DIAGNOSTIC VOMITOXIN (DON) SERVICES IN 1999-2000

Howard H. Casper

INTRODUCTION

Fusarium Head Blight (FHB) emerged in the 1990's as an important problem for American agriculture. Resolving the FHB problem involves cooperative efforts and a multidisciplinary approach, including analytical assays for vomitoxin in the new wheat and barley varieties. In 1999, 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 information provides an insight into the methods, quality assurance and number of samples processed by each of these laboratories.

MATERIALS AND METHODS

The following information provides names, addresses and analytical techniques for the 4 laboratories. The 3 laboratories in Minnesota and North Dakota all use the method of Tacke (1), followed by GC/EC or GC/MS quantitation.

1. 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)

2. 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 (12.4 ppm)

3. 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), Malt Pool (1.5 ppm DON), Barley Pool (3.2 ppm DON).

4. NORTH DAKOTA
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Method: Acetonitrile: water extraction, silylation and DON quantitation by GC/EC
Samples Types: Barley and malt products
Intralab Quality Assurance: Malt Pool (2.0 ppm DON)

5. PROFICIENCY CHECK SAMPLES

During the FHB meeting in Michigan in 1998, there was some discussion on the exchange of check samples between the 4 laboratories. In August of 1999, North Dakota State University (NDSU) 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 and whether corrective actions were necessary.

RESULTS AND DISCUSSION

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

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. This is a reasonable precision considering the objective of separating the high versus low producers of vomitoxin. The interlab proficiency check samples demonstrated that the 4 laboratories are getting similar results, and there were no major differences between the labs. Considering the intralab coefficient of variation, it is unlikely that there is a significant statistical difference between the different labs. The ELISA kit (2) provided a reasonable intralab coefficient of variation and the overall data was

Table I. Intralab Vomitoxin Quality Assurance (QA) Data for July November, 1999.

1.	MICH P. Hart	QA: Wheat Pool;	n = 24,	Ave = 2.2 ppm,	cv = 9%
2.	MINN W. Xie	QA: Wheat Pool;	n = 31,	Ave = 12.4 ppm,	cv = 6%
3.	ND P. Schwarz	QA: Malt Pool;	n = 98,	Ave = 2.0 ppm,	cv = 16%
4.	ND H. Casper	QA: Wheat Pool;	n = 42,	Ave = 1.8 ppm,	cv = 9%
		: Malt Pool;	n = 42,	Ave = 1.5 ppm,	cv = 10%
		: Barley Pool;	n = 42,	Ave = 3.2 ppm,	cv = 9%

Table II. Estimated Vomitoxin Assays for 1999 2000.

	Method	PIs	States	Samples	CV
MICH P. Hart	ELISA	~9	~8	~2,200	~9%
MINN W. Xie	GC/MS	~13	~1	~3,400	~6%
ND H. Casper	GC/ECD	~8	~4	~3,800	~9%
ND P. Schwarz	GC/ECD	~7	~2	~3,500	~16%

PIs = Research scientists supported by DON assays
CV = Coefficient of variation for control pools

Table III. Interlab Vomitoxin Proficiency Check Samples.

			ppm DON				
Lab	**Method**	**Grain**	**Aug. 99**	**Sep. 99**	**Oct. 99**	**Nov. 99**	**Ave.**
MICH P. Hart	Elisa	Wheat	3.7	1.6	4.8	12.5	5.6
MINN W. Xie	GC/MS	Wheat	2.6	1.0	4.0	14.4	5.5
ND H. Casper	GC/EC	Wheat	3.0	0.9	3.2	12.2	4.8
ND P. Schwarz	GC/EC	Wheat	3.0	0.8	2.0	9.6	3.9
Ave.			3.1	1.1	3.5	12.2	
MICH P. Hart	Elisa	Barley	1.1	7.4	6.4	2.4	4.3
MINN W. Xie	GC/MS	Barley	1.0	6.8	6.5	3.3	4.4
ND H. Casper	GC/EC	Barley	1.0	6.2	5.9	3.0	4.0
ND P. Schwarz	GC/EC	Barley	0.9	5.0	5.2	2.2	3.3
Ave.			1.0	6.4	6.0	2.8	

not significantly different from the chromatographic techniques. One NDSU lab (P. Schwarz) might be coming in slightly lower than the other 3 labs and this difference is being evaluated by Dr. Schwarz. Similarly, the other NDSU lab (H. Casper) experienced a higher coefficient of variation (~9%) for 1999 than he saw in 1998 (~6%) and will be evaluating this factor. The vomitoxin assay by chromatographic techniques does have certain steps (i.e., glassware) that may cause variation, but the cause and effect are not well defined. Information that is accumulated at each of the chromatographic laboratories, on improved precision, will be shared. In the research campaign for 1999-2000, we estimate that ~13,000 samples will be processed by the 4 laboratories for ~37 principal investigators in ~15 states. The 4 laboratories have met the criteria of the grants from the US Wheat and Barley Scab Initiative and probably processed more samples than originally planned.

The interlab proficiency check samples will probably be continued in the FHB research campaign for 2000-2001. Each laboratory will be evaluating means of refining the analytical techniques for improved precision, speed and broader scope of mycotoxin analysis.

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2. 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.

VALIDATING SAMPLING STRATEGIES FOR VOMITOXIN IN THE MIDWESTERN US

L. Patrick Hart[1], Oliver Schabenberger[2], and Fanzhi Kong[3]

Previously, we investigated the bias and inconsistency due to spatial heterogeneity in toxin distribution in estimating deoxynivalenol (DON) concentrations when sampling kernels from truck loads of wheat with standard seed probes (Schabenberger et al, 1998). A probabilistic model was 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 were compared with data gathered throughout Michigan from fourteen trucks during the 1996 Fusarium Head Blight epidemic. This comparison provided supporting evidence that vomitoxin distribution throughout a truck load may be clustered rather than homogeneous. Therefore, if spatial heterogeneity is not properly represented in the sample, confidence intervals for DON are centered around the wrong value and are less informative for larger sample sizes. In 1998, a more intensive statistical study of sampling was used to validate the 1996 study. Spatial heterogeneity in toxin distribution was greater in 1998 compared to 1996. The importance and possible sources of the differences are discussed. Developing and supervising appropriate sampling strategies remains of critical importance.

INTRODUCTION

Wheat scab, also known as Fusarium Head Blight (FHB) is a serious problem in grain producing regions of the Midwestern U.S. (McMullen et al 1997). An estimated 90 million bushels of wheat were lost in North Dakota during head blight epidemics in 1993.

Contamination of the grain with deoxynivalenol (DON, vomitoxin) is the most serious consequence of infection apart from reductions in grain yield and quality. The toxicological properties and mammalian toxicity of deoxynivalenol (Rotter et al. 1995) prompted the U.S. Food and Drug Administration in 1993 to revise earlier advisory levels for deoxynivalenol in wheat entering the milling process, shifting emphasis to concentrations in finished products (FDA 1993). Since the advisory levels create thresholds for marketing of wheat, detecting the deoxynivalenol contamination level in scab infected wheat accurately and precisely has gained critical importance in terms of food quality and agricultural economics (Hart et al. 1998).

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 cannot be followed with seed probes of standard length. In addition, based on the 1996 statistical study (Hart et al, 1998) a minimum of four probes per truck were necessary to predict levels of DON within 1 ppm of the upper limit with ninety-five percent confidence.

In this contribution, we discuss the findings of a 1998 statistical study of sampling designed to validate the 1996 study, and to provide additional information on deoxynivalenol distribution. The following changes were made in the 1998 study: 1) spring wheat from N. Dakota was used in place of winter wheat; 2) five trucks were sampled instead of fourteen; 3) twenty-five

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probes per truck instead of ten were collected, and the contents of each probe were divided into four equal parts based on depth.

RESULTS AND DISCUSSION

Approximately one hundred samples per truck were analyzed by ELISA (Hart et al, 1998). The probe to probe variance for DON distribution for 1996 and 1998 data is shown in Figure 1. The between probe variance for three out of the five trucks sampled in 1998 was outside the range of variances from 1996. Why the variation was greater in the 1998 study is not known, but it could be related to specific factors associated with this particular epidemic, or there may be greater variability associated with spring wheat when compared to winter wheat. Figure two shows the actual distribution of DON within the "Orange" and "Red" trucks as examples of distribution in all of the trucks. To determine coverage probabilities for DON estimates we examined all possible probe combinations of n samples from twenty-five truck probes assuming the overall sample mean DON is the true concentration. When N=4 there are 12,650 possible combinatons, and when N=7 there are 480,700 (Table 1). As in 1996, a minimum of four probes was required to obtain 95% coverage probability, but the interval width was three times higher than in 1996. Therefore, in 1998 at the 95% confidence level the mean is within 3 ppm of the estimated truck mean (1.5 ppm above and below the estimated mean) vs 1 ppm (0.5 ppm above and below the estimated mean) in 1996. The two trucks with mean DON levels below 10 ppm achieved 95% coverage probability for 2 ppm (1 ppm above and below the mean). This is in accordance with the higher variability of the trucks with mean DON concentrations above 10 ppm.

Increasing the number of probes to six per truck increases the 95% confidence level to 1 ppm above and below the mean for four of the five trucks. Economically, six probes may not be an acceptable practice. We have not determined if DON concentrations in a truck can be accurately estimated by pooling wheat from the individual probes, milling the entire sample, and testing a specific volume sub sampled from the pooled wheat. Year to year and regional variability needs to be addressed further. The affect of storage and grain handling may contribute to decreased variability.

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Table 1. Confidence Interval Probabilities (above and below the mean DON concentration for each truck) for N=4, 5, 6, and 7 probes. Standard deviation for DON (stddon) is the probe-to-probe variance for each truck, the DON average (mndon) is the average DON of 25 probes (in ppm) assumed to be the true concentration on that truck.

