USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY10 Final Performance Report
July 15, 2011

Cover Page

PI:	David Schmale
Institution:	VA Tech.
Address:	Dept. of Plant Pathology, Physiology, Weed Science 403 Latham Hall Blacksburg, VA 24061
E-mail:	dschmale@vt.edu
Phone:	540-231-6943
Fax:	540-231-7477
Fiscal Year:	FY10
USDA-ARS Agreement ID:	59-0790-7-078
USDA-ARS Agreement Title:	Contribution of Local Inoculum Sources to Regional Atmospheric Populations of *G. Zeae*.
FY10 USDA-ARS Award Amount:	$ 99,044

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
FSTU-S	Diagnostic Testing Services for Deoxynivalenol in the Eastern United States.	$ 64,980
PBG	Mycotoxin Potential and Aggressiveness of FHB Pathogens used for USWBSI Research.	$ 21,854
MGMT	Within-Field Inoculum from Corn Debris and the Management of FHB/DON.	$ 12,210
	Total ARS Award Amount	**$ 99,044**

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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 Soft Winter Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: *Diagnostic Testing Services for Deoxynivalenol in the Eastern United States.*

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

   There is a growing need to develop and expand USWBSI diagnostic testing services for mycotoxins throughout the United States. DON testing services are vital to the development of new varieties of wheat and barley with reduced mycotoxin potential and are necessary to identify and/or exclude appropriate strategies for managing FHB. FY10 DON testing services at Virginia Tech provided analytical services necessary to develop new cultivars of wheat and barley with reduced potential for DON contamination and to improve chemical and cultural practices necessary to reduce DON contamination in wheat and barley.

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:
   In FY10, DON was quantified from 9,750 samples (runs, internal checks (controls), cooperative samples, and re-runs) of wheat and barley from three USWBSI investigators (Schmale, Griffey, and Glover) in two states (Virginia and South Dakota). Most of the samples tested in FY10 were 100g kernel lots from FHB field trials (Schmale, Griffey, and Glover), but some were ground 0.25-5g samples from greenhouse experiments (Schmale). We also processed samples associated with barley ethanol production in Virginia and a cooperative project associated with the USDA ARS Sustainable Biofuels and CoProducts Research Lab to reduce DON in barley ethanol co-products. Extraction, clean-up, and quantification of DON were conducted following standard protocols using a GC/MS. DON testing services were managed by a talented scientist (Niki McMaster) who was hired in August, 2010 to manage USWBSI testing services. Niki McMaster and Diane Reaver visited Michelle Mostrom's lab and Paul Schwartz's lab the week of September 20, 2010. Schmale continues to be committed to the long-term management of a successful and productive mycotoxin testing lab for the USWBSI.

   Impact:
   The goals of this work were to provide analytical services necessary to develop new cultivars of wheat and barley with reduced potential for DON contamination and to facilitate DON testing that will improve chemical and cultural practices necessary to reduce DON contamination in wheat and barley. This work directly addresses Goal #1 of the Action Plan to 'Provide analytical support for DON/trichothecene quantitation for Initiative's stakeholders'. We are providing DON testing services for wheat and barley samples from USWBSI investigators. Schmale routinely interacts with stakeholders in VA to discuss new diagnostic technologies for DON and related management strategies for FHB, an effort aligned with Goal #2 of the Action Plan to 'Provide requisite information on DON/ trichothecene safety issues to producers, millers, researchers, risk assessors and regulators'.

Project 2: *Mycotoxin Potential and Aggressiveness of FHB Pathogens used for USWBSI Research.*

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

   USWBSI investigators often rely on a 'favorite' set of strains of FHB pathogens for their field and greenhouse trials, yet little is known about the mycotoxin potential and aggressiveness of these strains. Knowledge of the aggressiveness and mycotoxin potential of FHB pathogens used in USWBSI research may assist in selecting appropriate strains for field and greenhouse experiments and may help explain observed variations in FHB and/or DON among field and greenhouse trials. We screened strains that have been used (or are currently being used) in inoculations of field and/or greenhouse trials to support USWBSI VHDR research. The aims of the project were to: (1) measure the aggressiveness of individual strains on a series of susceptible and moderately-resistant cultivars of wheat and barley, (2) determine the relative concentrations of trichothecene mycotoxins produced by these strains following a series of controlled inoculations, and (3) identify individual strains to the level of species, based on both biological and phylogenetic species recognition.

