultivars which accumulate less DON in the grain when infected is a useful approach to solving the problem. However, toxin analysis is laborious and expensive, and is not feasible for routine breeding applications where thousands of lines need to be screened every year. An indirect selection criterion needs to be established to facilitate breeding applications. In addition, a survey of DON accumulation in scab resistant germplasm would help us to identify lines with low DON contamination which would be useful parents for breeding programs. The objectives of this study were to identify wheat germplasm that accumulates less DON under greenhouse infection conditions, and to investigate the relationship between visual scab ratings and DON level in order to identify parameters for indirect selection for low DON level in wheat.

MATERIALS AND METHODS

A total of 115 wheat cultivars and breeding lines were selected from different breeding programs to represent various degrees of resistance to scab. Wheat plants were grown in the greenhouse at the University of Illinois at Urbana-Champaign in 1997. At early anthesis, a droplet of conidia (about 1000 spores) was injected with a hypodermic syringe into a central floret of selected spikes. The inoculum of *F. graminearum* was a mixture of field isolates that originated from scabbed seeds collected in 1996 at the Crop Sciences Research and Education Center of the University of Illinois. Mung bean liquid medium was used to produce conidial inoculum (Bai and Shaner, 1996). The inoculated plants were misted with tap water from overhead misting nozzles installed inside an inoculation chamber that consisted of a polyethylene sheet covered frame on a greenhouse bench. The misting schedule was 10 sec every 30 min. for three days. Temperatures within the moist chamber were 23 to 25 C and relative humidity was 100%. On the fourth day after inoculation, plants were returned to their original positions on the greenhouse benches. Greenhouse temperatures averaged 25C during the day with a range of 19 to 30 C, and 19 C at night with a range of 17 to 21C.

Spikelets with symptoms, ranging from light-brown, water-soaked spots on the glumes to bleached spikelets, were counted 3, 9, 12, 15, 18, and 21 days after inoculation. On the 21st day, total spikelets on each inoculated spike were counted. Disease severity was calculated as the proportion of scabbed spikelets per infected spike (PSS). From these proportions we calculated area under the disease progress curve (AUDPC) for each plant according to Shaner and Finney (1977). The inoculated and uninoculated spikes from each pot were harvested and threshed separately. Seed grade (SG) was visually scored on a 1-5 scale where 1 indicates the lowest and 5 the highest incidence of scabby seeds.

Seeds from 3 inoculated spikes and uninoculated spikes from each pot were pooled and ground for DON analysis. Because sample size ranged from 1 to 5 g kernels, no subsampling was done. The total ground sample was extracted with acetonitrile/water (5 ml/g sample) for three hours with shaking. The extract was filtered and kept refrigerated until analyzed. DON in the extract was measured by high performance liquid chrematography (HPLC) with detection by electrospray ionization mass spectroscopy (MS). The small sample size precluded sample cleanup, so crude extracts were diluted appropriately and 10 ul aliquots were injected directly. Quantification was by comparison with a standard curve obtained from injection of pure DON. The detection limit of the method was approximately 0.5 mg/kg. The greenhouse experiment was conducted in a completely randomized block design with three replications (pots). Each replication had 3 inoculated spikes with one inoculated spike per plant. Visual scab ratings were analyzed on a single spike basis. Seed grade and DON measurement was based on bulked seeds from 3 spikes of each pot. Data were analyzed by using Microsoft Excel according to Steel and Torrie (1980).

RESULTS AND DISCUSSIONS

Based on single floret inoculation in the greenhouse, wheat cultivars differed significantly in area under disease progress curve (AUDPC), percentage of scabbed spikelets (PSS), thousand seed weight (TSW), seed grade (SG), and DON level (Table 1). Resistant cultivars had as low as 5% infected spikelets, but susceptible cultivars had up to 100% of spikelets infected (Tables 1 and 2). The scab infection significantly reduced the seed quality as reflected by poor seed grade and low seed weight (Table 2). Significant contrasts in scab ratings were observed for known resistant and susceptible genotypes, which indicates that the disease evaluation conditions were appropriate for the study.

No DON was detected in uninoculated wheat kernels. However, for inoculated heads, almost all cultivars tested accumulated DON in the infected kernels. Large variation in DON level was observed among the cultivars (Tables 1 and 2). Cultivars resistant to spread of visual scab symptoms within a spike accumulated less DON than susceptible and moderately susceptible cultivars. Highly significant correlation coefficients were observed among D21, AUDPC, TSW and SG (Table 3). These four scab ratings all correlated significantly with DON levels. Among them, the highest positive correlation coefficient was detected between AUDPC and DON level (Table 3). Therefore, selecting for low AUDPC in a breeding program is most likely to obtain plants with relatively low DON levels.

Seeds from inoculated spikes of 17 cultivars had less than 2 ppm DON and 28 additional cultivars had less than 5 ppm DON (data not shown). When more than one wheat spikelet was infected, the DON level was usually higher than 2 ppm. In general, resistant and moderately resistant cultivars with about 20% or less infected spikelets had relatively low DON level and variation among these cultivars was small. For moderately susceptible and highly susceptible cultivars with scab severity of 60-100%, DON level was high (76 ppm in average over 24 cultivars) with a large amount of variation among the cultivars (ranging from 4 to 283 ppm). Most cultivars in this category had high DON levels, but there were a few exceptions. Cultivars Foster, Kaskaskia, and PA8769-160 had about 70% scabbed spikelets, but DON levels ranged from 4 to 9ppm which was far lower than the average (76 ppm). This result suggests that severe visual symptoms may not always be associated with high DON levels.

Although no cultivars are completely free of DON when infected, cultivars Shinchunaga, Sumai 3, Ning 7840, Fumai 3, Fu 5125, Sumai 49, P93D1-10-2, IL9634-24851 and IL 95-1966 had very low DON levels (<1 ppm). These cultivars may be good sources for breeding resistant cultivars that do not accumulate significant levels of DON when environmental conditions favor scab development. Most cultivars with low DON levels came from China, but some were from Japan, Argentina, Austria and breeding programs in the US (Table 2). Cultivars with low DON levels were identified from several areas with frequent scab epidemics. Most of cultivars with low DON levels are breeding lines, only a few are landraces. For example, cultivars from University of Illinois with low DON levels are all advanced breeding lines. Among them, 8 lines had less than 5 ppm DON and 2 had less than 1 ppm. Low DON levels in these cultivars resulted from selection for scab resistance based on visual symptoms, not from direct selection for low DON levels. Therefore, in early generations of the breeding process, wheat lines with low DON levels can be selected by selecting plants or lines with low levels of visual scab symptoms. However, the DON levels in more advanced resistant lines need to be measured before cultivars release to commercial production.

Reference

Bai G-H., and Shaner, G. 1996. Plant Disease. 80: 975-979.
Shaner, G., and R. E. Finney. 1977. Phytopathology 67:1051-1056.
Steel, R. G. D., and J.H. Torrie. 1980. Principles and procedures of statistics. 2nd edition.