			Confidence Interval above and below the mean							
Obs	YEAR	TRUCK	0.5	1.0	1.5	2.0	2.5	3.0	mndon	stddon
1	98	Black	0.54	0.87	0.98	1.00	1.00	1.00	11.7	1.43
2	98	Blue	0.55	0.89	0.99	1.00	1.00	1.00	11.8	1.38
3	98	Brown	0.68	0.96	1.00	1.00	1.00	1.00	9.3	1.07
4	98	Orange	0.79	0.99	1.00	1.00	1.00	1.00	5.9	0.87
5	98	Red	0.50	0.84	0.97	1.00	1.00	1.00	14.6	1.55
		Confidence Interval Probabilities for N=5 probes								
1	98	Black	0.61	0.92	0.99	1.00	1.00	1.00	11.7	1.43
2	98	Blue	0.62	0.93	1.00	1.00	1.00	1.00	11.8	1.38
3	98	Brown	0.75	0.99	1.00	1.00	1.00	1.00	9.3	1.07
4	98	Orange	0.85	1.00	1.00	1.00	1.00	1.00	5.9	0.87
5	98	Red	0.57	0.89	0.99	1.00	1.00	1.00	14.6	1.55
		Confidence Interval Probabilities for N=6 probes								
1	98	Black	0.66	0.95	1.00	1.00	1.00	1.00	11.7	1.43
2	98	Blue	0.68	0.96	1.00	1.00	1.00	1.00	11.8	1.38
3	98	Brown	0.81	1.00	1.00	1.00	1.00	1.00	9.3	1.07
4	98	Orange	0.89	1.00	1.00	1.00	1.00	1.00	5.9	0.87
5	98	Red	0.62	0.93	1.00	1.00	1.00	1.00	14.6	1.55
		Confidence Interval Probabilities N=7 probes								
1	98	Blakc	0.75	0.98	1.00	1.00	1.00	1.00	11.7	1.43
2	98	Blue	0.72	0.98	1.00	1.00	1.00	1.00	11.8	1.38
3	98	Brown	0.85	1.00	1.00	1.00	1.00	1.00	9.3	1.07
4	98	Orange	0.93	1.00	1.00	1.00	1.00	1.00	5.9	0.87
5	98	Red	0.70	0.97	1.00	1.00	1.00	1.00	14.6	1.55

Figure 1. Probe-to-probe variances for data from 1996 and 1998.

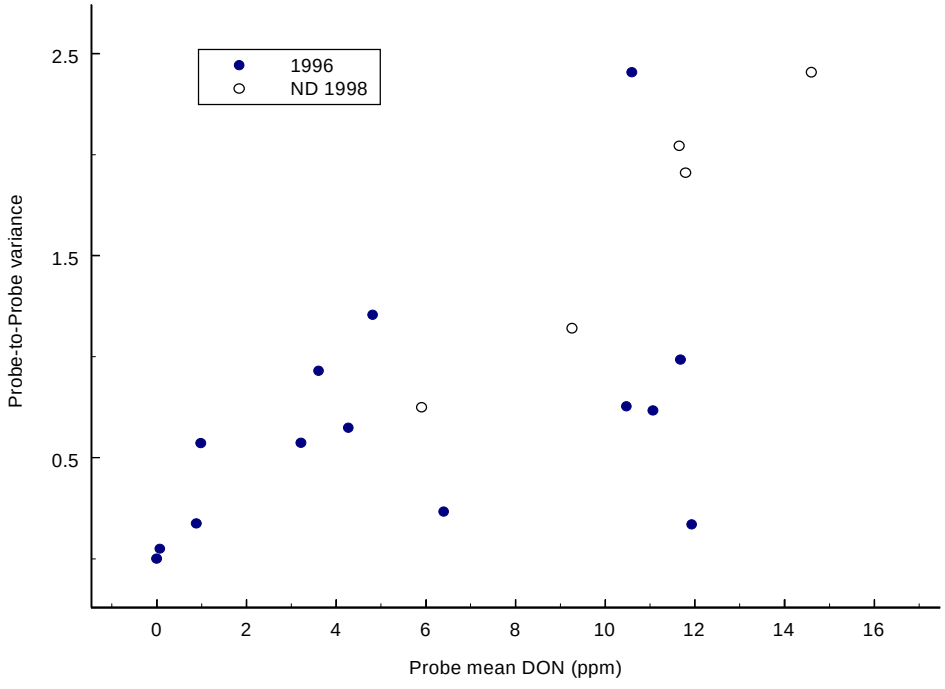
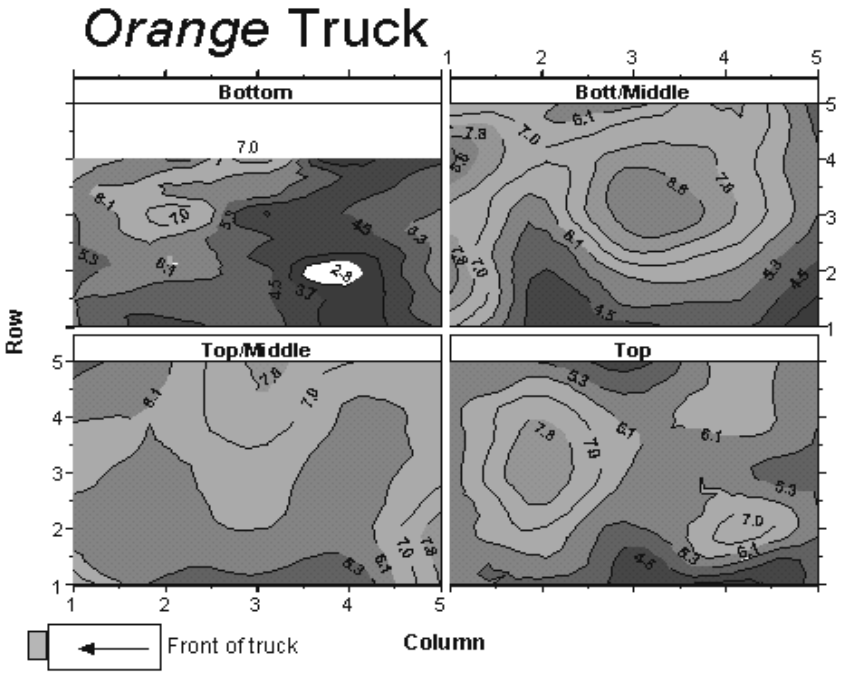
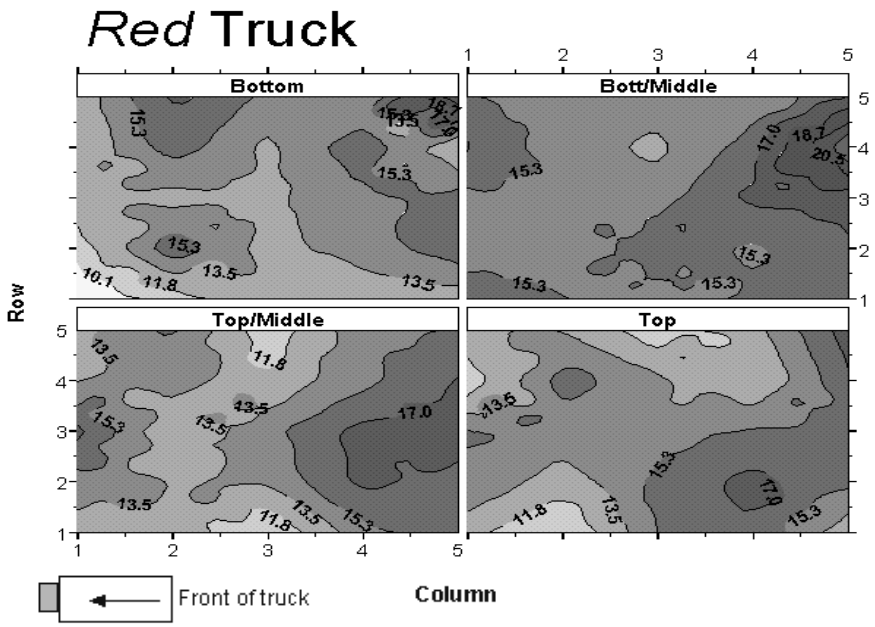


Figure 2. Distribution of deoxynivalenol through four depths, and at different probe insertion points for the "Orange Truck". Deoxynivalenol mean was 5.9 ppm, based on the analysis of one hundred samples (twenty five proes/truck, and four subsample/probe) with a range between 2.8 and 8.6 ppm. "Red Truck" similar to "Orange Truck". Mean deoxynivalenol was 14.6 ppm, with a range between 10.1 and 20.5 ppm.





VOMITOXIN (DEOXYNIVALENOL) INDUCES CYTOKINES AND APOPTOSIS IN HUMAN T CELLS

J.J. Pestka*, R. Uzarski, S.Ross, A.Randell, and G-H. Yang

ABSTRACT

In various well-characterized experimental rodent models, the primary cellular target for vomitoxin (VT) and other trichothecenes produced by *Fusarium* are leukocytes of the immune system. We have hypothesized that the levels of VT and closely related 8-ketotrichothecenes required for toxicity will be identical in mouse and human leukocytes. We have initiated experiments using cloned human Jurkat cells. First, it was observed that VT was indeed capable of inducing apoptosis in this cell line in vitro as determined by changes in cell morphology. Second, it was also observed in T cells that VT could superinduce expression of the cytokines IL-2, IL-4 and IL-5. These results mimic those observed in the mouse model. We are now performing detailed dose response and structure function studies. Third, it was observed that VT rapidly turns on a group of stress-activated protein kinases known as MAP kinases. The results presented here suggest that VT can affect human and mouse leukocyte cell function and viability in a similar fashion. Although the levels required for cytotoxicity (ie. apoptosis induction) were similar, the human Jurkat T cells appeared to be slightly more sensitive to cytokine superinduction then has been previously observed for rodent cell cultures. MAP kinases are involved in signaling cascades that can turn on or off many cellular activities such as apoptosis or cytokine production.

