USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY15 Final Performance Report
Due date: July 15, 2016

Cover Page

Principle Investigator (PI):	David Schmale
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Fiscal Year:	2015
USDA-ARS Agreement ID:	59-0206-2-082
USDA-ARS Agreement Title:	Diagnostic Testing Services for Deoxynivalenol in the Eastern U.S.
FY15 USDA-ARS Award Amount:	$ 117,061
Recipient Organization:	Virginia Polytechnic Institute and State University 1880 Pratt Drive, Suite 2006 Blacksburg, VA 24060
DUNS Number:	003137015
EIN:	54-6001805
Recipient Identifying Number or Account Number:	422288
Project/Grant Reporting Period:	06/25/15-06/24/16
Reporting Period End Date:	06/24/16

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
FST-S	Diagnostic Testing Services for Deoxynivalenol in the Eastern U.S.	$ 77,772
PBG	Enzymatic Detoxification of Deoxynivalenol.	$ 39,289
	FY15 Total ARS Award Amount	**$ 117,061**

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
EC-HQ – Executive Committee-Headquarters
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 Soft Winter Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: *Diagnostic Testing Services for Deoxynivalenol in the Eastern U.S.*

1. **What are the major goals and objectives of the project?**
   The overall goals of our project were to (1) provide diagnostic testing services for DON for wheat and barley samples associated with USWBSI-supported research projects in the eastern U.S. and (2) reduce DON contamination in wheat and barley.

2. **What was accomplished under these goals?**
   a. Major activities. In FY15, DON data was delivered for 6,074 wheat and barley samples from USWBSI investigators (Laskar, Glover, Griffey, Grybauskas, Wegulo, Mehl and Schmale). The testing number does not include controls, checks, and re-runs. Most of the samples tested in FY15 were 100g kernel lots from FHB field trials, but some were smaller lots (~5g samples) from laboratory experiments. We also processed samples associated with DON during detoxification studies. Extraction, clean-up, and quantification of DON were conducted following standard protocols using a GC/MS. Research associate Niki McMaster and PI David Schmale attended the 2015 USWBSI meeting in St Louis, MI.
   b. Specific objectives. The specific objectives of the proposed research were to (1) provide analytical services necessary to develop new cultivars of wheat and barley with reduced potential for DON contamination and to (2) facilitate DON testing that will improve chemical and cultural practices necessary to reduce DON contamination in wheat and barley.
   c. Significant results. The proposed project provided essential DON testing services for the USWBSI and supported the only USWBSI-associated DON testing lab in the eastern U.S. Many of the wheat and barley lines had not been tested previously for mycotoxins.
   d. Key outcomes or other achievements. The research has contributed to the development and release of new FHB-resistant wheat and barley varieties, (2) ensured rigorous testing of both new and historical wheat and barley varieties for mycotoxin contamination. The Schmale Lab at Virginia Tech continues to be committed to the long-term management of a successful and productive mycotoxin testing lab for the USWBSI. DON testing services were coordinated, supported, and managed by a talented research associate (Niki McMaster).

3. **What opportunities for training and professional development has the project provided?**
   Research associate Niki McMaster attended a three-day training workshop on GC at Virginia Tech to enhance her skills in methods for mycotoxin detection. The course consisted of nine lectures and six labs. Topics ranged from FID and TCD detectors, autosamplers, gas sampling valves, and the latest software for analyses. Methods were developed an optimized for some of the samples from the Schmale Lab.

4. **How have the results been disseminated to communities of interest?**
   Schmale gave a series of lectures on mycotoxins for about 200 undergraduate students at Virginia Tech. McMaster communicated with USWBSI stakeholders via phone and email to coordinate sample collection, processing, and testing. Results were disseminated to stakeholders at the 2015 USWBSI meeting in St. Louis.

Project 2: *Enzymatic Detoxification of Deoxynivalenol.*

1. **What are the major goals and objectives of the project?**
   The goals of our project are to (1) reduce mycotoxin contamination in wheat and barley used for feed and food and (2) provide an efficient control strategy for reducing mycotoxins in wheat and barley throughout the United States.

