DIAGNOSTIC VOMITOXIN (DON) SERVICES IN 2000-2001

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INTRODUCTION

Fusarium Head Blight (FHB) is an important problem for American agriculture. Resolving the FHB problem involves cooperative efforts, including analytical assays for vomitoxin in the new wheat and barley varieties. In 2000, the US Wheat and Barley Scab Initiative provided grants, for diagnostic vomitoxin (DON) services, to 4 laboratories in Michigan, Minnesota, and North Dakota. The following provides an insight into the methods, quality assurance and number of samples processed by each laboratory.

MATERIALS AND METHODS

The 3 laboratories in Minnesota and North Dakota all use the method of Tacke (1), followed by GC/EC or GC/MS quantitation. Michigan used the Neogen Veratox system.

MICHIGAN
Lab Director: L. Patrick Hart, Ph.D., Dept. of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824, Phone: 517-353-9428, Fax: 517-353-5598, e-mail: hartl@pilot.msu.edu
Method: Water extraction and DON quantitation with the Neogen Veratox kit
Sample Types: Wheat and barley
Intralab Quality Assurance: Wheat Pool (2.2 ppm DON)

MINNESOTA
Lab Director: Weiping Xie, Ph.D., Dept. of Plant Pathology, University of Minnesota, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, Phone: 612-625-2751, Fax: 612-625-9728, e-mail: weipingx@puccini.crl.umn.edu
Method: Acetonitrile: water extraction, silylation and DON, 15 A-DON, 3 A-DON quantitation by GC/MS, plus a screen for 8 other trichothecenes.
Sample Types: Wheat and barley
Intralab Quality Assurance: Wheat Pool (12.9 ppm)

NORTH DAKOTA
Lab Director: Howard H. Casper, Ph.D., Dept. Vet. and Micro. Science, North Dakota State University, Fargo, ND 58105, Phone: 701-231-7529, Fax: 701-231-7514, e-mail: hcasper@ndsuext.nodak.edu
Method: Acetonitrile: water extraction, silylation and DON, Nivalenol, 15 A-DON, quantitation by GC/EC. Full screens for 17 mycotoxins can also be done by GC/MS.
Sample Types: Wheat and barley
Intralab Quality Assurance: Wheat Pool (1.8 ppm DON), Barley Pool (3.2 ppm DON).

NORTH DAKOTA
Lab Director: Paul B. Schwarz, Ph.D., Dept. Cereal Science, North Dakota State University, Fargo, ND 58105, Phone: 701-231-7732, Fax: 701-231-7723
e-mail: nubarley@badlands.nodak.edu
Method: Acetonitrile: water extraction, silylation and DON quantitation by GC/EC
Samples Types: Barley and malt products
Intralab Quality Assurance: Barley Pool (3.4 ppm DON), Wheat Pool (2.0 ppm DON)

PROFICIENCY CHECK SAMPLES
In August of 2000, H. Casper collected wheat and barley samples from local sources and distributed these samples on a monthly basis. A wheat sample and a barley sample was sent on each occasion and the data was collected from each laboratory within one week. Each laboratory did the DON analyses in their normal fashion. These check samples allowed each laboratory to evaluate the accuracy and precision of their system.

RESULTS AND DISCUSSION

Information from the 4 laboratories, pertaining to quality assurance, number of samples analyzed and proficiency check samples is listed in Tables I, II and III.

Table I. Intralab Vomitoxin Quality Assurance (QA) Data for May – November, 200

1.	MICH – P. Hart	QA: Wheat Pool;	n = 56,	Ave = 1.6 ppm,	cv = 7%
2.	MINN – W. Xie	QA: Wheat Pool;	n = 25,	Ave = 12.9 ppm,	cv = 11%
3.	ND – P. Schwarz	QA: Wheat Pool;	n = 38,	Ave = 2.0 ppm,	cv = 12%
		QA: Barley Pool:	n = 48,	Ave = 3.4 ppm,	cv = 18%
4.	ND – H. Casper	QA: Wheat Pool;	n = 59,	Ave = 1.8 ppm,	cv = 8%
	" "	" : Barley Pool;	n = 59,	Ave = 3.2 ppm,	cv = 7%

Table II. Estimated Vomitoxin Assays for 2000 – 2001.