INTRODUCTION

Most food-borne 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, (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.

In various well-characterized experimental rodent models, the primary cellular target for VT and other trichothecenes produced by *Fusarium* are leukocytes of the immune system. We have hypothesized that the levels of VT and closely related 8-ketotrichothecenes required for toxicity will be identical in mouse and human leukocytes. To examine the effects of trichothecenes on human leukocytes, we have initiated experiments using cloned human Jurkat T cells.

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EXPERIMENTAL

1) Cell lines and Cell Culture. Human Jurkat T cells were obtained from American Type Culture Collection (Rockville, MD). These will be maintained at 37^0C, 5% humidified CO_2 in RPMI-1640 medium supplemented with 10% fetal bovine and 2 mM glutamine, plus penicillin (100U/ml) and streptomycin (100mg/ml). Experiments were carried out in the absence of or graded doses of VT.

(2) Cytotoxicity Assay. Cytotoxicity was evaluated by th eMTT assay. Briefly, cells were cultured for 24 hr with and without activating agent in the presence or absence of VT in 96 well plates (100 μl/well) as described above . MTT (5 mg/ml) will then be added and plates will be incubated for another 5 hr. SDS (10% in 0.01 N HCl) (100 μl/well) will then be added, wells incubated for 16 hr, and then OD590 will be determined using ELISA reader.

(3) Apoptosis. Acridine orange-ethidium bromide staining was used to determine apoptotic and necrotic cells in the above described cultures.

(4) Cytokine Quantitation. Supernatant was collected from appropriate plates at different time points. Cytokine production was determined by ELISA using corresponding human recombinant cytokine standards, purified anti-human cytokine antibodies, and biotinylated anti-human cytokine antibody R&D Systems.

RESULTS

1. VT induced cytotoxic effects at 500 ng/ml (Fig. 1).

2. VT induced apoptosis at 1000 ng/ml (Fig. 2).

3. VT could superinduce expression of the cytokines IL-2, IL-4 and IL-5 (Fig. 3 and 4). These results mimic those observed in the mouse model.

4. It was observed that the VT rapidly turns on a group of stress-activated protein kinases known as MAPkinases. These are involved in signaling cascades that can turn on or off many cellular activities such as apoptosis or cytokine production.

DISCUSSION

Potential regulations that would lower the tolerance level for VT in wheat and wheat products either in the U.S. or other countries could threaten the ability of U.S wheat, barley and resultant products to compete in the national and global economies. Should such regulations be proposed, it is absolutely essential that basic information be available relative to human sensitivity. Despite the importance of this issue, to our knowledge, other than our research group, there are no other labs in the U.S. or world that are currently studying toxicity of VT and related 8-ketotrichothecenes at the cellular/ molecular level or have plans for evaluating its potential effects on humans.

The results presented here suggest that VT can affect human and mouse leukocyte cell function and viability in a similar fashion. Although the levels required for cytotoxicity (ie. apoptosis induction) were similar, the human Jurkat T cells appeared to be slightly more sensitive to cytokine superinduction then has been previously observed for rodent cell cultures. These studies will be repeated using additional dose levels of VT and time points to extend this comparison.

It was particularly striking that we have observed that VT rapidly turns on a group of stress-activated protein kinases known as MAPkinases.

These are involved in signaling cascades that can turn on or off many cellular activities such as apoptosis or cytokine production. Interestingly, MAP kinases or evolutionarily conserved and thus can be found in plant and fungi. The ability of trichothecenes to alter cell function in this manner may have relevance to virulence in plants and to signaling within the fungus.

Figure 1. Effect of VT on MTT Conversion By Human Jurkat T Cells

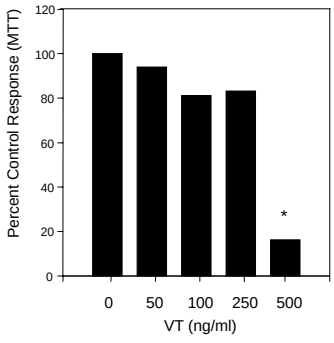


Figure 2. Effect of VT on Apoptosis of Human Jurkat T Cells

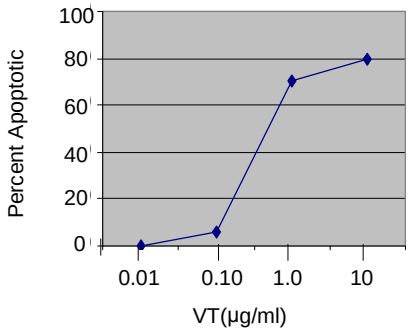


Figure 3. Effect of VT on Interleukin 2 Production By Human Jurkat T Cells

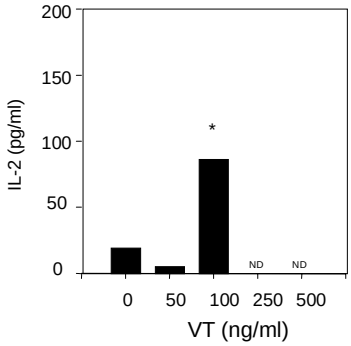
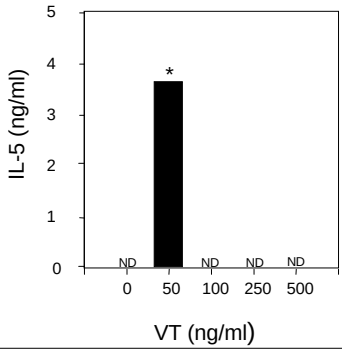


Figure 4. Effect of VT on Interleukin 5 By Human Jurkat T Cells



WHEAT AND BARLEY UTILIZATION RESEARCH, 1999-2000

Paul B. Schwarz

INTRODUCTION

Fusarium Head Blight (FHB) can damage grain quality, and contamination with mycotoxins can lower the acceptability of infected samples for food and feed. The widespread occurrence of FHB since the early 1990's has consequently reduced the amount of wheat and barley available to end-users. The ultimate solution to FHB problems will be development of resistant or tolerant varieties. However, in the interim, methods which can lead to greater utilization of FHB infected grain are of significance.

In 1999, the US Wheat and Barley Scab Initiative provided grants, for utilization research to four laboratories in Michigan, Nebraska and North Dakota. The following provides information as to the objectives and approaches of each of these laboratories.

USE OF FOOD PROCESSING TECHNIQUES TO ELIMINATE/LOWER DEOXYNIVALENOL IN INFECTED WHEAT

Perry Ng, Department of Food Science and Human Nutrition, Michigan State University.

Importance

The three most common treatments to remove/lower deoxynivalenol (DON) in grain are mechanical, heat and chemical treatments, and it appears the chemical treatment is the most promising decontamination process. However, in general, grain treated with chemicals has detrimental effects on the end-use quality, for example in baked products. Thus, new approaches for processes to decontaminate infected grain are needed.

Objective

To explore cleaning and milling techniques and/or techniques for processing of wheat grain into food to eliminate or acceptably lower DON levels of grain infected by *Fusarium.*

Progress

In the preliminary studies, samples of highly infected wheat grain (7.3 ppm of DON) were soaked in sodium bisulfite solutions. Soaked wheat grain was extruded via an extrusion process to produce puffed products. DON levels in the puffed products were reduced to as low as 0.3 ppm via the soaking and extrusion processes. The proposed approach appears viable not only for reducing DON levels, but also for removing moisture and volatile chemicals from the soaking solution, and producing acceptable puffed products.

Planned Activities

The following are studies targeted to meet the objective based on the preliminary work.

- Reducing vomitoxin levels of infected wheat during the cleaning process prior to milling.
- Reducing vomitoxin levels of infected grain during the washing and polishing processes.
- Milling of cleaned and washed wheat; analysis of DON levels of various mill streams.

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- Development of novel processes for utilizing washed and wetted grain, e.g., expand the extrusion conditions used in the preliminary studies to produce better-extruded products and a wider variety of extruded products.
- Utilization of milled materials for baked and steamed-products, analysis of DON levels in the products, and evaluation of product qualities.
- Utilization of chemically soaked grain by extrusion, analysis of DON levels in extrudates, and quality evaluation of extruded products.

FUSARIUM HEAD BLIGHT RESEARCH/ EXTRUSION PROCESSING AS A MEANS OF REDUCING DEOXYNIVALENOL IN CEREAL-BASED FOODS

Lloyd B. Bullerman, Department of Food Science & Technology, University of Nebraska-Lincoln.

Importance

DON is a fairly heat stable compound, and it survives many thermal food processes, such as baking and canning. However, extrusion is a thermal process that can be more destructive to DON than other thermal processes. Extrusion processing reaches higher temperatures and utilizes other destructive forces, such as shear forces and chemical reactions. Using the right set of processing parameters, extrusion processing of scab infected wheat contaminated may result in a processed product with a reduced DON content.

Objective

The purpose of this research is to determine the optimum conditions of extrusion processing that will destroy and detoxify DON in scab infected wheat. Since the same fungus, *F. graminearum*, produces zearalenone (ZEN), some of the work may also address ZEN. Various extrusion parameters will be studied to determine the best combination of extrusion variables (single vs. double screw, screw speed, temperature) and conditions (moisture, additives) for the destruction and detoxification of DON and possibly ZEN.

Progress

- Corn grits were spiked with 4 Fg/g of DON. Spiked corn grits were extruded at 22% moisture, 120, 140 and 160°C. Alpha-Amylase treatment improved recovery of DON from extruded product. The conditions used resulted in no loss of DON.
- Extrusion of a dog food extrusion mixture contaminated with 5.5 Fg/g of deoxynivalenal at 100°C likewise resulted in no loss of DON.