2. **What was accomplished under these goals?**
   a. Major activities. Research activities were focused on (1) characterizing functional enzymes and microorganisms that reduce the toxicity of DON and (2) analyzing breakdown products of the toxins.
   b. Specific objectives. The specific objectives of the project were to (1) identify and engineer functional enzymes (epoxide hydrolases and cycloisomerases) to reduce the toxicity of DON and (2) engineer yeast to express DON-detoxification enzymes in wheat and barley samples.
   c. Significant results. Microbes were isolated from the environment (DON detoxifying microbes, DDMs) that consistently detoxified DON in laboratory experiments. DNA sequencing analyses have indicated that the pure cultures were *Pseudomonas* and *Achromobacter*. NMR analysis from one of the cultures showed that DON is converted to 3-keto-DON. We have used unique yeast strains (that we have engineered to be sensitive to DON) to screen enzymes and fragments of DNA from different microorganisms for their ability to detoxify DON. Two enzymes and three different library fragments show growth in the presence of high amounts of DON, demonstrating that these candidates are likely detoxifying DON. Sequencing of one fragment has revealed a possible resistance gene.
   d. Key outcomes and other achievements. This research offers unique strategies for detoxifying DON in wheat and barley products. We have initiated collaborations with Vireol Bio Energy and AgBiome to explore opportunities to transfer this technology to the commercial sector in the future.

3. **What opportunities for training and professional development has the project provided?**
   Ph.D. candidate Nina Wilson learned new techniques to culture DON-detoxifying microbes, identify and express candidate enzymes for DON detoxification, and analyze toxin byproducts following transgenic yeast feeding assays. Research associate Regina Hanlon was trained in expressing candidate enzymes for DON detoxification.

4. **How have the results been disseminated to communities of interest?**
   Schmale gave a series of lectures on mycotoxins for about 200 undergraduate students at Virginia Tech. Ph.D candidate Wilson disseminated the results to stakeholders at the 2015 USWBSI meeting in St. Louis.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY15 award period. The term "support" below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student's stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY15 award period?**

   If yes, how many? No

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY15 award period?**

   If yes, how many? Yes, 1

3. **Have any post docs who worked for you during the FY15 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**

   If yes, how many? No

4. **Have any post docs who worked for you during the FY15 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**

   If yes, how many? No

Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY15 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.*

Name of Germplasm/Cultivar	**Grain Class**	**FHB Resistance** (S, MS, MR, R, where R represents your most resistant check)	**FHB Rating** (0-9)	**Year Released**

Add rows if needed.

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Abbreviations for Grain Classes

Barley - BAR
Durum - DUR
Hard Red Winter - HRW
Hard White Winter - HWW
Hard Red Spring - HRS
Soft Red Winter - SRW
Soft White Winter - SWW

Publications, Conference Papers, and Presentations

Refer to the FY15-FPR_Instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY15 grant. If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.

Books or other non-periodical, one-time publications.

Other publications, conference papers and presentations.

Griffey, C., Ullrich, J., Malla, S., Brooks, W., Van Sanford, D., Clark, A., Murphy, J., Brueggeman, R., Cowger, C., McMaster, N., Schmale, D., Chao, S., Brown-Guedira, G. (2015). Identification of quantitative trait loci for resistance to Fusarium head blight in winter barley cultivar Eve. In: S. Canty, A. Clark, S. Vukasovich and D. Van Sanford (Eds.), *Proceedings of the 2015 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. Barley Dec. 6-8; Saint Louis, MO. p. 113.
Status: Abstract published and poster presented.
Acknowledgement of Federal Support: YES

Griffey, C., Malla, S., Murphy, J., Milus, E., Clark, D., Van Sanford, D., Costa, J., McMaster, N, Schmale, D., Chao, S., Brown-Guedira, G. (2015). Characterization of FHB resistance QTL in SRW wheat cultivar Tribute. In: S. Canty, A. Clark, S. Vukasovich and D. Van Sanford (Eds.), *Proceedings of the 2015 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. Barley Dec. 6-8; Saint Louis, MO. p. 94.
Status: Abstract published and poster presented.
Acknowledgement of Federal Support: YES

Wilson, N., Gantulga, D., McMaster, N., Senger, R., and Schmale, D. (2015). Using enzymes and microorganisms to modify the mycotoxin deoxynivalenol. In: S. Canty, A. Clark, S. Vukasovich and D. Van Sanford (Eds.), *Proceedings of the 2015 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. Barley Dec. 6-8; Saint Louis, MO. p. 62.
Status: Abstract published and poster presented.
Acknowledgement of Federal Support: YES

PI: Schmale, David
Project: Diagnostic Testing Services for Deoxynivalenol in the Eastern U.S.