Table 1 Statistical description of visual disease scores and DON content.

	TSW	D21	AUDPC	SG	DON
Min	4.9	0.05	0.59	1.00	0.00
Max	38.4	1.00	10.94	5.00	283.00
Mean	22.4	0.37	3.27	3.09	21.39
LSD	9.5	0.33	2.54	1.22	45.64

Table 2 Scab ratings and DON content for selected wheat cultivars from different origins

Cultivar	Origin	DON(ppm)	PSS	AUDPC	TSW(G)	SG
Shinchunaga	Japan	0.00	0.12	1.13	19.67	1.7
Ning 7840	China	0.00	0.08	1.37	26.50	1.7
P93D1-10-2	Indiana	0.01	0.17	1.80	24.61	1.8
F 5125	China	0.38	0.05	0.59	38.42	1.3
Sumai3	China	0.53	0.08	1.13	27.30	1.7
IL95-1966	Illinois	0.67	0.08	0.99	25.84	1.0
IL9634-24851	Illinois	0.85	0.16	1.31	22.8	1.7
Sumai 49	China	0.99	0.08	1.13	36.51	2.0
Spartakus	Austria	1.18	0.07	0.77	26.08	1.7
Sumai 3	China	1.61	0.11	1.29	32.18	1.7
113.92	Argentina	1.76	0.31	2.69	21.56	3.7
Roane	Virginia	1.97	0.17	1.57	25.49	1.4
Wangshuibai	China	2.14	0.19	1.78	29.70	1.8
Bacup	Minnesota	2.17	0.14	1.03	27.15	2.0
Ning 8331	China	2.19	0.12	1.03	36.76	2.0
111.92	Argentina	2.21	0.28	2.22	25.65	3.3
WZHHS	China	2.29	0.13	1.48	31.01	2.2
Karat	Austria	2.56	0.31	1.73	28.64	2.0
Poncheau	France	2.82	0.10	0.91	30.63	2.3
FSW	China	2.88	0.17	1.54	24.05	2.3
IL93-2283	Illinois	3.28	0.15	1.22	28.76	3.0
IL94-2426	Illinois	3.33	0.21	1.97	29.64	2.3
OH 552	Ohio	3.37	0.37	3.34	28.79	2.0
Sanshukomugi	Japan	3.68	0.34	2.52	17.81	3.7
PA-8769-160	Pennsylvania	3.97	0.64	4.26	18.9	3.3
Ernie	Missouri	6.57	0.57	3.43	17.43	3.0
IL94-1911	Illinois	8.51	0.59	5.24	21.65	3.7
Kaskaskia	Illinois	8.70	0.71	3.80	18.6	3.0
Freedom	Ohio	19.33	0.29	2.41	20.01	3.7
Pontiac	Agripro	21.63	0.71	5.89	11.57	4.7
Pio 2553	Pioneer	73.67	0.74	6.78	14.14	4.5
Mo-94-193	Missouri	154.00	1.00	10.94	7.71	4.7
Cardinal	Indiana	216.27	0.76	6.30	12.73	4.3
Clark	Indiana	225.00	0.94	8.94	8.08	5.0
IL94-6280	Illinois	283.33	0.94	9.73	4.89	5.0

Table 3 Correlation between visual disease scores and DON content

	AUDPC	TSW	SG	DON
D21	0.94	-0.74	0.84	0.65
AUDPC		-0.72	0.80	0.75
TSW			-0.76	-0.52
SG				0.56

P

Studies of inheritance of Fusarium Head Blight resistance in wheat.

George Fedak, Jeannie Gilbert, K.C. Armstrong, J.D. Procunier, N. Tinker, G. Butler and R. Pandeya,
Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario K1A 0C6;
Cereal Research Centre, A.A.F.C., Winnipeg, Manitoba R3T 2M9

ABSTRACT

Severity of head blight symptoms, frequency of fusarium infected kernels (FIK) and vomitoxin (DON) content were studied in six doubled haploid populations derived from crosses of resistant and susceptible parents. The frequency distributions for all traits in all populations showed nearly normal distributions implying quantitative inheritance for these traits. The best correlations between the three traits were obtained from a Sumai-3 x HY368 population where r = .65 for symptoms - FIK kernels r = .68 for symptoms - DON and r = .85 for FIK-DON.

INTRODUCTION:

Fusarium Head Blight caused by *Fusarium graminearum* occurs with increased regularity in wheat, barley and corn. Severity of infection is largely influenced by environment and occurs primarily in Eastern Canada and the Red River valley of Manitoba. The components of resistance currently being examined include head blight symptoms from either spray inoculation (Type I) or floret inoculation (Type II), fusarium infected kernels and deoxynivalenol (DON) content of the seed.

Studies on inheritance of resistance to FHB at various centres have given somewhat variable results. The estimates for number of genes controlling resistance are: additive interaction of a minimum of 3 minor genes in Frontana (Singh et al. 1995), two dominant genes in each of Frontana and Sumai-3 (van Ginkel et al., 1996); one to six genes that differed between parents and affected resistance differently (Snijders, 1990). Ban and Suenaga (1997) showed that Sunai-3-derived resistance is controlled by two major loci with additive effects. One of these loci is linked to one of two awn suppressor genes B1 or B2 and located on either chromosome 5A or 6B. By means of reciprocal monosomic analysis (Buerstmayr et al., 1997) it was shown that chromosomes 5A, 1B, 3B, 4B, 6B and 6D carried resistance genes in one resistant accession and chromosomes 3A, 3B, 6B and 4D in another. In summarizing the above results with previously-published data 17 of the 21 wheat chromosomes are implicated in FHB resistance. In the present report spikelet infection, frequency of infected kernels vomitoxin (DON) content and relationships between these parameters were studied.

MATERIALS AND METHODS:

Screening of parents and progeny for Type I resistance was conducted in field and growth chamber plantings. Spikes of growth chamber grown plants were sprayed at anthesis with a 50,000 spores/ml. suspension; maintained in a mist bed at high relative humidity for 48 hours then scored for symptoms at 14 and 28 days after inoculation. Seed from inoculated spikes was ground for DON analysis (Sinha *et al.*, 1995). Field plots were inoculated at the mid-anthesis stage and 4 days later with a suspension of 50,000 spores per ml. High relative humidity was maintained for 14 days with an overhead mist irrigation system. Composites of 20 spikes for determination of FHB indicies were taken at 21 days after inoculation and the remainder of the plot was harvested at maturity. Frequency of infected kernels was determined on threshed samples and one gram aliquots ground for DON analysis. In screening for Type II resistance central florets of growth chamber-grown plants were inoculated at anthesis with a suspension of 5,000 spores per ml. and maintained at high humidity in a mist bed for 48 hours. Disease symptoms were scored at 14 and 28 days after inoculation. Threshed seed from inoculated spikes was ground for DON analysis after the frequency of Fusarium infected kernels was scored (Fedak *et al.*, 1997a).