Planned Activities

- Extensive extrusion studies will be done.
- Higher temperatures (160, 180, 200, 220°C) will be studied.
- Screw speeds of 40, 80, 120, 160 rpm will be tested.
- Moisture contents of 18, 22, 26% will be studied.
- Effects of certain food additives and chemicals will be studied.
- Chemicals such as salt, sulfur dioxide, sugars, ammonium and sodium hydroxide, phosphates and others will be studied.

POST-HARVEST CONTROL OF *FUSARIUM* MYCOTOXINS IN GRAIN-BASED FOODS

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Importance

When grains are processed into finished products, the chemical structure of mycotoxins such as DON may be changed due to the heat

and chemical reactions that occur in the food matrix. Chemically altered forms of DON and other mycotoxins may still have toxigenic characteristics. There is a definite need to be able to detect and isolate chemical products from mycotoxin degradation to determine their toxicity, prevalence in human and animal food supplies, and to find means by which this toxicity can be reduced or eliminated. An understanding of the physiology of the mold, which produces mycotoxins, is also important in studies involving pre- and post-harvest contamination of foods and feeds. Several factors, such as the growth substrate, temperature, moisture, and competing organisms have been reported to influence DON synthesis. Most importantly, strain to strain variation in DON synthesis has been observed .

Objective

The long-term goal of this project is to develop methods to control the content of *Fusarium* mycotoxins in cereal grains and their resulting foods or feeds. This may allow the utilization of FHB infected cereals by preventing preformed toxins and toxins formed during processing from ending up in the final foods and feeds. The supporting objectives of this research are to improve mycotoxin detection and quantification procedures for use on cereals and processed cereal products; to determine the fate of these mycotoxins during food processing; to develop treatments to reduce levels of mycotoxins in FHB infected cereals; and to study the metabolism and regulatory mechanisms of *F. graminearum.*

Progress

- *Fusarium* was decreased by 78% with irradiation at 10 kilogray (KGy) with only a 20 % reduction in germination. At 8 KGy there was still significant reduction in *Fusarium* growth, but an actual increase in the germination ability of the treated infected barley.
- Dry heat, steam and microwave treatments were found to significantly reduce *Fusarium*, but the concomitant reduction in grain germination was unacceptable.

Planned Activities

- Evaluate the effects of growth conditions and strain on mycotoxin production.
- Continue to evaluate current analytical methods for DON detection and quanitiation in processed food samples.

UTILIZATION OF FUSARIUM INFECTED MALTING BARLEY

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Importance

Since 1993, up to 85% of the Midwestern malting barley crop has been contaminated with DON. As FHB infection and DON present a number of potential problems to maltsters and brewers, much of the regional crop has not been purchased or utilized for malting. This has caused regional barley growers significant economic hardship. Resistant or tolerant varieties are not anticipated for several years, and development of methods by which some FHB infected barley could be utilized would be of significance to both barley growers, and to the industry. Problems associated malting and brewing FHB infected barley include, reduced germination, lower malt quality, contamination of beer with my mycotoxins, and beer gushing.

Objective

The objectives of this project are to determine the level of FHB infection that causes irreparable damage to grain quality, and to evaluate physical, chemical and biological treatments for control of *Fusarium* growth during malting.

Progress

- Malting equipment was purchased.
- Dry heat, steam and microwave treatments were found to significantly reduce *Fusarium*, but the concomitant reduction in grain germination was unacceptable.
- Evaluated the impact of *Fusarium* enzymes on malt quality. An increase in soluble nitrogen, due to *Fusarium* proteases, appears to be the principal negative impact on grain quality.

Planned Activities

- Determine the relationship between indicators of *Fusarium* infection and malt soluble protein.
- Evaluate malting process control changes on *Fusarium* growth
- Evaluate chemical biological control agents for control of *Fusarium growth during malting.*

EVALUATION OF PHYSICAL TREATMENTS TO PREVENT FUSARIUM GROWTH DURING BARLEY MALTING

Charlene Wolf-Hall*, Paul Schwarz, Jurgen Schwarz, and James Gillespie

ABSTRACT

Contaminated barley or adjunct grains used in the beer making process may lead to the presence of mycotoxins in the final beer and by-products. This is a problem with Fusarium head blight infected barley. During the germination period an increase in deoxynivalenol (DON) occurs as the mold grows in the moist, warm environment. The objective of this project is to evaluate physical treatments to reduce the amount of DON produced during the barley malting process. The methods compared in this project included dry heat, steam, microwaves, and gamma irradiation. Results, so far, indicate that dry heat, steam heat, and conventional microwaving are too harsh. These treatments tend to kill off the germination prior to affecting the survival of the *Fusarium* in contaminated barley. The irradiation treatments show promise the *Fusarium* growth being decreased by approximately 78% at 10KGy with only a 20% reduction in germination. Further testing is needed to optimize a physical treatment process which will result in killing the *Fusarium* without affecting germination.

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RESISTANCE IN WHEAT CULTIVAR CHOKWANG TO *FUSARIUM GRAMINEARUM*

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Genetic resistance to Fusarium head blight is regarded as an essential component of a management program for this disease. Although a few effective sources of resistance have been identified and are being incorporated into hexaploid wheat cultivars, they do not provide complete control of the disease nor of development of DON in grain. Moreover, nearly all wheat breeding programs in the eastern soft wheat region and in the northern plains spring wheat area of the U.S. are using two related sources of resistance from China: Sumai 3 and Ning 7840. There is no evidence at present for specific virulence in *Fusarium graminearum* toward these sources of resistance, but it is known that isolates of the fungus differ in ability to cause head blight symptoms (Bai and Shaner, 1996). Given the notorious variability within species of *Fusarium*, it is conceivable that if the same source of resistance were to be used widely in the U.S., strains of *F. graminearum* that could overcome this resistance, at least to some degree, might develop. It is prudent to seek other genes for resistance and incorporate these into cultivars intended for commercial wheat production. Additionally, the sources of resistance currently being used (Sumai 3 and Ning 7840) may not provide sufficient protection under conditions conducive for severe disease. Other sources of resistance may contain genes that would interact with the genes from Sumai 3 or Ning 7840 to confer a greater degree of resistance than provided by any of the currently available sources. It is important that sources of resistance other than the few being currently used be identified, characterized phenotypically and genetically, and made available to wheat breeders.

Several types of resistance to Fusarium head blight in wheat have been described (Mesterhazy, 1995). The two types represented in the resistant sources used commonly in the U.S. are type 2 (resistance to spread of the fungus throughout an infected spike), and to some extent, type 1 (resistance to primary infection). A wide range of variation of type 2 resistance has been found in wheat. The best expression of this type of resistance is found in a related group of cultivars from Nanjing, China (Bai and Shaner, 1996). Type 2 resistance has proven to be less sensitive to environment and easier to manipulate in breeding programs and genetic studies (Bai and Shaner, 1994). It has reasonably high heritability and is conferred by a few genes (Bai et al., 1999; Moreno-Sevilla et al., 1997; Van Ginkel et al., 1996).

The purpose of our work was to identify sources of resistance that may contain genes different from those in Sumai 3 and Ning 7840. We identified a Korean winter wheat cultivar, Chokwang, as having resistance. We originally studied this cultivar because of its partial resistance to *Puccinia triticina*. A recombinant inbred population from the cross Chokwang x Clark was being developed for leaf rust work when we discovered that Chokwang also showed resistance to *Fusarium graminearum*.

MATERIALS AND METHODS

Seed of F_2-derived F_8 families from the cross Chokwang x Clark was planted in flats and allowed to germinate, then flats were placed in a 5 °C coldroom for 65 days to allow plants to

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vernalize. After vernalization, plants were transplanted into 10-cm-diameter pots and placed on a greenhouse bench. High intensity lights supplemented daylight. Plants were fertilized at the tillering stage (GS 23) with Miracle Gro Plant Food 15-30-15 and at stem elongation (GS 31) with Co-Op Farm Gro 9-44-9. The experiment was set up as a randomized complete block on the greenhouse bench, with eight replicates. There were 80 recombinant inbred families, each parent and a Patterson check. Because of powdery mildew, fewer than eight plants were available for inoculation with *F. graminearum* for some families.

Conidia of *F. graminearum* on were produced in Mung bean extract (Bai and Shaner, 1996). After filtration through cheesecloth, conidia were adjusted to a concentration of 10^4 spores per ml. Each plant was inoculated by the point method when it reached the mid flowering stage of growth (GS 63). A droplet of approximately 20 ml was placed into a floret at the middle of the spike. Inoculated plants were incubated in a moist chamber for three successive nights. The chamber was partially open during the day to prevent temperature buildup.

Blighted spikelets on each spike were counted at 5-day intervals, from days 7 through 27 after inoculation. A count of all spikelets on the spike was used to convert number of blighted spikelets to a percentage of blighted spikelets (severity). Area under the curve for the progress of severity over time was the statistic used to characterize resistance (Shaner and Finney, 1977).

RESULTS

There was no association between the mean and standard deviation for area under disease progress curve (AUDPC). Therefore, no transformation was applied to this statistic. The distribution of family mean AUDPCs was bimodal (Fig. 1). Neither mode corresponded to the parental mean, although the higher mode was only slightly below the mean for Clark. The lower mode was considerably higher than the mean for Chokwang. Based upon an analysis of variance, 17 families had an AUDPC not significantly different from the AUDPC for Chokwang. Fifty-three families were not different from Clark. One family had a significantly greater AUDPC than the AUDPC for Clark, but its value barely exceeded the limit established by the LSD.

DISCUSSION

Until the evaluation of the recombinant inbred population is repeated, a thorough analysis of the data, with an attempt to generate genetic models is premature. However, the pattern of inheritance is quite distinct from that observed for an inbred recombinant population derived from the cross Ning 7840 x Clark (Fig. 2). This leads us to conclude that the genes for resistance in Chokwang are different from those in Ning 7840.