			ppm DON					
Lab	**Method**	**Grain**	**Aug. 00**	**Sep. 00**	**Oct. 00**	**Nov. 00**		**Ave.**
MICH – P. Hart	Elisa	Wheat	10.8	1.5	0.6	7.2		5
MINN – W. Xie	GC/MS	Wheat	11.4	0.6	0.6	5.6		4.6
ND – H. Casper	GC/ECD	Wheat	10.6	0.8	0.6	5.8		4.5
ND – P. Schwarz	GC/ECD	Wheat	13.2	0.8	0.3	4.2		4.6
Ave.			11.5	0.9	0.5	5.7		
MICH – P. Hart	Elisa	Barley	2	6.6	7.9	14.5		7.8
MINN – W. Xie	GC/MS	Barley	2	5.6	7.4	14.7		7.4
ND – H. Casper	GC/ECD	Barley	2	5.7	7.8	14.2		7.4
ND – P. Schwarz	GC/ECD	Barley	1	4.4	9.5	10		6.2
Ave.			1.8	5.6	8.2	13.4		

Table III. Interlab Vomitoxin Proficiency Check Samples.

			ppm DON					
Lab	**Method**	**Grain**	**Aug. 00**	**Sep. 00**	**Oct. 00**	**Nov. 00**		**Ave.**
MICH – P. Hart	Elisa	Wheat	10.8	1.5	0.6	7.2		5.0
MINN – W. Xie	GC/MS	Wheat	11.4	0.6	0.6	5.6		4.6
ND – H. Casper	GC/ECD	Wheat	10.6	0.8	0.6	5.8		4.5
ND – P. Schwarz	GC/ECD	Wheat	13.2	0.8	0.3	4.2		4.6
Ave.			11.5	0.9	0.5	5.7		
MICH – P. Hart	Elisa	Barley	2.0	6.6	7.9	14.5		7.8
MINN – W. Xie	GC/MS	Barley	2.0	5.6	7.4	14.7		7.4
ND – H. Casper	GC/ECD	Barley	2.0	5.7	7.8	14.2		7.4
ND – P. Schwarz	GC/ECD	Barley	1.0	4.4	9.5	10.0		6.2
Ave.			1.8	5.6	8.2	13.4		

The data in Table I shows that the intralab coefficient of variation for the 4 labs varies from 6 to 16% on the control pools that were analyzed with the test samples. The interlab proficiency check samples demonstrated that the 4 laboratories are getting similar results. The ELISA kit (2) provided a reasonable intralab coefficient of variation and the overall data was not significantly different from the chromatographic techniques. In the FHB campaign for

2000-2001, we estimate that ~23,000 samples will be processed by the 4 laboratories for ~42 principal investigators in ~16 states.

The interlab proficiency check samples will be continued in the FHB campaign for 2001-2002. Each laboratory will be evaluating means of refining their analytical techniques.

REFERENCES

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

Hart, L. P., H. Casper, O. Schabenberger, and P. Ng. 1998. Comparison of Gas Chromatography and Enzyme Linked Immunosorbent Assay for Deoxynivalenol in Milled Fractions of Naturally Contaminated Wheat. J. Food Protec. 61:1695-1697.

DON LEVEL IN GRAIN FROM WHEAT INOCULATED WITH *F. GRAMINEARUM* IS NOT CORRELATED TO THE DON PRODUCING POTENTIAL OF INDIVIDUAL CULTURES

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ABSTRACT

A collection of twelve cultures of *F. graminearum* isolated from corn and wheat were previously tested for production of deoxynivalenol (DON). The isolates produced a range of low (<1ppm) to high (>20) DON levels under standard test conditions. (*Wolf-Hall and Bullerman. 1998. J.Food Mycol. 1:171-180.*) Some researchers have claimed that DON is a pathogenicity factor in Fusarium Head Blight (FHB). We used this set of isolates to test that hypothesis. Isolates were tested for disease causing ability using methods used previously (*Stack, 1989. Can. J. Pl. Path. 11:137-142*). The isolates were grown on half-strength PDA under ambient room lighting for two weeks; conidia were washed from plates and suspensions adjusted to 50k/ml. Droplets (10µl) were placed into single spikelets on flowering wheat heads. After 3.5 weeks of incubation, individual heads were scored for FHB severity on a 0-100% scale. Plants matured naturally and inoculated heads were harvested and threshed. Grain was examined for tombstone kernels and was analysed for DON and other mycotoxins by GC-MS. The cultures varied in disease-causing ability. Three isolates were weakly pathogenic while four others were highly pathogenic, comparable to standard tester isolates; the remaining isolates were intermediate. FHB severity and tombstone kernels in grain were highly correlated to each other but neither was correlated to the toxin production potential previously determined for these cultures. The DON level in the grain was most highly correlated to the percent of tombstones and was not correlated to the isolate toxin potential determined previously. This study does not support the hypothesis that DON plays a major role in FHB disease development. These results agree with a similar study recently reported elsewhere (*J. Gilbert et al. 2000. Proc. Int. Symp. Wheat Improv. for Scab Resist., Suzhou and Nanjing, China. p218-223.*).