F_1 hybrids from intercrosses of resistant and susceptible cultivars were pollinated with maize (Fedak *et al.*, 1997b) to produce segregating doubled haploid populations for inheritance and molecular marker studies. A total of 15 doubled haploid populations have been produced to date with those involving Wongshiubai, Sumai-3x Biggar and Wuhan ´ Maringa having been screened most extensively.

RESULTS

Parental Screening: In screening of various wheat accessions for FHB resistance, none was found to be immune to the disease and the best sources of resistance were in

accessions originating in China, Brazil, Hungary and Ukraine. On a scale of 1-10 the following scores were assigned following floret inoculation (Table 1).

Frequency Distribution: The frequency distribution of floret infection, Fusarium infected (tombstone) kernels and DON concentrations in doubled haploid populations involving the FHB resistant parent Wongshiubai are shown in Figures 2,3 and 4 respectively. This data represents results obtained from spray inoculation under controlled conditions. The cultivar Fundulea shows a moderate degree of resistance under our conditions whereas Frontana shows a high level of Type I resistance but not so for Type II. The lower quadrant of Figs. 2, 3 and 4 represents a scoring of a backcross population from crosses to the susceptible parent.
A similar distribution pattern is shown for all three parameters, floret infection, frequency of tombstone kernels and DON content (Fig. 2, 3 and 4). The Frontana x Wongshiubai population was obtained for two resistant parents and is skewed towards the lower levels for all three parameters, with higher frequencies of transgressive segregants. The backcross population showed the lowest frequency of resistant type of segregates.

The frequency distribution of FHB symptoms on doubled haploid populations of Sumai x HY368 and Wuhan x Maringa following point inoculation are shown in Fig. 1. The distributions in both populations are fairly normal but the former is skewed towards lower infection with higher frequencies of lines with higher levels of resistance. This is expected since both Wuhan and Maringa are resistant parents, whereas H4368 is a susceptible parent. These observations would suggest that Wuhan and Maringa may not carry the same resistance loci and transgressive segregants are expected.

However, the fairly normal distributions shown for all 3 traits analyzed in a total of 6 populations in our studies would suggest quantitative inheritance with a more than two loci involved. The ongoing studies with molecular markers on the above populations will help to resolve the number of loci controlling the various traits and whether resistance in different accessions is controlled by different loci.

CORRELATIONS AMONG TRAITS: The correlation coefficients among head blight symptoms, Fusarium infected kernels (FIK) and DON content are shown in Table 2. The Sumai-3x Biggar population represents only floret infection whereas Wongshiubai progenies represent data acquired from several hybrid combinations involving that source of resistance. In the latter it is interesting to note that the coefficients for the two methods of inoculation are quite similar. It is probably premature at this point to speculate on the nature of the inheritance of the three traits but the low coefficients suggest control by different factors.

The DH progeny derived from the Wuhan ´ Maringa hybrid combination were screened for head blight symptoms and several morphological and physiological traits. The correlation coefficients between percent floret infection (floret inoculation) and date of anthesis was r^2 = -0.35* and between floret infection and awn length was r^2 = 0.33*. The coefficients between floret infection and plant height, spike length and number of florets were not significant. The awn suppressor loci B_1 and B_2 are known to be located on chromosomes 5AL and 6BL. It has been shown (Ban and Suenaga, 1997) that one of the resistance genes of Sumai-3 was linked to one of the awn suppressor genes in the repulsion phase with recombination values of 15-20%. It was therefore assumed that one of the resistance genes was located on the long arm of chromosome 5A or 6B. Buerstmayr *et al.*, 1997 have shown that two of the six scab resistance loci obtained from Sumai-3 are located on chromosomes 5A and 6B. Therefore chromosomes 5A and 6B appear to be critical to FHB resistance derived from Sumai-3. RFLP markers linked to a FHB resistance locus on chromosome 6B have already been defined in the above Sumai-3 derived population (Armstrong et al., unpublished).

Based on the significant correlation obtained between spikelet infection and awn length in the Wuhan ´ Maringa population, the same loci could be involved. There may also be a FHB resistance locus associated with delayed anthesis or maturity in the Wuhan ´ Maringa population.

The molecular marker studies currently underway should assist in defining the loci controlling head blight symptoms, FIK and DON content and their relationship to morphological and physiological traits.

REFERENCES

Ban, T. and K. Suenaga, 1997. Cereal Res. Comm. 25: 727-728.
Buerstmayr, H. M. Lemmens and P. Ruckenbauer, 1997. Cereal Res. Comm. 25: 731-732.
Fedak, G. K.C. Armstrong, R.C. Sinha, J. Gilbert, J.D. Procunier, J.D. Miller and R. Pandeya, 1997a. Cereal Res. Comm. 25: 651-654.
Fedak, G. M. Burvill and H.D. Voldeng, 1977b. J. Appl. Genet. 38: 407-414.
Singh, R.P., H. Ma and S. Rajaram. 1995. Plant Disease 79: 238-240.
Sinha, R.C. and M.E. Savard, 1996. Can. J. Plant Path. 18: 233-236.
Snyders, C.H.A. 1990. Euphytica 50: 11-18.
Van Ginkel, M., W. Van Der Schaar and Y. Zhuping. Plant Disease 80: 863-867.

Table 1. FHB ratings of wheat cultivars following floret inoculation

Wuhan-2-37E	0.4	Nanying 7840	3.2
Wongshiubai	1.0	Ning 8343	3.3
Wuhan-1	1.5	Praag 8	3.7
Ankra	1.7	Frontana	5.4
Novokrumka	2.7	Glenlea	5.7
Maringa	2.7	Roblin (check)	9.0
Sumais 3	3.1		

Table 2. Correlations among traits

	Sumais 3x Biggar	**Wongshiubai progeny**	
Traits	Floret	Floret	Spray
Symptoms – FIK	0.65	0.80	0.80
Symptoms – DON	0.68	0.57	0.48
FIK – DON	0.85	0.61	0.66

O

Toxicity Assessment of Trichothecene Mycotoxins Associated With *Fusarium* Head Scab

James J. Pestka, Department of Food Science and Human Nutrition
National Food Safety and Toxicology Center Submitted October 12, 1998

1. Background

Most foodborne illnesses in developed countries are attributable to microbiological contamination. Not surprisingly, there has been a sharp upsurge in national public interest about microbial and chemical food safety during the past few years. The trichothecene mycotoxins are naturally and frequently-occurring contaminants frequently found in grain-based foods. They are a group of sesquiterpenoid metabolites produced by *Fusarium* and other fungi that include some of the most potent eukaryotic protein synthesis inhibitors known. Concern over the trichothecene mycotoxins is due primarily to (1) their potential adverse effects on human and animal health, (2) their unavoidable capacity to contaminate agricultural commodities, (3) their recalcitrance to degradation during milling or processing , (4) economic losses associated with reduced efficiency of livestock production and through the discarding of highly-contaminated wheat or corn [20, 21].