Because heterozygosity is largely eliminated by the F_8 generation, our data provide no information about degree of dominance of the genes for resistance. If genes at two loci accounted for most of the resistance in Chokwang, then one-fourth of the families should have the same resistance phenotype as Chokwang. Statistically, 17 of 80 families did not differ from Chokwang, which tends to support a hypothesis of only two independent genes. If these two genes were additive (i.e. if AAbb had the same resistance phenotype as aaBB), there should be three phenotypic classes representing 0, 1, or 2 loci homozygous for the plus allele, in frequencies of 1, 2, and 1, respectively. Clearly, this model does not fit the data (See Fig. 1).

A more complicated model, involving genes of unequal effect, epistatis, or complementation, would seem to be required to explain the distribution. Bai et al. (1999) found evidence that genes for resistance in Ning 7840 were of un-

equal effect. Shaner et al. (1997) were able to discern unequal effects of genes for partial resistance to Puccinia triticina in a recombinant inbred wheat population, and we intend to apply the same method of analysis to the Chokwang x Clark population once the experiment is repeated.

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Fig. 1. Frequency distribution of area under the disease progress curve for Fusarium head blight severity for family means for a recombinant inbred population of Chokwang/Clark. The AUDPCs for Chokwang (4.0) and Clark (13.2) are indicated by arrows.

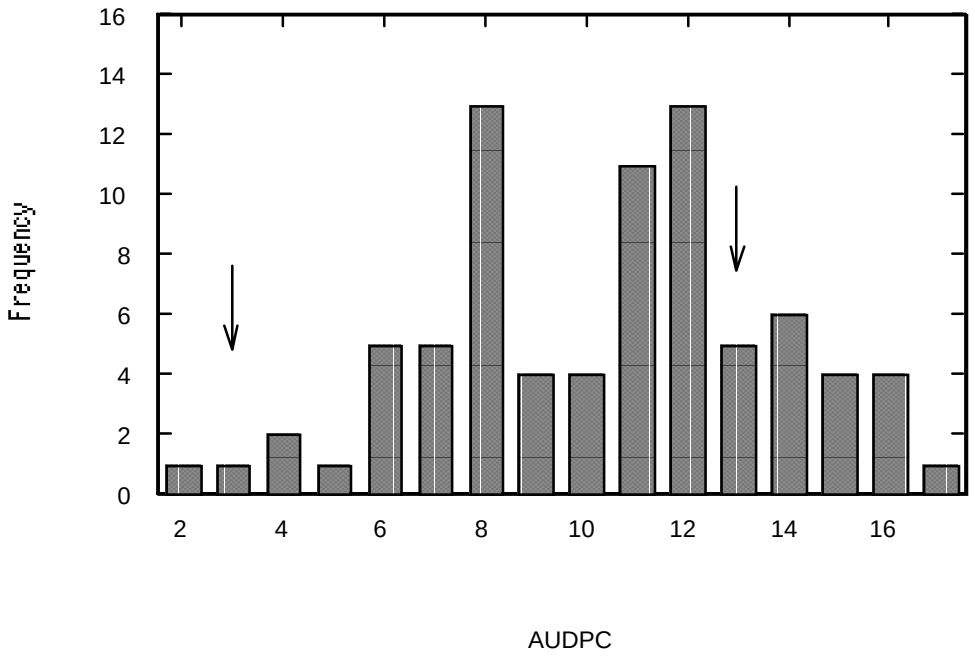
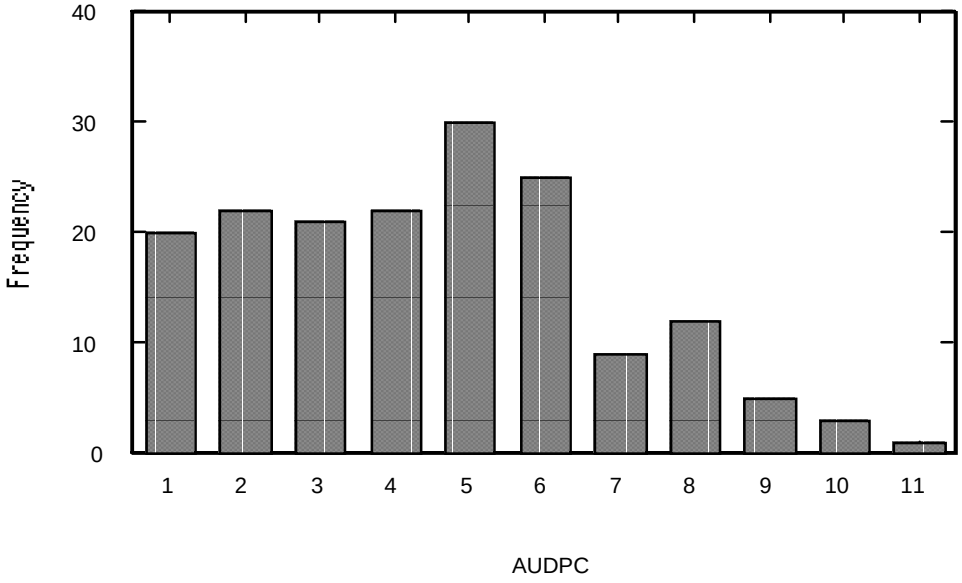


Fig. 2. Frequency distribution of area under the disease progress curve for Fusarium head blight severity for family means for a recombinant inbred population of Ning 7840/Clark.



EVALUATION OF DURUM GERMPLASM FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

Elias M. Elias

Fusarium head blight has been seriously attacking the spring, winter, and durum wheat crop in 12 states in the midwest area. Economic losses in wheat have been in billions of dollars from 1993-1999. Two states, North Dakota and Minnesota, account for two thirds of these dollar losses. North Dakota is the number one producing durum wheat state in the U.S. These losses are disastrous to the farm economy and has direct national impact as alternative sources of supply are sought by importing countries. The search for sources of resistance is essential to insure the development of Fusarium head blight (FHB) resistant durum cultivars. Identified sources of resistance will be incorporated to the currently susceptible durum wheat germplasm to develop resistant cultivars. These cultivars will insure the stability of good quality durum wheat production for the producers, domestic pasta industry, and the international export market.

The main objective of this project is to identify sources of resistance to FHB. Several durum wheat genotypes including durum accessions from the world collection were evaluated for FHB resistance at Prosper, ND and Shanghai China. In 1998, durum wheat from the world collection tested at Prosper, ND were lost because they were out of their adaptation area and were very susceptible to foliar diseases such tan spot *Pyrenophora tritci-repentis* and *Septoria spp*. Therefore the accessions were tested only in China and France in 1999. A total of 500 accessions were sent to the Academy of Agricultural Sciences, Plant Protection Institute (AASPPI) Shanghai, China to be evaluated for FHB in the 1998-99 growing season. Also 50 accessions were sent to Groupment Agricole Essonnois (GAE) in France for evaluation. These evaluations at various sites will allow germplasm exchange and provide international evaluation to a large array of Fusarium strains to determine the effectiveness of incorporated resistance in the germplasm.

The 500 accessions were successfully evaluated at AASPPI. A variation in infection existed among these genotypes, few lines had a very moderate level of resistance to FHB. The identified resistant genotypes will be re-tested in the in the 2000 spring greenhouse to confirm their resistance. Two thousand new accessions were sent to AASPPI to be evaluated in the 1999-00 growing season FHB nursery.

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A POINT INOCULATION METHOD FOR EVALUATING SCAB RESISTANCE IN WHEAT

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ABSTRACT

A point-inoculation technique was evaluated in greenhouse and field studies to improve the efficiency of inoculation for evaluating scab resistance. Seeds of foxtail millet (*Setaria italica*) were soaked in distilled water for 24 hours. A thin layer (ca. 1cm) was placed in a glass petri plate (or a larger tray when desired) and autoclaved for 20 minutes. Autoclaved seeds were inoculated with agar pieces of *Fusarium graminearum* cultures. Inoculated plates were incubated for 10-12 days. Colonized seeds were dried and stored at 4°C. Plants were inoculated by placing a colonized seed between lemma and palea of a floret using a pair of fine forceps. Greenhouse experiments were conducted to compare the "millet-inoculation" method with floret-injection using conidial suspension. Five spring wheat cultivars, ranging from moderately resistant to highly susceptible, were used as testing materials. Results indicated that the millet inoculation was as effective as floret injection with or without mist incubation. Both inoculation methods could result in a high level of infections without any mist incubation when relative humidity was high during the incubation period. Soaking the inoculum in sterile water for 20 minutes prior to inoculation promoted infection. An interaction between genotypes and inoculation methods was observed in the greenhouse experiments. This method was also used to evaluate 14 lines in the advanced yield trials at three spring wheat breeding nursery sites where irrigation was not available. Three replications were used at each site. Twenty spikes in each plot were tagged for growth stages and inoculated with colonized millets between the heading and flowering stages. Disease on the inoculated spikes was evaluated 14-17 days after inoculation. Disease on a random sample of 20 non-inoculated spikes was surveyed at the same time. At two of the three locations, scab indices on the inoculated spikes ranged from 33.4% on a resistant genotype to 69.1% on a susceptible genotype, whereas scab indices due to natural infections in these plots ranged from 7.9 to 26.4%. At the third site, scab development was minimal with scab indices ranging from 9.0 to 21.3% on inoculated spikes and 4.0 to 11.6% on non-inoculated spikes. In two winter wheat breeding nurseries where natural scab infections were minimal, inoculated spikes of 12 selected genotypes had scab indices ranged from 5.9 to 39.6%, and 10.3 to 54.0%, respectively, for the two locations. An apparent interaction between genotype and location was observed using this method. Both greenhouse and field experiments suggested that the millet inoculation method could reliably induce scab infection. The advantages of this inoculation seemed apparent: 1) inoculum can be prepared efficiently with minimal preparation and stored for future use; 2) it is portable to be used in breeding nurseries where mist-irrigation is not available; 3) plants can be inoculated anytime between heading and flowering stages; and 4) minimal training is required to carry out the inoculation.