FY15 FPR – USWBSI ADDENDUM
DON Service Labs – Quality Control Data

Insert below Quality Control Data/Results from the FY15 Award Period (06/25/15-06/24/16):

Quality control data were collected at Virginia Tech through (a) the blind testing of samples with unknown DON levels (coordinated by the USWBSI through Trilogy Analytical Laboratories), and (b) the testing of subsamples of grain lots in each GC/MS run (to test for consistency among GC/MS runs). Known standards are run throughout the the GC/MS run to establish our standard curves.

a. QC data for blind testing of samples from Trilogy Labs (coordinated by Trilogy Labs, and communicated through Sue Canty; scabusa@scabusa.org). Lab ID 'Lab3' is the Virginia Tech lab (highlighted in grey). Lab IDs 1-4 are other USWBSI labs. Data are in ppm.

	Trilogy Sample	Trilogy Quant	Lab1	Lab2	Lab3	Lab4-1	Lab4-2
August 2015	**Low**	0.70	0.60	0.70	0.55	0.53	0.55
	High	6.40	4.68	5.60	5.5	5.03	5.57
	Med	2.10	1.65	2.00	1.95	1.64	1.76
Sept 2015	**Low**	0.50	0.52	0.50	0.78	0.40	0.41
	High	7.00	5.91	6.20	5.58	4.80	4.78
	Med	2.10	1.95	2.20	1.82	1.45	1.51
Oct 2015	**Low**	1.60	1.10	1.30	1.38	1.07	1.15
	High	7.90	5.74	6.20	6.15	5.48	5.84
	Med	2.60	1.94	2.10	2.15	2.03	2.12
Nov 2015	**Low**	1.50	1.38	1.30	1.16	1.18	1.24
	High	8.90	8.03	7.10	6.07	5.79	6.69
	Med	3.50	3.09	3.10	2.48	2.37	2.37
Dec 2015	**Low**	0.50	0.72	0.50	0.38	0.44	0.45
	High	6.40	6.34	5.40	5.71	4.48	5.02
	Med	2.10	2.10	1.60	1.21	1.54	1.62
Jan 2016	**Low**	0.7	0.84	0.60	0.76	0.50	0.54
	High	7.9	9.28	6.30	7.90	5.77	6.11
	Med	2.4	2.61	1.70	2.01	1.43	1.51
Feb 2016	**Low**	0.50	0.74	0.40	0.62	0.44	0.46
	High	8.90	7.91	6.20	8.35	6.45	6.50
	Med	2.60	2.75	1.70	2.64	1.95	1.95
March 2016	**Low**	1.00	1.37	0.80	0.91	0.78	0.75
	High	7.00	7.46	5.10	5.93	4.90	4.71
	Med	3.80	4.40	2.90	3.31	2.42	2.51
April 2016	**Low**	0.50	0.51	0.60	0.56	0.44	0.47
	High	6.40	4.82	4.30	4.91	4.26	4.15
	Med	2.10	2.06	1.30	1.87	1.47	1.55

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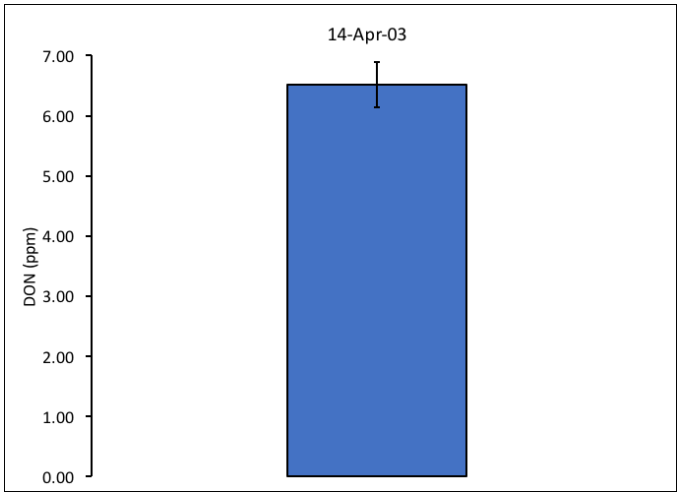
b. QC data from internal checks of subsamples of grain lots from Trilogy (14-Apr-03) in each GC/MS run (to test for consistency among GC/MS runs). Trilogy sample 14-Apr-03 was measured 300 times and determined to have an average DON concentration of 6.51 ppm with a standard error of 0.38.



July 15, 2016