The trichothecene vomitoxin (VT, deoxynivalenol) has occurred with alarming frequency in wheat, corn and barley produced Michigan and Midwest. In a graphic example of this problem cereal processors refused to buy Michigan wheat in 1996 because of widespread *Fusarium* head scab and VT contamination. Several economic concerns are associated with the occurrence of foodborne trichothecenes. First, while a current level of concern of 1-2 ppm has been recommended by the FDA, there is intensive pressure on the U.S. and other nations to establish lower, enforceable levels. 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 VT but posing no risk. Motivation for such an action might be based on overzealousness of government regulators or an attempt by other nations to place a trade barrier to U.S. products. Secondly, from a legal standpoint, litigation may arise as a result of low level VT contamination in the absence of valid human toxicity data. A third problem is that lower levels of other trichothecenes such as 3- and 15-deoxynivalenol, nivalenol and fusarenon (ie. the "8-keto trichothecenes") may also occur with VT and these may complicate potential regulation and litigation issues.

Trichothecene mycotoxins can cause a multisystem shock-like syndrome including dermal irritation, nausea, vomiting, diarrhea, hemorrhage, leukopenia and anemia in farm animals and humans. Although VT and other trichothecenes such as T-2 toxin have been demonstrated to cause acute human toxicity such as alimentary toxic aleukia in China, India, Japan, Russia, and Korea, nothing is known about the dose threshold for human effects. While human feeding studies are impossible because of ethical considerations, it is possible to extrapolate human susceptibility by evaluating the effects of VT on animals and human cell culture systems. Research in our lab over the past 15 years has focused on toxicologic effects of VT and the cellular/molecular mechanisms mediating these toxicities. Using the mouse model, we have determined the threshold VT doses for induction of acute and chronic toxicity as well as a autoimmune kidney disease, IgA nephropathy, which is extremely common in humans. Furthermore, we have identified specific cellular and molecular targets of VT using *in vivo* and *in vitro* murine models . These observations are particularly important to human health because of the high similarities in immune system between human and mouse. We believe that the most critical step for VT toxicity induction is its action on leukocytes (white blood cells) either by activation of cytokines and inflammatory mediators or, at higher doses, by the inductionof apoptosis. *If human leukocyte cytokine dysregulation and/or apoptosis induction are indeed targeted by the same levels of VT as are their mouse equivalents, then the risk of low ppm levels of VT to humans will be extremely small when one considers the diversity of the human diet and the actual potential level of VT exposure in human tissues.* Such evidence is critical because it would support the argument against establishing lower action levels than those currently set for VT.

2. Project goals

Leukocytes (or white blood cells) are primary targets of the trichothecenes. We have hypothesized that the levels of VT and closely related 8-ketotrichothecenes required for

cytokine and apoptosis induction will be identical in mouse and human leukocytes. Three critical leukocyte types- the macrophage, T cell and B cell are being assessed in this study. We are currently evaluating murine cell clones as sources for these leukocyte types and will follow these studies up by reconstituting these findings with purified subsets obtained from spleens and lymph nodes of mice. Using the above approaches, we will develop quantitative structure-activity relationship(QSAR) models for members of the 8-ketotrichothecenes including 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol, nivalenol and fusarenone as well as their specific detoxification products. Specifically, we will correlate biological activities with structural features (Free Wilson analysis) or with molecular properties such as lipophilicity, polarizabilty, electonic and steric properties. With the availability of Fusarium Head Scab research funds, we will expand these models to include human macrophage, T cell and B cell clones and subsequently evaluate primary cultures derived from peripheral blood mononuclear cells (PBMC) of human volunteers.

3. Progress

The RAW 264.7 murine cell line was used as a macrophage model to assess effects of the VT on proliferation and the production of nitric oxide (NO), hydrogen peroxide (H_2O_2) and cytokines. Using the MTT cleavage assay, VT at concentrations of 50 ng/ml or higher was found to significantly decrease proliferation and viability of RAW 264.7 cells without stimulation or with stimulation by lipopolysaccharide (LPS) or interferon (IFN)-. In the absence of an activation agent, VT (25-250 ng/ml) had negligible effects on the production of NO, H_2O_2 and cytokines. Upon activation with LPS at concentrations of 10 to 100 ng/ml, VT at 25 to 100 ng/ml markedly enhanced production of H_2O_2 but was inhibitory at 250 ng/ml. VT enhancement of H_2O_2 production was observed as early as 12 h after LPS stimulation. When IFN- was used as the stimulant, VT (25 to 250 ng/ml) delayed peak H_2O_2 production. VT (25-250 ng/ml) also markedly decreased NO production in cells activated with LPS or IFN-g. Interestingly, VT superinduced TNF-a and IL-6 production in LPS-stimulated cells and also elevated TNF-a in IFN-stimulated cells. These results suggest that VT can selectively and concurrently upregulate or downregulate critical functions associated with activated macrophages.

4. Publications

Wong, S.S., H.R. Zhou, M.L. Marin-Martinez, R.H. Brooks, and J.J. Pestka. 1998. Modulation of IL-1 beta, IL-6, and TNF-alpha secretion and mRNA expression by the trichothecene vomitoxin in the RAW 264.7 murine macrophage cell line. Food Chemical Toxicol. 36:409-419.

Ji, G.E., S.Y. Park, S.S. Wong, and J.J. Pestka. 1998. Modulation of nitric oxide, hydrogen peroxide, and cytokine production in a clonal macrophage model by the trichothecene vomitoxin (deoxynivalenol). Toxicology 15:203-214.

Roth, R.A., J.R. Harkema, J.J. Pestka and P.E. Ganey. 1998. Does exposure to bacterial endotoxin determine susceptibility to intoxication from xenobiotic agents? Toxicol. Appl. Pharmacol. 147:300-311.

Yan, D., H.R. Zhou, K.H. Brooks and J.J. Pestka. 1998. Role of macrophage in elevated IgA and IL-6 production by Peyer's Patch cultures following acute oral vomitoxin exposure. Toxicol. Appl. Pharm. 148:261-273.

Li, S., Y.L. Ouyang, W. Dong and J.J. Pestka. 1997. Superinduction of IL-2 gene expression by vomitoxin (deoxynivalenol) involves increased mRNA stability. Toxicol. Appl. Pharmacol. 147:331-342.

Yan, D., H.R. Zhou, K.H. Brooks, and J.J. Pestka. 1997. Potential role of IL-5 and IL-6 in enhanced IgA secretion by Peyer's patch cells isolated from mice acutely exposed to vomitoxin. Toxicology 122:145-158.

Zhou, H.R., D. Yan, and J.J. Pestka. 1997. Differential cytokine mRNA expression in mice of oral exposure to vomitoxin dose response and time course. Toxicol. Appl. Pharmacol. 144:294-305.

Pestka, J.J. 1997. Mechanisms of trichothecene-induced murine IgA nephropathy. Mucosal. Immunol. Update. September p. 49-52.