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EVALUATION OF ASIAN, ITALIAN AND BRAZILIAN WINTER WHEAT GERMPLASM FOR TYPES II AND III RESISTANCE TO FUSARIUM HEAD BLIGHT

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INTRODUCTION

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), also known as scab, is an increasingly important problem in the north-central region of the United States because of the emphasis on conservation tillage, (Wilcoxson et al., 1988; Bai and Shaner, 1994), rotations with corn (Windels and Kommedahl, 1984), the lack of effective cultural and/or fungicide control (McMullen et al., 1997) and the lack of effective sources of genetic resistance. Yield losses in Missouri alone have exceeded $250 million dollars since 1990. In addition to reduced kernel density and color at harvest, associated deoxynivalinol (DON) accumulation in the grain prevents it from being marketed. Host resistance has long been considered the most practical and effective means of control (Schroeder and Christensen, 1963; Martin and Johnston, 1982), but breeding has been hindered by a lack of effective resistance genes and by the complexity of the resistance in identified sources (Mesterházy, 1997). No source of complete resistance is known, and current sources provide only partial resistance, therefore, the identification of different sources of resistance and their incorporation into adapted wheat varieties is critical to the continued improvement of Fusarium head blight resistance in winter wheat. Screening of germplasm collections has confirmed that accessions from China, Brazil and Europe carry resistance genes (Fedak et al., 1997). Wild and related species of the Triticeae have also been identified as potential sources of resistance genes for wheat breeding (Baier et al., 1980; Liu et al., 1990; Ban, 1997; Rubiales et al., 1996).

OBJECTIVES

This research is a component of the aggressive world-wide search for resistance to scab initiated in 1998 with support from the National Wheat and Barley Scab Initiative. Regions that have been targeted include those geographical areas where resistance has been identified or where environmental conditions are conducive to scab development and include: China, Korea, Japan, Brazil, Italy and Eastern Europe. Approximately 4,200 winter wheat accessions from these target geographical areas were identified in the USDA National Small Grains collection. The purpose of this research was to evaluate, under greenhouse conditions, accessions from China, Korea, Japan, Brazil and Italy for Types II and III resistance (Mesterházy, 1995) to *Fusarium graminearum.*

MATERIALS AND METHODS

In the fall of 1998, 937 accessions representing winter wheat landraces, breeding lines, cultivars and cultivated genotypes from China, Korea, Japan, Brazil and Italy were acquired from the USDA-ARS Small Grains Collection at Aberdeen, Idaho.

Disease Resistance Screening

Vernalized seedlings (4 per accession) were planted in the greenhouse over a two month

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period in the fall and winter of 1998 and 1999, respectively. At first anthesis, plants were inoculated with 10µL of a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was placed in a single central floret using an Oxford 8100™ repeat dispensing syringe. For all inoculations, a single isolate was used which had been previously determined to be the most aggressive Missouri isolate on our most resistant cultivar, Ernie. Previous research had also determined that this Missouri isolate was more aggressive in causing disease than similar isolates acquired from Indiana, Michigan, Ohio and Virginia. Plants were incubated in a mist chamber (100% relative humidity) for 72 h post-inoculation to promote disease development and then returned to the greenhouse bench. Ratings for Type II resistance (disease spread in the spike) were made at 14 and 21 d after inoculation. At maturity, heads were harvested, kernels were counted and evaluated for the degree of shriveling and the presence of tombstone kernels. Seeds were counted and each was given a value on a 5 point scale as follows: 1 (sound): 2 (slightly shriveled): 3 (moderately shriveled): 4 (very shriveled): 5 (tombstone). Lines meeting the following criteria for resistance are currently being progeny tested to verify resistance.

Definition of Resistance

For the purposes of greenhouse screening, Type II resistance was compared to the resistant checks Sumai 3, Ning 7840, and Ernie. The susceptible check was MO 94-317. Lines are retained for verification that met the following criteria:

1. Disease spread in the head # 2 spikelets.
2. A low kernel quality score based on the 5 point scale outlined above. Lines are retained where the majority of the kernels had a score of 1 on this scale.
3. High kernel retention ($\geq$ 75% compared to an uninoculated head).

Lines were eliminated, regardless of spread, if inoculated heads had low kernel numbers and/or a high kernel quality score.

RESULTS AND DISCUSSION

Table 1 provides information on country of origin and improvement status of accessions screened in the 1998/99 greenhouse season. The majority of the accessions evaluated (627) were from the People's Republic of China with 406 being classified as cultivated accessions. The improvement status of entries in this class is unclear as the term cultivated is used as a "catch-all" to describe those accessions where passport data on improvement status is poor (Dr. Harold Bockelman, USDA-ARS, Aberdeen, Idaho - personal communication). In the greenhouse, many of these "cultivated" accessions had a desirable agronomic type with dense heads, long spikes and multiple seed set across the spikelet. In addition, many were early to mature with 8 weeks of artificial vernalization. Of the 627 accessions from China that were screened, 4% were classified as resistant in the preliminary screen. The frequency of resistant accessions from other countries was lower, however, a total of 6 accessions from diverse geographical origins were also classified as resistant in the preliminary screen. If verified, these may provide breeders with different sources of resistance. Progeny evaluations of each of these accessions are ongoing.

In addition to the 31 accessions classified as resistant, 111 accessions had one or more plants showing high levels of Type II and Type III resistance. Resistant plants from these accessions are also being progeny tested in the greenhouse. Once verification of Type II resistance is completed, inoculated heads will be harvested and kernel quality will be assessed to verify Type III resistance in these lines. Data for completed

verifications will be made available at the 1999 scab forum in South Dakota. Seed harvested from resistant accessions will be increased for distribution and entered into a soft red winter wheat crossing program at the University of Missouri during the winter of 2000. Concurrently, crosses will be made with the susceptible cultivar MO 94-317 to initiate the development of populations for genetic study.

All accessions being verified in the 1999 fall greenhouse have been planted as head rows in the field at Columbia, Missouri for assessment of Type I resistance. Plants will be sprayed at 75%

Table 1. Origin and improvement status of germplasm screened for Type II and III resistances in the greenhouse at Columbia, Missouri during the fall and spring of 1998 and 1999, respectively.

Country	Improvement status	Total number of accessions screened	Number of resistant accessions	Total number of accessions re-screened in 1999/2000
Brazil	Breeding	3	-	-
	Cultivar	5	1	2
	Cultivated	-	-	-
	Landrace	-	-	-
China	Breeding	22	2	5
	Cultivar	75	1	10
	Cultivated	406	13	65
	Landrace	124	9	23
Italy	Breeding	16	-	2
	Cultivar	118	2	11
	Cultivated	28	-	-
	Landrace	13	-	4
Japan	Breeding	9	-	-
	Cultivar	62	2	10
	Cultivated	6	-	-
	Landrace	8	-	1
South Korea	Breeding	-	-	-
	Cultivar	25	1	5
	Cultivated	12	-	2
	Landrace	5	-	2
Totals		937	31	142

† Accessions with one or more plants having excellent Type II (disease spread in the head) and III (kernel quality) including the 38 accessions classified as resistant. Resistant plants in these accessions are being progeny tested in the greenhouse and field in 1999/2000.

‡ Cultivated is a “catch-all” term used to describe improvement status when passport data on the accession is poor. Improvement status is unclear for these accessions.

heading with a macroconidial suspension concentrated to 50,000 macroconidia/mL. Head rows will be maintained under overhead mist irrigation through heading and evaluated for scab incidence and severity18 - 21 d after inoculation. In addition, accessions will be evaluated for winter hardiness, resistance to other relevant diseases, height, maturity and yield under disease pressure.

Field and greenhouse screening of approximately 1000 accessions from Yugoslavia obtained from the National Small Grains Collection is continuing in the 1999/2000 season. This work is being done in cooperation with Dr. Paul Murphy, North Carolina State University.

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EVALUATION OF DIPLOID, TETRAPLOID, HEXAPLOID, AND SYNTHETIC WHEATS FOR TYPE II RESISTANCE TO FUSARIUM HEAD BLIGHT

J. P. Murphy[1*], R. A. Navarro[1], and S. Leath[2]

OBJECTIVES

To evaluate a range of diploid and tetraploid *Triticum* and *Aegilops* species, synthetic (*T. turgidum* sp. *durum* x *Aegilops tauschii*) hexaploids and exotic and adapted cultivated hexaploids for resistance to FHB.

INTRODUCTION

The Southeastern U.S. soft red winter wheat-producing states have, so far, been spared Fusarium Head Blight (FHB) epidemics on the scale experienced in the North-Central and Midwestern States since 1993 (McMullen et al., 1997). Nevertheless, in both the 1997 and 1998 seasons an increase in the incidence of FHB was observed associated, in particular, with minimum/no-till cultural practices in the corn-wheat-soybean rotation that is common in the region. Southeastern breeders, producers, and millers are anxious that proactive measures be taken to avoid a repeat of the Midwestern/North-Central experience with FHB. In order to provide Southeastern breeders with an array of sources of resistance to FHB, we initiated a screening program involving cultivated and related species accessions.

