USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY09 Final Performance Report
July 15, 2010

Cover Page

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Fiscal Year:	2009
USDA-ARS Agreement ID:	59-0206-9-068
USDA-ARS Agreement Title:	Determination and Characterization of Deoxynivalenol in Barley.
FY09- USDA-ARS Award Amount:	$ 180,177

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
FSTU	Determination of Bound Deoxynivalenol in Barley Breeding Lines.	$ 24,725
FSTU	Malting Barley Deoxynivalenol Services.	$ 155,452
	Total Award Amount	**$ 180,177**

Principal Investigator Date

* MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Winter Wheat Region
SWW – Southern Sinter Wheat Region

Project 1: *Determination of Bound Deoxynivalenol in Barley Breeding Lines.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Wheat and barley have been shown to have the ability to detoxify deoxynivalenol (DON) by forming glycosides. The presence of these DON-glucosides, or bound DON in barley and wheat are a cause for concern, as by definition, bound DON is that which escapes detection by the routine analytical methods. The evidence that suggests bound DON may be released into the free form under some food processing conditions, through enzymolysis in malting and brewing, or in digestion raises concerns that the potential toxicity of samples is being underestimated. Breeder's lines that show partial resistance or lower DON accumulation through the formation of DON-glucosides may be of questionable value, if free DON is simply being offset by bound DON. The objectives of this project are to determine the prevalence of bound DON in commercial samples of wheat and barley that have been infected with Fusarium Head Blight (FHB), and to determine how differences in plant resistance to FHB and DON accumulation impact the levels of total and bound DON.

2. List the most important accomplishment and its impact (i.e. how is it being used) to minimize the threat of Fusarium head blight or to reduce mycotoxins. Complete both sections (repeat sections for each major accomplishment):

Accomplishment: Published methods for the determination of DON-glucosides have employed solvent extraction and analysis by LC-MS/MS. Our laboratories, as well as many others, have no access to this equipment, and our initial objective was to develop alternative means for measuring DON-glucosides. Both the use of GC-MS and HPLC with UV/Vis detection were evaluated. Analysis of DON-glucoside by GC-MS was not successful as the derivatization procedure results in the fragmentation of DON-3-glucoside into unquantifiable fragments. HPLC was evaluated as the procedure requires no derivatization. However, detection by UV/Vis does not have the sensitivity or selectivity required for quantitation. Numerous interfering peaks were present in the extract matrix. We are currently evaluating the use of LC-MS. However, problems with equipment have delayed these efforts. The NDSU Agricultural Experiment Station provided $10,000 for repair of this equipment in May 2010.

Impact: This project will provide much needed data on the occurrence of DON-glucoside in regional barley and wheat samples, and its relevance in mycotoxin measurement. Should DON-glucoside be found to account for a significant portion of the total DON, it may be necessary to change testing protocols.

Project 2: *Malting Barley Deoxynivalenol Services.*

1. **What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?**

   Mycotoxin analyses are essential for most researchers working on FHB of cereals. However, in barley DON is a major economic factor, and new varieties must display increased resistance to DON accumulation as well as to FHB. Screening barley lines for DON is requisite for any breeding program intending to develop varieties for the upper Midwestern USA. DON analytical services are primarily provided to three barley varietal developmental programs. These breeding programs stated a need for the analysis of approximately 13,000 samples in FY09 (but only 10,700 were submitted). Supporting research and extension work required an additional 1,600 samples. In total, approximately 13,000 samples were analyzed, and eleven collaborating scientists were served. The major issue is to provide DON analytical services in a cost effective, timely and accurate manner. Funds provided by the USWBSI have allowed us to hire additional personnel and to subsidize the cost of analysis.

2. **List the most important accomplishment and its impact (i.e. how is it being used) to minimize the threat of Fusarium head blight or to reduce mycotoxins. Complete both sections (repeat sections for each major accomplishment):**

   Accomplishment: Approximately 13,000 samples (excluding standards) were analyzed from May 2009 to May 2010. These analyses were primarily from barley varietal development programs (n=10,696). Additional analysis were conducted for personnel involved in extension/crop production work (n= 1,701) and for barley FHB research projects. Samples analyzed as part of the 2009 regional barley crop quality survey indicated that average levels of DON (0.30 mg/kg) were comparable to those seen in recent years, and were among the lowest observed since testing began in 1993. Periodic check samples (barley and malt) are analyzed by all USWBSI funded diagnostic laboratories as a means of quality assurance, to help assure that comparable results are obtained in each laboratory

   Impact: This project provides essential support to all barley breeding programs working on the development of FHB-resistant varieties for the Midwestern USA. The occurrence of FHB and DON is a primary factor in the dramatic decrease in barley acreage that has been observed over the past 15 years/

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Delgado, J.A., Schwarz, P.B., Gillespie, J., Rivera-Varas, V.V., Secor, G.A. Trichothecene mycotoxins associated with potato dry rot caused by *Fusarium graminearum*. PHYTOPATHOLOGY 2010,100(3), 290-296.

Lamb, K.E., Gonzalez-Hernandez, J.L., Zhang, B.X., Green, M., Neate, S.M., Schwarz, P.B., Horsley, R.D. Identification of QTL conferring resistance to Fusarium Head Blight Resistance in the breeding Line C93-3230-24. CROP SCIENCE 2009, 49(5), 1675-1680.

PI: Schwarz, Paul
Project: Determination of Bound Deoxynivalenol in Barley Breeding Lines.

FY09 FPR – USWBSI ADDENDUM
DON Service Labs – Quality Control Data

Insert below Quality Control Data/Results from the FY09 Award Period (May 09-May 10):

Inter-laboratory check samples are mailed three times/year. Four samples (2 barley, 2 malt) are sent with each mailing. Collaborating labs include the four USWBSI funded labs as well, as eleven other industry labs. Our performance relative to the other laboratories is presented in the table below:

Sample	**Date**	**NDSU**	**Mean of all labs**	**No. of labs**	**Std Dev.**
Barley82	January 2009	0.16	0.17	12	0.28
Malt82		10.73	6.49	12	1.62
Barley83		0.52	0.30	12	0.10
Malt83		17.62	10.28	12	5.29
Barley84	June 2009	0.58	0.54	12	0.14
Malt84		0.00	0.06	12	0.06
Barley85		0.00	0.06	12	0.05
Malt 85		0.00	0.03	12	0.04
Barley86	April 2010	0.22	0.21	10	0.10
Malt86		0.56	0.57	10	0.10
Barley87		0.21	0.20	10	0.06
Malt87		0.54	0.64	10	0.11

We also include two internal check samples with each set of 46 samples analyzed. These two are selected from a total of six check samples (four barley and two malt samples). Mean DON values on these checks are from 0.70 to 25.00 mg/kg DON. The internal checks are run in duplicate (one duplicate/detector). In the event that 3 of the 4 DON values are outside 1 standard deviation of the mean, the set will be reanalyzed. From May 2009 to May 2010 approximately 1100 internal checks were analyzed. Data for each of the six checks is shown in the table below

Check	No.	Mean	Std. Dev.	C.V.
Barleyii	216	25.65	3.34	13.02%
Barleyjj	228	5.92	1.04	17.51%
Barleykk	95	8.52	1.31	15.38%
Maltll	192	2.96	0.42	14.04%
Maltmm	176	14.30	1.73	12.08%
Barleynn	166	0.71	0.17	24.55%