P

A Machine Vision and Neural Network Based Automatic Scabby Wheat Estimation System

Roger Ruan, Liuqing Luo, Shu Ning, Xia Chen, and Paul Chen, Dept. of Biosystems and Agricultural Engineering, University of Minnesota, St. Paul, MN 55108.Roger Jones, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Objective

The objective of this research was to develop an automatic system for rapid determination of scabby rate in wheat samples.

INTRODUCTION

Fusarium head blight or "scabby" is a disease that affects the quality and consequently the price of wheat. In order to determine appropriate dockage on lots offered for sale, the scabby rate of wheat samples must be estimated. Researchers also need a method for rapid determination of scabby wheat percentage during their research. Currently this is done by manually picking out the scabby kernels one by one from the samples, and then calculating the scabby weight percentage. This is very time consuming. Another approach that has been explored is to measure deoxynivalenol (DON) produced by infected kernels, using chromatography methods, based on the assumption that DON level is related to scabby rate (Stack et al. 1993, Tekauz, 1993). However, the low correlation coefficient between DON and scabby rate does not permit an accurate estimation of scab rate, and the chromatography for measurement of DON is expensive and time consuming.

The combination of machine vision and neural network techniques has been proved effective in estimating scabby rate of wheat kernels (Ruan et al. 1995). The machine vision technique is used to capture the visual features unique to the scabby kernels (Figure 1), and the neural network technique to quantitatively relate the visual features to scabby rate. Substantial work has been carried out by Ruan and his co-workers to obtain visual features most closely related to scab through a series of image processing techniques, and to build neural network structures suitable for this application (Ruan et al. 1998). This report describes authors' effort to automate the operations involved in estimating scabby rate of wheat kernels using the machine vision and neural network based system.

MATERIALs AND METHODs

Wheat samples

One hundred and eighty Red Sprint wheat samples were used in this study. For each sample, the scabby kernels were picked out manually, and the weight of the scabby kernels divided by the total weight of the sample was expressed as '*SWP*' to indicate the scabby rate of the sample. The 180 samples were divided into two portions, namely the training portion (101 samples with 27 different *SWP*) for development of the trained neural network, and the predicting portion (79 samples with 23 different *SWP*) to evaluate the performance of the trained network.

The System

The system (Figure 2) features several components including a sample carrier (or feeder, step motor driven), an image capturing and processing device (light box, camera, digitizer, and image processor), and neural network program. A Visual C++ program was written to control the operations of all these components. The system can be commanded to execute following tasks in sequence: moving sample into the light box one frame length at a time, capturing image of the sample, extracting features from the image, passing extracted features to the trained neural network, computing the data and making estimation by the trained neural network, displaying result, and readying the system to take next sample.

The sample carrier is about eight frames long therefore eight duplicate samples could be imaged continuously. From each captured image, twenty-six color features were extracted, and passed to the neural network. The neural network was trained using the extracted features

and known *SWP*s from about three fifth of the 180 samples. When the trained network was fed with the features extracted from the rest of the samples (that had not been used for training of the network), it produced estimated *SWP*s for these samples.

In current research, Two new features extracted from the captured images, namely approximate scabby kernel area (*ASKA*) and approximate scabby kernel number (*ASKN*), were used. Potential errors from several sources such as light fluctuation, overlapping kernels, and signals from non-kernel areas have been reduced through online color calibration and image processing techniques to improve the quality of the extracted color features.

RESULTS AND DISCUSSION

Fig. 3 shows that neural network-estimated *SWP* followed the trend of the actual *SWP* very well. The statistical analysis (Table 1) indicates that the correlation coefficient between the network-estimated and actual *SWP*s was 0.96 with a mean absolute error of 1.32% and maximum absolute error of 5.22%. In order to evaluate the repeatability of the system, we performed the estimation procedure 20 times for each of the three samples with actual *SWP* of 5%, 15% and 24%, respectively. It was found that the coefficients of variation of the estimation results were 7.3%, 5.4%, and 4.0%, respectively. The total estimation time using two images for every sample is approximately 30 seconds.

References

Ruan, R., J. Z. Xu and R. K. Jones 1995. Rapid Analysis of Scabby Wheat Using Machine Vision and Neural Networks. Proceedings of the Food Processing Automations IV Conferences ASAE. Chicago, IL

Ruan, R. , S. Ning, A. Song, A. Ning, R. Jones, and P. Chen. 1998. Estimation of Fusarium Scab in Wheat Using Machine Vision and a Neural Network. Cereal Chem. (Accepted)

Stack, R. W. 1993. Relationship Between Vomitoxin Levels and the Scabby Seed in 1991

Tekauz, A. 1993. Analysis of Fusarium Tricothecene Data from Canada. The Scab Proceedings. Mn Wheat Res. And Promotion Council, Red Lake Falls, MN.

Figure 1. Images of wheat samples with 5% (L) and 30% (R) visual scabby kernels.

Figure 2. Schematic diagram of the machine vision and neural network based system for estimation of scabby rate of wheat kernels.

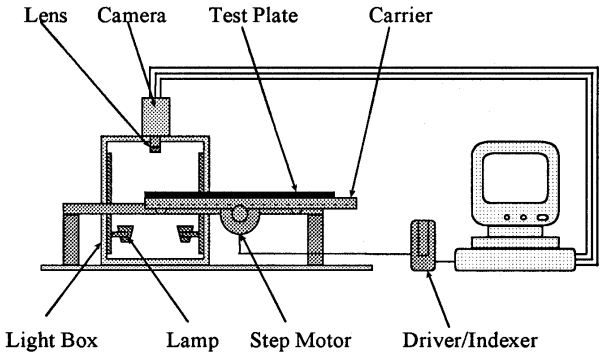

Figure 3. Comparison of actual *SWP* and neural network-estimated *SWP*. Features were extracted from color calibrated images of single layer samples.

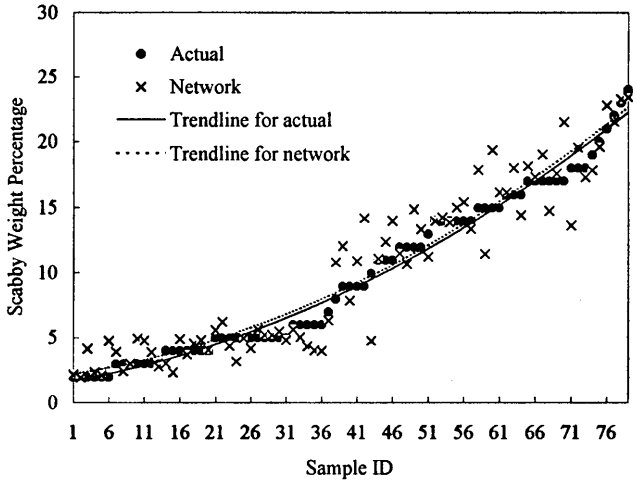

Table 1. Statistical analysis of the estimation result

R squared:	0.913
r squared:	0.922
Mean squared error:	3.26
Mean absolute error:	1.323
Min. absolute error:	0.026
Max. absolute error:	5.221
Correlation coefficient r:	0.960
Relative error<= 5%:	26.58
Relative error within 5% to 10%:	17.72
Relative error within 10% to 20%:	22.78
Relative error within 20% to 30%:	15.19