MATERIALS AND METHODS

Sept.-Dec. 98

Seedlings of 83 accessions of 10 diploid and tetraploid species (Table 1) were vernalized in peat pots at 3°C for 65 days in a growth chamber with a 10/14-hour light/dark cycle. Seeds of winter type *T. aestivum* accessions from China, Japan, Italy, The Balkans, and breeding lines and cultivars from the southeastern United States were vernalized in moistened paper towels for 50 days at 3°C. All vernalized and spring growth habit entries were planted in the greenhouse on Sept. 25, 1998. Supplemental lighting to 16-hour days was provided from Oct. 25. Two pots (replications) were planted per entry. Pots were overplanted and later thinned to two plants per pot. Tiller production was high, and three heads per pot, at approximately the same stage of anthesis, were inoculated with 50 ml of a macroconidial suspension (50,000 spores/ml) using a pipette. The inoculation suspension consisted of four aggressive North Carolina isolates of *Fusarium graminearum* identified by Walker et al. (1998). Following inoculation, the plants were placed in a mist chamber for 3 days. The chamber was opened from 9:00 AM to 5:00 PM to prevent excessive heat build-up. Twenty-one days post-inoculation, heads were rated on a 0-5 scale describing infection type as follows: 1.0 - only inoculated floret infected, 2.0 - only inoculated spikelet infected, 3.0 - inoculated spikelet and rachis infected, 3.2 - inoculated and one adjacent spikelet infected, 3.4 - inoculated and two adjacent spikelets infected, 3.6 - inoculated and three adjacent spikelets infected, 3.8 - inoculated and four adjacent spikelets infected, 4.0 - half of spike infected, and 5.0 - whole spike infected.

A square root transformation was utilized and data were analyzed using the PROC GLM procedure in SAS.

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Sixty-six of the 316 entries that appeared resistant in the first screening were re-evaluated using the protocols described above. Selfed seed from resistant plants were utilized in this second screening to avoid complications that could arise from intra-accession variability.

RESULTS

All diploid and tetraploid accessions exhibited high levels of susceptibility in the first run of the experiment and none were retested. Infection types at 21 days post-inoculation were generally in the 4 to 5 range. Jauhar and Peterson (1998) and Miller et al. (1998) reported on *Thinopyrum junceiforme, Lophopyrum elongatum,* and *T. turgidum* var. *dicoccoides* as sources of resistance to FHB. Although we evaluated relatively few accessions per species in this initial test, we will likely concentrate on the species reported to have resistance to FHB in the immediate future.

Six of the seven Asian cultivated sources with resistance were Chinese in origin (Table 2). The seventh, 'Shinchunaga' was from Japan. Three of the four European cultivated sources with resistance were Italian in origin. The fourth, 'NS 18-99' was from Serbia. The pedigree of 'Mentana' was one-half 'Akagomughi', a Japanese line. The pedigrees of 'Luizia Strampelli', 'Inallettabile 3', and NS 18-99, were unavailable through the Germplasm Resources Information Network (GRIN). The CIMMYT synthetic wheats each had a different *Ae. tauschii* parent, but three of the five lines had the durum cultivar Altar 84 in their pedigree.

The North Carolina breeding lines exhibiting resistance had a mean of 12.5% eastern European germplasm utilized as a source of powdery mildew resistance in their pedigrees. The resistance of these lines to FHB is unknown.

Field and greenhouse screening of eastern European germplasm obtained from the National Small Grains Collection is continuing in cooperation with Dr. Anne McKendry, University of Missouri.

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Table 1. Species, genome designation, and number of genotypes evaluated for Type II resistance to Fusarium Head Blight.

Species	Genome	No. accessions/breeding lines
T. monococcum sp. *monococcum*	A	25
T. monococcum sp. *aegilopoides*	A	4
T. urartu	A	3
Ae. tauschii	D	8
Ae. speltoides	S	7
Ae. sharonensis	s	7
T. timopheevii sp. *armeniacum*	AG	10
Ae. neglecta	UM	5
Ae. cylindrica	CD	11
Ae. triuncialis	UC	3
CIMMYT Synthetic wheats	ABD	77
T. aestivum	ABD	
1997 Uniform Southern Nursery		30
Asian and European accessions		67
Southeastern U.S. breeding lines		59
TOTAL		316

Table 2. Origin and performance of genotypes exhibiting Type II resistance to FHB based on two separate evaluations during the 1998-99 greenhouse season.

	Infection type	
	Untransformed	Transformed
Asian		
Sumai 3	1.3	0.96
Sho Chou	1.5	1.21
Futai 8944	1.8	1.31
Ning 7840	1.9	1.34
Wan Nian #2	2.0	1.40
Shinchunaga	2.0	1.40
JG 1	2.1	1.41
European		
Mentana	1.5	1.21
NS 18-99	2.0	1.40
Luizia Strampelli	3.0	1.71
Inallettabile 3	3.1	1.75
CIMMYT Synthetics		
TA 4064.200	2.6	1.60
TA 4073.200	2.8	1.67
TA 4094.200	2.8	1.64
TA 4054.200	3.0	1.73
TA 4069.000	3.0	1.72
North Carolina		
NC96-13374	2.4	1.55
NC96-13965	2.7	1.62
NC96-14629	2.7	1.63
NC96-13848	3.2	1.79
Checks		
Ernie	2.8	1.64
Roane	3.0	1.71
Freedom	3.1	1.75
NK-Coker 9663	4.3	2.1
Mean	2.5	1.55
LSD (0.05)	--	0.36
CV (%)	--	16.5%

EVALUATION OF SIX-ROWED SPRING BARLEY ACCESSIONS FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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OBJECTIVE

In the Upper Midwest, six-rowed cultivars are the preferred type for malting. Unfortunately, most of the Fusarium Head Blight (FHB) resistant germplasm identified to date is in a two-rowed genetic background (Prom et al. 1997). To identify additional sources of resistance, accessions of six-rowed spring barley from the USDA-ARS National Small Grains Collection are being evaluated to FHB in the field.

INTRODUCTION

FHB threatens the existence of the malting barley industry in the Upper Midwest (Salas et al. 1999; Steffenson 1998). The deployment of resistant cultivars is the most effective and environmentally sound means of managing the disease. In an early screening effort at the University of Wisconsin, R. G. Shands (1939) identified Chevron, a six-rowed cultivar from Switzerland, as a good source of resistance to FHB. Recent evaluations indicate that Chevron is still the most resistant six-rowed accession to FHB, but is poor in malting quality and agronomic performance. Additional sources of FHB resistance in a six-rowed genetic background need to be identified and exploited in breeding programs.

MATERIALS AND METHODS

The first half of the spring six-rowed barley collection (4035 accessions) were planted in both Langdon and Osnabrock, North Dakota in the spring of 1999. The nurseries were inoculated using methods modified from Prom et al. (1997). Equal amounts of six regional *Fusarium graminearum* isolates (KB171, KB172, KB173, KB176, KB582, and KB672) were applied uniformly to plots in both nurseries. The first inoculation was made when the flag leaves of the earliest maturing plants were expanding. Four successive inoculations were made at weekly intervals to ensure that sufficient inoculum was available for infection of later maturing accessions. To maintain sufficient moisture on the spikes for optimal FHB infection (Paulitz, 1996), an automatic overhead misting system was used, operating twice per day during early morning and early evening. Irrigation began two weeks after the first inoculation and continued until the latest maturing accessions reached the late dough stage of development. FHB severity (average percentage of infected kernels on 5-10 spikes) was assessed on each accession at the mid-dough stage and then two weeks later to record possible changes in the infection level. In addition to FHB assessments, maturity data [Decimal code (Zadok *et al.*, 1974) at five ≤DC15, ≤DC25, ≥DC31] and eight weeks (≤DC15, ≤DC25, ≤DC45, ≤DC45, ≤DC59) and plant height (level 1 = 25cm, level 5 >100cm) at ten weeks post-planting were recorded on accessions in the Langdon nursery. FHB severity and quantitative assessments of these various traits were analyzed statistically to determine possible relationships between disease expression and plant type and development. The country of origin of each accession also was considered. To detect different groups of germplasm based on FHB resistance levels, cluster analysis was performed using

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the between-groups-linkage-method with squared-euclidian-distances-interval in the SPSS statistical package (version 9.0).

RESULTS AND DISCUSSION

In general, the disease pressure was higher in Langdon than in Osnabrock. This was likely due to longer wetness periods, earlier sowing date and higher plant densities in Langdon compared to Osnabrock.

1. Field selected germplasm

1.1 Accessions exhibiting high levels of FHB resistance

From 4035 tested accessions in Osnabrock, 12 exhibited a resistance level (less than 20% infection) comparable with Chevron, the resistant check (Table 1). These accessions also were in the same maturity group as Chevron and originated from the United States, Georgia, Mongolia, China, Yugoslavia and Romania. The widely grown susceptible six-rowed malting barley cultivar Foster had a FHB severity of 54% in Osnabrock.

1.2 Additional selected accessions exhibiting FHB resistance in Langdon or Osnabrock.

The level of FHB occurring on barley can be influenced by several traits like maturity and plant height (Steffenson et al. 1996). To avoid the loss of potentially resistant material, especially in early and in late maturing germplasm, 16 additional accessions that exhibited resistance levels marginally below that of Chevron were selected. These accessions were from Ethiopia, China, South Korea, Russia, and central Europe. Nearly one quarter of this group were semi-dwarf types.

1.3 Origin of accessions

The hierarchical cluster analysis based on one FHB severity assessment in Langdon and two assessments in Osnabrock resulted in five groups distinguishable at the 96% probability level:

A) ≤30% FHB infection in Langdon, ≤20% in Osnabrock (diverse origin of germplasm)
B) ≤40% Langdon, ≤20% Osnabrock (mostly European origin)
C) ≤35% Langdon and ≤10% Osnabrock (Europe and USA)

Table 1: Six-rowed barley accessions exhibiting FHB resistance comparable to Chevron in Langdon, ND and Osnabrock, ND in 1999.