Evaluating Sampling Strategies for Vomitoxin in the Midwestern US

Oliver Schabenberger, L. Patrick Hart, Fanzhi Kong

ABSTRACT

We investigate the bias and inconsistency in estimating deoxynivalenol (DON) concentrations when sampling kernels from truck loads of wheat with standard seed probes due to spatial heterogeneity in toxin distribution and complete or incomplete probe insertion. A probabilistic model is developed for distributing deoxynivalenol contaminated kernels throughout a lot or bin according to a fixed or random spatial pattern of toxin intensity. Results of a simulation study are compared with data gathered throughout Michigan from twenty-four trucks during the 1996 Fusarium Head Blight epidemic and the non-epidemic 1997 growing season. This comparison provides supporting evidence that vomitoxin distribution throughout a truck load is in fact not homogeneous but clustered. If spatial heterogeneity is not properly represented in the sample, the variability of the DON estimate will shrink with increasing number of sample probes, but the bias component remains constant. Confidence intervals for DON are then centered around the wrong value and are less informative for larger sample sizes, a dangerous and counterintuitive result. Developing and supervising appropriate sampling strategies is hence of critical importance.

ACKNOWLEDGMENTS

We gratefully acknowledge assistance provided by Dr. C. J. Mirocha, University of Minnesota, regarding the identification of deoxynivalenol distributions by kernels.

1. INTRODUCTION

Wheat scab, also known as Fusarium Head Blight (FHB) has been a serious problem in grain producing regions of the Midwestern U.S. for many years (McMullen et al. 1997). An estimated 90 million bushels of wheat were lost in North Dakota during head blight epidemics in 1993. In 1996, Michigan, Ohio, and Indiana experienced their worst scab epidemic of this century. Contamination of the grain with the secondary metabolite deoxynivalenol (DON, vomitoxin) is the most serious consequence of infection apart from reductions in grain yield and quality. The toxicological properties of deoxynivalenol were recently reviewed (Rotter et al. 1995) as well as the importance of wheat and barley scab in the United States (McMullen et al. 1997). Because of its mammalian toxicity the U.S. Food and Drug Administration in 1993 revised earlier advisory levels for deoxynivalenol in wheat entering the milling process, shifting emphasis to concentrations in finished products (FDA 1993). As a consequence of the advisory levels coupled with the availability of Enzyme Linked Immunosorbent Assay (ELISA) test kits for deoxynivalenol testing after sampling wheat from trucks, bins, railcars, etc. has dramatically increased (Hart et al. 1998). Since the advisory levels create thresholds for marketing of wheat, detecting the contamination level of scab infected wheat accurately and precisely has gained critical importance in terms of food quality and agricultural economics.

Currently, detection and estimation of deoxynivalenol in wheat on trucks is facilitated by extracting the kernels trapped in a (small) number of randomly inserted probes and determining DON contamination through ELISA or gas chromatography (Hart et al. 1998). Probes are placed on the surface of the truck in a random, or more conveniently, systematic arrangement. It is presumed that samples so collected will lend themselves naturally to unbiased estimates of vomitoxin concentration if systematic errors in post-sampling processing are avoided. It can be shown quite easily, however, that ignoring the spatial distribution of toxin within a lot, bin, or truck, can introduce substantial bias if the sampled material does not adequately represent the truck as a whole. Schabenberger et al. (1999) investigated the effects of spatial toxin heterogeneity on DON estimates as a function of various sampling designs. Assuming for the time being, that probe locations are randomly determined on the truck surface, incomplete insertion of the probe will create non-ignorable bias unless the distribution of toxin is completely homogeneous throughout

O. Schabenberger(*) is Assistant Professor of Applied Statistics, Dept. of Crop and Soil Sciences, Michigan State University, East Lansing, MI, 48824-1325 (E-Mail:schabenb@pilot.msu.edu); L. Patrick Hart is Professor, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI, 48824 (E-Mail: hartl@pilot.msu.edu). Fanzhi Kong is Graduate Student, Department of Statistics and Probability, Michigan State University. The DON Simulator used herein can be downloaded from http://www.msu.edu/user/schabenb. * Corresponding author

the truck. A general recommendation is that manual probes should be inserted to at least 75% of the lot depth. When sampling from large containers such as railcars or ships, this recommendation can not be followed with seed probes of standard length.

In this contribution we demonstrate how severely ignoring the spatial distribution of toxin in combination with sloppy sampling techniques can bias the results of vomitoxin analysis. A comparison of random processes built on describing the distribution of infected kernels among DON-free kernels by probabilistic techniques with data gathered from twenty-four trucks in the field, in fact suggests that the distribution of toxin on trucks is heterogeneous and must be accounted for through proper sampling. A Windows95/WindowsNT software product for self-evaluation of sampling bias and variance is available from the senior author's web page at http://www.msu.edu/user/schabenb.

2. A RANDOM PROCESS FOR INFECTED KERNELS

Mathematical details of the development of a probabilistic model for the distribution of infected kernels throughout a lot of wheat can be found in Schabenberger et al. (1999) and are not repeated here. For this contribution we simplify the setup discussed there as follows. A truckload is assumed to consists of è kernels per cubic meter which can be determined from, e.g., thousand kernel weight and bulk density. A proportion π of ø is infected with DON. Based on personal communication with Dr. Mirocha (Univ. of Minnesota) and preliminary data provided on deoxynivalenol load by kernel, we choose to model the distribution of DON by kernels as an exponential random variable with mean ì (in ppm). By varying the frequency (intensity) with which infected kernels appear in any sub-volume of the truck, either according to some random law or systematically, different spatial toxin distributions can be generated. When this intensity is varied in a systematic fashion, for example linearly with the depth of the lot, distribution patterns as displayed in Figure 1 emerge. For the interested reader, the underlying process distributing infected kernels throughout the truck such that the overall infection percentage is è and a particular spatial arrangement is achieved, is known as an inhomogeneous Poisson point process (Cox and Isham 1980, Cressie 1993) and the actual toxin load of each kernel serves as a random mark variable. For implementation of the simulation algorithm see Lewis and Shedler (1979).