CIho or PI Number	Development 10 weeks after planting	Height (cm)	% FHB-severity Langdon*	Osnabrock** 1.	and 2.	Name	Country of Origin
2236	≤DC25	76-100	40		10	Reids Triumph	USA
4095	≤DC25	51-75	40		20		Georgia
4339	≤DC25	≤25	60	20	S***		Mongolia
4530	≤DC25	51-75	40	10	20	7603	China
5809	≤DC25	51-75	30		20	484	
6610	≤DC25	51-75	30		20	Zander 1	USA
6611	≤DC25	51-75	30	10	10	Hietpas 3	USA
6613	≤DC25	51-75	30		10	Seed Stocks 1148-1	USA
7163	≤DC25	51-75	50	10	20	Beaver Dam 8	USA
9114	≤DC25	51-75	40		20	184	Yugoslavia
11526	≤DC25	76-100	10		10	Chevron Sel.	USA
15258	≤DC25	76-100	40		20		Romania
1111	≤DC25	76-100	17	10	14	Chevron	Switzerland
592758	-****	-	-	39	54	Foster	USA

* single reading in Langdon
** two readings in Osnabrock
*** plants were senescent
**** data not available

D) ≤15% Langdon and ≤10% Osnabrock (Ethiopia)
E) ≤60% Langdon and ≤20% Osnabrock (Mongolia, Russia, USA)

2. Database selected germplasm

To increase the genetic diversity for FHB resistance, additional germplasm was selected for further analysis based on FHB severity assessments in both nurseries and country of origin.

Based on the ranked average FHB severity assessments from the Osnabrock nursery, the five most resistant lines from different countries with accessions exhibiting <30% FHB infection were subjected to cluster analysis. Only lines with one FHB reading in Langdon and two FHB readings in Osnabrock were considered; thus lines with late head emergence were excluded. Accessions from Mongolia, Canada, and China had the best FHB resistance (<12% FHB), followed by a major group consisting of western, northern, and central European germplasm (13-15% FHB). Slightly higher levels of FHB infection (>15%) were found for a germplasm group from Australia, United Kingdom, Japan, and Algeria. No significant differences in height and maturity between the grouped accessions were detected. In total, 52 more lines have been selected for further screening.

3. Outlook

Evaluation of the first half of the spring six-rowed barley collection resulted in the identification of 12 accessions with FHB resistance comparable to the standard resistant cultivar Chevron. An additional 80 accessions also were identified that exhibited a level of FHB resistance marginally below that of Chevron. This research will result in the widening of genetic diversity for FHB resistance in barley breeding programs. Data for FHB severity of all accessions will be entered in the Germplasm Information Resource Network (GRIN) to benefit all interested researchers. The resistance performance of the selected germplasm will be re-evaluated under controlled conditions in the greenhouse and also under field conditions in China and North Dakota in 2000. The second half of the six-rowed spring barley collection will be evaluated for FHB resistance in the coming field season.

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SCREENING OF SPRING WHEAT SCAB RESISTANCE FROM THE USDA GERMPLASM COLLECTION

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INTRODUCTION

A few known scab resistant materials have been used as the primary sources of resistance in most breeding programs. The level of the resistance in these materials is moderate, and significant loses can be expected when scab pressure is high. Moreover, the widespread use of these materials will likely result in considerable genetic uniformity, which may lead to potential genetic vulnerability to disease and other biotic or abiotic stresses. Additional sources of resistance are needed to build up higher levels of resistance and to introduce genetic diversity. The wheat germplasm in the USDA National Small Grains Collection (Aberdeen, ID) is a valuable genetic resource for various agronomic traits, disease, and pest resistance for wheat improvement. Over the past two years, we initiated evaluations of scab reaction in USDA spring wheat collections from regions where sources of resistance had been identified. This paper reports the results of germplasm screening in 1998 and 1999.

MATERIALS AND METHODS

Preliminary evaluation of scab reaction in the field nursery. Accessions were first tested in the Preliminary Screening Nursery. The number of accessions and their origin are given in Table 1. Spring wheat cultivars/lines Sonalika (susceptible, early), Wheaton (susceptible, late), Backup (resistant, early), and ND 2710 (resistant, late) were used as checks. Check-to-entry ratio was 1:30. In 1999, checks were planted at three different dates at weekly intervals, starting from the first field plot planting. Materials were planted in one meter single row plots. Growth stage of the test entries was monitored twice a week, and individual plots were tagged when at least 50-75% of the plants reached anthesis. Entries were inoculated with a mixture of 10 *Fusarium graminearum* isolates by spraying 50 ml of conidial suspension (75,000 conidial ml^{-1}) on each tagged plot followed by a second inoculation one week later. Plots were mist-irrigated for two minutes with a 30-minute recess between 8:00 p.m. and 8:00 a.m. Mist-irrigation was continued till the last readings. In addition to conidial suspension inoculation, starting from the jointing stage, 7.5 g of corn and 5 g of oat kernels colonized by *F. graminearum* were spread into each row weekly for three consecutive weeks. Scab reactions were evaluated about 14-17 d after the first spray inoculation. A sample of 20 spikes were evaluated following a 0-9 scale, where 0 indicated free of disease, and 9 indicated over 90% spikletes in a spike were infected. The increment "1" represented 10% disease severity on a spike. Disease incidence represented the percentage of infected spikes in the sample. Resistance to kernel damage of each entry was evaluated at maturity by squeezing the spikes. Entries with good seed set were harvested for further evaluation. After threshing, percent tombstone kernels of each selected entry was evaluated based on a 0-9 scale, where 0 indicated free of tombstone kernels and 9 indicated over 90% of scab infected kernels. Entries or plants within an entry with a low scab index and/or low kernel damage were selected for further evaluation in the greenhouse.

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Greenhouse re-evaluation and characterization

To confirm and characterize scab resistance from the field selections, selected accessions were evaluated by spray and point inoculation in the fall and spring greenhouse seasons. Approximately 40 to 80 plants of each selection were evaluated by point inoculation to assess resistance to spread. A similar number of plants were spray-inoculated to assess resistance to initial infection. At anthesis, flowering spikes were tagged and sprayed with a spore suspension (75,000 spores ml^{-1}). Inoculated plants were incubated in a misting chamber for 48 hr with 8 hr photoperiod, then moved to a greenhouse bench. Disease data were collected 14-17 d after inoculation, depending on the scab development of the susceptible checks (Sonalika and Wheaton). Total number of spikelets and number of diseased spikelets were recorded.

Elite Germplasm Nursery

Based on greenhouse screening, a portion of the preliminary selections was advanced to the field Elite Germplasm Nursery. Entries were replicated three times and arranged in a randomized complete block design. To avoid disease escape due to late maturity, entries with late maturity (later than Wheaton) were pre-germinated in germination paper four weeks prior to planting, and transplanted into the field. Sonalika, Wheaton, Backup, and ND 2710 were used as checks. Check-to-entry ratio was 1:30. The checks were planted at three different dates at a weekly interval. The nursery management was the same as that in the Preliminary Screening Nursery except that all the entries were harvested for seed evaluations.

RESULTS AND DISCUSSION

Preliminary screening for scab resistance

Many entries showed a wide range of reactions to scab between plants. In 1998, based on the field visual disease severity and kernel rating, and uniformity of scab reaction, 57 accessions were selected for greenhouse evaluation. In the 1999 Preliminary Screening Nursery, 55 accessions were selected. In addition, single plant selections were made from 70 accessions. Most of these selections were from South America (primarily from Brazil and Argentina) and Europe (Yugoslavia, Hungary, and Switzerland). The 1999 field selections are being evaluated in the greenhouse to characterize the type and level of resistance. Selections from the greenhouse evaluation will be advanced to the 2000 Elite Germplasm Nursery.

Scab reactions in the Elite Germplasm Nursery

After greenhouse evaluation, 51 accessions from the 1998 Preliminary Screening Nursery were advanced to the 1999 Elite Germplasm Nursery. Most of the test entries in the Elite Germplasm Nursery were found to be moderately susceptible. Five lines, however, consistently exhibited lower disease severity and low kernel damage in various tests (Table 2). Among these lines, Tokai 66 ranked the lowest in severity and very low percentage of damaged kernels (2 on a 0-9 scale). Low kernel damage was observed on 16-52-9 and Mentana. Seed of these selections is available upon request.

Introgression of scab resistance into adapted materials

A total of 17 crosses were made between the newly identified resistant lines and Wheaton or Russ for introgressing the resistance into adapted materials and for developing populations for genetic studies. Backcrossing will continue for those lines that continue to show good resistance.

Table 1. Number and origin of spring wheat accessions screened for scab resistance in 1998 and 1999.

Country of origin	Number of accessions evaluated 1998	1999
Argentina		130
Austria		174
Bosnia and Herzego		22
Brazil	168	11
Bulgaria		12
China	70	3
Czech Republic		28
Greece		43
Hungary		32
Poland		45
Romania		29
Switzerland		291
Italy	113	10
Japan	73	3
Ukraine		26
Uruguay		21
Yugoslavia		62
Others (less than 10 accessions)		39
Total	424	981

Table 2. Average scab index and kernel damage score of two-year evaluation in the field and greenhouse (GH) by spray and point inoculation (point inoc) methods in spring wheat accessions selected for scab resistance.

ID	Accession	Country of origin	Days to flowering	Scab index field	Scab index GH-spray	Scab index GH-point inoc	Kernel damage score§
Tokai 66	PI 382161	Brazil	56	23.2	25.4	10.3	2.3
Sapporo Haru.	PI 81791	Japan	63	29.4	11.9	8.8	2.0
Nobeoka Bozu	PI 382153	Japan	59	31.2	38.4	9.9	5.5
16-52-9	PI 382167	Brazil	64	33.7	37.2	39.0	1.0
Mentana	PI 182416	Italy	61	62.2	20.0	27.0	1.3
ND 2710		ND	55	27.4	22.3	9.0	3.0
Backup		MN	55	40.7	--	--	1.3
Wheaton		MN	58	78.0	90.0	93.3	8.2
Sonalika		Mexico	51	85.7	87.0	76.0	8.2

Days to flowering was estimated in a 1998 field nursery at Brookings, SD.

§ Average of seed scores from the field 1999 Elite Germplasm Nursery.