3. EFFECTS OF SAMPLING WITH INCOMPLETE INSERTION

Assume that probes are inserted randomly on the surface in a population structured as the linear inhomogeneous process in Figure 1. Heterogeneity of the toxin distribution exists only in the depth dimension. If an inserted sampling probes does not capture the entire cross-section of the lot, the material in it will not properly represent the population at large, since material from the top is always extracted, but material from the bottom only with lesser frequency. If the rate changes linearly in such a way that the concentrations on the surface exceed those in the center of the lot by 70%, Figure 2 shows the toxin concentration represented by the sample as a function of infection percentage. Even if the probe is inserted at least two thirds of the lot depth, deoxynivalenol concentration in the sample will overestimate the true concentrations systematically. This effect increases with the infection percentage. Skimming samples off the top of the lot, as was suggested elsewhere, is an extremely hazardous practice if any heterogeneity of the toxin distribution exists. Being aware of these pitfalls, one should attempt to insert the probe completely. Due to the resistance of the material, especially when probing manually, this may not always be possible, although the sampler may have the impression of success. If each probe is inserted at least two-thirds of the lot depth, but the remainder is left to uniform random variation, that is, some probes will hit the bottom, while others do not, bias is smallest in Figure 2, but not necessarily ignorable. Especially near one of the F.D.A. threshold levels, a bias of 0.3 ppm may not be acceptable.

4. SIMULATION VS. REALITY

Hart et al. (1998) analyzed data from fourteen trucks sampled throughout Michigan during the 1996 harvest season. Ten probes were sampled from each truck. Material from four of these was furthermore subsampled in 50g units. Hart et al. (1998) examine the probe-to-probe variability of deoxynivalenol concentration in these data and report confidence intervals as a function of the number of probes sampled. Using additional data collected on ten trucks in 1997 not included in above mentioned work, Figure 3 shows probe-to-probe variability as a function of average deoxynivalenol concentration. It is interesting to note, that the variability of the more regular, spatially diverse processes, such as linear intensity functions (Figure 1), layered distributions, or sigmoidal rate functions, as well as homogeneous toxin distributions produced processes with considerably less variability than was observed in the field. One explanation is the effect of variability of ELISA results which contributes to the data shown in Figure 3 but is absent from the simulated data. However, this could not explain the differences in variability between simulated and empirical data alone. A simulated process which resembled more closely the variability pattern observed in the field is het-

erogeneous in all three dimensions of space and toxin appears in clusters throughout the truck (Figure 4).

While the clustered process may not appear much different from the smooth but inhomogeneous process in Figure 1 to the naked eye, sampling from this process exhibits considerably more variability. In addition, since the disease intensity changes in either direction, an appropriate sampling design on the surface of the truck is critical. The dashed line in Figure 2 shows the estimated vomitoxin concentrations if the probe is inserted two-thirds of the truck depth based on randomly generating one hundred trucks. A different set of trucks was generated for each infection percentage. The large variability of the estimates is an artifact of the truck-to-truck variability in the clustered process. Although for some infection percentages, bias is negligible, it is considerable for example, around $\partial = 0.2$.

5. INCONSISTENCY AND CONFIDENCE INTERVALS

An interesting fact, elaborated upon by Schabenberger et al. (1999), is the inconsistency of the above mentioned sampling strategy. This term, defined statistically, says that with increasing sample size the bias of the DON estimate will not shrink, although its variability does. As an interesting upshot, 95% confidence intervals for DON, as reported in Hart et al. (1998) have decreasing coverage probability with increasing sample size (Table 1). If samples are skimmed from the top only , the bias is substantial. The target value of 2 ppm is estimated accurately only if the probes are inserted completely. Within a given sample depth the standard error of the DON estimate decreases with sample size, as expected. The confidence intervals are centered around a biased value and the center of the intervals does not move towards the true value, even if sample size is increased. Only for complete insertion do the 95% confidence intervals actually include the target value of 2 ppm. Otherwise, the recommendation should be to collect **as few** samples as possible, since that increases the odds that the confidence intervals cover the true value. For example, at 66% insertion depth the lower bound of the interval for n=2 probes is closer to the target than the intervals for n=4 or n=10. In other words, if each probe sample estimates is an equally poor estimate of the target, collect as little poor samples as possible, i.e., **decrease** sample size.

6. RESULTS AND DISCUSSION

If toxin is distributed homogeneously throughout a lot being sampled, all sampling designs provide unbiased estimates of average deoxynivalenol concentration. Recommendations regarding sample size, probe placement, and insertion depth can then be developed based on infection percentages, toxin distribution per kernel, etc. Whether probes are inserted completely or not is not critical. If toxin is not distributed homogeneously, the placement of probes is essential if the sample is to represent the lot as a whole. Deriving sampling recommendations on the basis of sample size alone can lead to erroneous conclusions, since the bias component does not depend on the sample size, but the DON estimator's variability does (Table 1). Emphasis in this contribution was on the effects of incomplete insertion which does not represent the DON distribution properly in the depth dimension of the lot. A comparison of the variability of simulated point processes with field data gathered in Fusarium Head Blight epidemic and non-epidemic years suggested that heterogeneity is present in all three dimensions. That implies, that the placement of the probes on the surface is also critical. Sampling only the reachable edges of a truck bed will increase the sampling bias in this case. Investigations are currently under way by authors that examine the spatial toxin distributions in a large number of trucks empirically to gain more detailed insight into the spatial dependencies of deoxynivalenol distribution. Reports on this investigation are forthcoming.

REFERENCES

Cox, D. R. and Isham, V. (1980), *Point Processes*. London: Chapman and Hall.

Cressie, N. A. C. (1993), *Statistics For Spatial Data*, Revised Edition. John Wiley & Sons, Inc.

FDA, (1993), U.S. *Food and Drug Administration Advisory Levels - Deoxynivalenol* Sept. 16, Associate Commissioner for Regulatory Affairs, Dept. of Health and Human Services, Rockville, Md.

Hart, P. L. and Schabenberger, O. (1998), "Variability of Vomitoxin in Truckloads of Wheat in a Wheat Scab Epidemic Year," Plant Disease, 82:625-630

Hart, P. L., Casper, H., Schabenberger, O., and Ng. P. (1998), "Comparison of gas chromatography and enzyme linked immunosorbent assay for deoxynivalenol in milled fractions of naturally contaminated wheat," Plant Pathology, In print.

Lewis, P.A.W. and Shedler, G.S. (1979), "Simulation of non-homogeneous Poisson processes by thinning," Naval Research Logistics Quarterly, 26:403-413

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

Ratkowsky, D.A. (1990) *Handbook of Nonlinear Regression Models*. Marcel Dekker, New York

Rotter, B., Prelusky, D. and Pestka, J. (1996), "Toxicology of deoxynivalenol (vomitoxin)," J. Toxicol. Environ. Health, 48, 1-34

Seber, G.A.F. and Wild, C.J. (1989) Nonlinear Regression. John Wiley and Sons, New York.

Schabenberger, O., Kong, F., Hart, P.L. and Gregoire, T.G. (1999) Sampling Random Volumes from Inhomogeneous Point Processes: An Application to Wheat Scab Epidemics in the Midwestern U.S. Submitted to Journal of the American Statistical Association

Figure 1. Simulated lot (truck) of kernels where the intensity of kernel infection changes linearly with the depth of the lot, whereas the distribution of infected kernels is homogeneous in horizontal dimensions. Inserted graphic shows change in infection intensity. Realistic densities of infected kernels are much higher. Small densities chosen for plotting purposes.

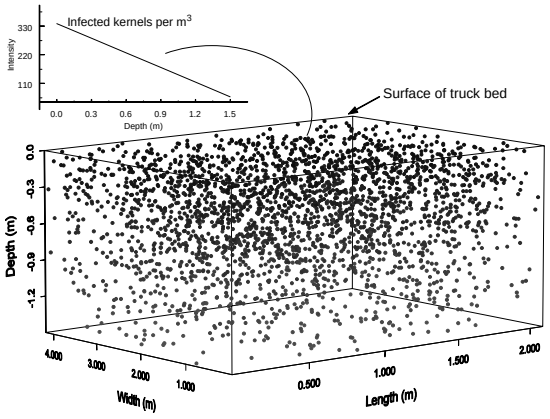

Figure 2. Bias in vomitoxin concentrations as a function of infection percentage if probes are not inserted to the bottom of the truck and disease intensity changes linearly with depth (see Figure 1). The dotted line represents the true deoxynivalenol concentration. The jagged line is discussed in subsequent text.

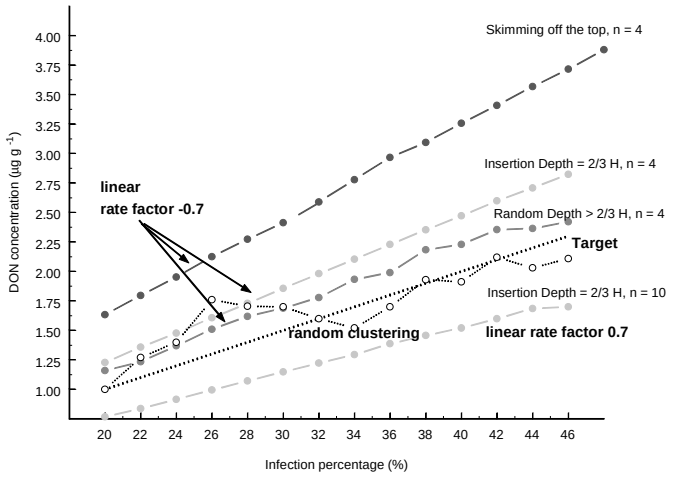

Figure 3. Probe-to-probe variability in twenty-four trucks sampled in 1996 (epidemic year) and 1997 (non-epidemic year) throughout Michigan. Solid line represents fitted, nonlinear von Bertalanffy (Mitscherlich law) function (Seber and Wild 1989, Ratkowsky 1990).

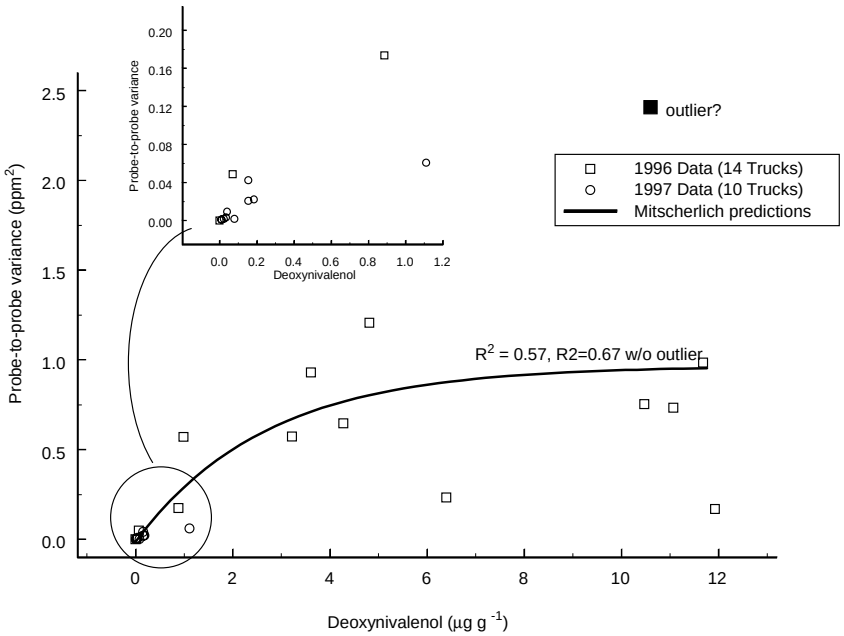

Figure 4. A clustered process where the change in toxin levels is not smooth, but abrupt in three dimensions of space. The lot consists of 64 individual (regular) clusters whose infection percentages change randomly from cluster to cluster.

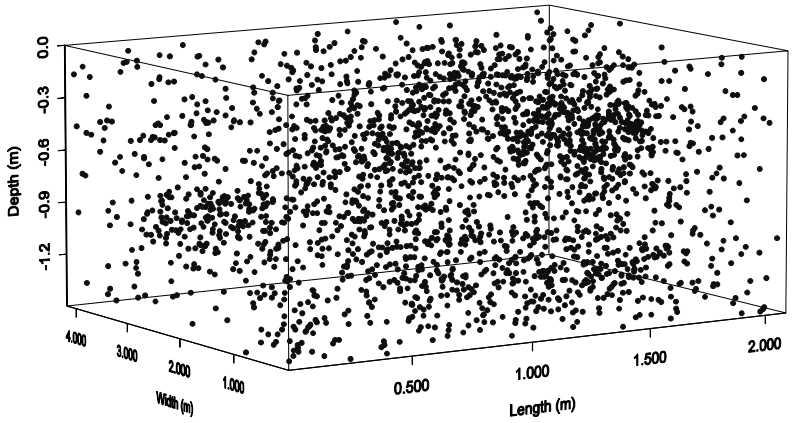

Table 1. Empirical 95% confidence intervals for mean deoxynivalenol concentration on a single truck when sampling from a clustered point process. The infection percentages between clusters change independently and exponentially. The truck consists of 64 clusters (Figure 4). Bulk density 50 lbs./cuft, 1000 kernel weight 31.83g, average DON load of infected kernels 5 $\mu g g^{-1}$. Infection percentage = 40%. Based on 200 independent simulations.

Depth of insertion (%)	No. of probes	DON estimate (.11)	Rel. Bias %	Std. Error	*&% Confidence Interval Lower Bound	Upper Bound
5	2	7.82	291%	0.363	6.25	9.38
5	4	7.82	291%	0.256	6.76	8.88
5	10	7.80	290%	0.126	6.44	7.72
50	2	3.62	81%	0.146	3.12	4.13
50	4	3.62	81%	0.104	3.28	3.96
50	10	3.63	81%	0.065	3.41	3.83
66	2	2.39	19%	0.068	2.06	2.73
66	4	2.40	20%	0.048	2.18	2.62
66	10	2.39	19%	0.030	2.26	2.53
100	2	1.95	2.6%	0.048	1.75	2.15
100	4	1.95	2.6%	0.034	1.81	2.09
100	10	1.95	2.6%	0.021	1.87	2.04