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:
   We received 44 strains of FHB pathogens from seven USWBSI investigators (P. Murphy, S. Zong, P. Paul, T. Friesen, G. Bergstrom, C. Griffey, and G. Milus). Receipt, handling, and transport of the strains were conducted under USDA-APHIS permit P526P-09-02605. Trichothecene mycotoxin genotyping of the strains showed that all three genotypes (3ADON/DON, 15ADON/DON, and NIV) were present in the collection of strains. Two greenhouse experiments were conducted in FY10 to measure the aggressiveness of individual strains on a susceptible cultivar of wheat (Glenn) and barley (Robust) and to determine the relative concentrations of trichothecene mycotoxins produced by these strains following a series of controlled inoculations. Three heads of wheat and three heads of barley were point-inoculated (a single spikelet in the center of the head) with each of the strains. Wounded non-inoculated plants served as controls. FHB severity ranged from 2 to 39% (mean of 10.3% across all strains) for the barley trials 9 days following inoculation, and 0 to 90% (mean of 20% across all strains) for the wheat trials 14 days following inoculation. For the strains that produced DON, concentrations of DON ranged from 0.7 to 117.8 ppm (mean of 20.2 ppm) from harvested heads following the barley trials, and 0.2 to 164.4 ppm (mean of 29.4 ppm) from harvested heads following the wheat trials. For the strains that produced NIV, concentrations of NIV ranged from 1.0 to 12.0 ppm (mean of 4.8 ppm) from harvested heads following the barley trials, and 0.2 to 2.6 (mean of 1.6 ppm) from harvested heads following the wheat trials. Portions of three genes have been amplified for all of these strains, and sequencing and phylogenetic analysis continue to associate these strains with the proper species of *Fusarium* (e.g., *Fusarium graminearum sensu stricto*).

Impact:

The project directly addresses Goal #1 of the Action Plan to 'Characterize genetic variation in the pathogen population with regard to aggressiveness toward plants and mycotoxin potential'. We screened strains representing nearly a decade of FHB research in the U.S. Our greenhouse assays and mycotoxin profiling suggest that the strains used by USWBSI researchers (at least those tested in this study) vary in aggressiveness and mycotoxin potential. This knowledge may assist in selecting appropriate strains for field and greenhouse experiments in the future and may help explain observed variations in FHB and/or DON among field and greenhouse trials.

Project 3: *Within-Field Inoculum from Corn Debris and the Management of FHB/DON.*

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

Our experimental objective was to quantify the relative contribution of within-field corn debris as an inoculum source of *Gibberella zeae* for Fusarium head blight and DON contamination in eleven variable wheat environments in 2010, all in regions where corn is the predominant crop in the agricultural landscape and corn debris is left on the land surface over large areas. Our research is based on the hypothesis that spores of *Gibberella zeae* that are deposited on wheat spikes and that result in Fusarium head blight come primarily from well-mixed, atmospheric populations in an area. The research was conducted in commercial-scale wheat fields in Illinois, Missouri, Nebraska, New York, and Virginia, each following a non-susceptible crop. Replicated (six) microplots containing corn debris from a nearby field or no added debris were set out in each field and were separated by a minimum of 100 ft in each dimension. Wheat spikes above each microplot were rated at soft dough stage for FHB incidence, severity, and index. At grain maturity, at least 100 spikes from each microplot were harvested, dried and shipped to Cornell where grain was threshed from a subsample of spikes and sent to the assigned USWBSI Testing Lab for DON analysis. Mature spikes from each microplot were also suface-disinfested and plated on Fusarium selective media to determine the incidence of spikes infected by *G. zeae*.

Characterization of epidemics over the 11 environments differed through the lenses of visual symptom development, incidence of mature spike infection, and toxin contamination. At every location except Chatham, VA, more than 20% of mature spikes were infected by *G. zeae*, regardless of the degree of symptom development at soft dough stage or the level of DON observed. This suggests that post-anthesis infection was quite common across environments in 2010. Based strictly on FHB index at soft dough, we observed five moderate epidemics (in Illinois, Missouri, and Nebraska) and six mild epidemics (in Nebraska, New York, and Virginia). On the other hand, three of the moderate epidemics, based on symptoms, were associated with toxin levels above 2 ppm. Mean DON levels in the no-debris microplots were 2.9 ppm in Urbana, IL, 4.4 ppm in Columbia, MO, and 12.2 ppm in Novelty, MO, and there was detectable DON at every site except Chatham, VA. Across the 11 environments, there was significantly (P=0.05) higher DON in grain from corn debris microplots (1.8 ppm) than from no-debris microplots (0.2 ppm) only in Bath, NY. It is especially noteworthy that DON levels were not significantly higher in corn debris

microplots than no-debris microplots in any of the high DON locations, suggesting the predominance of regional atmospheric inoculum in those locations. FHB incidence, severity, or index was not significantly (P=0.05) higher in corn debris-containing than no-debris microplots in any of the 11 fields at soft dough stage. And only at Wilbur, NE did mature wheat spikes from microplots containing locally overwintered corn debris show a statistically significant increase in infection incidence by *G. zeae* over those from microplots with no corn debris.

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:

The astounding result is that DON levels did not differ significantly between corn debris and no debris microplots in 20 of the 21 winter wheat environments studied over two years. The single exception was in Bath, New York in 2010, an isolated valley environment with less surrounding grain corn acreage than other locations. It is especially noteworthy that DON levels were not significantly higher in corn debris microplots than no-debris microplots in any of the high DON locations, suggesting the predominance of regional atmospheric inoculum over within-field inoculum in severe epidemic circumstances.

Impact:

By inference of our results over two years and 21 winter wheat environments, it appears that elimination of corn debris from single wheat fields in major corn-producing regions may have rather limited benefits in terms of reducing FHB and especially of reducing DON contamination of grain. One caveat regarding this interim conclusion is that the microplot experimental design (small area sources of corn debris) we used may have resulted in an underestimation of the contribution of large area sources of corn debris to wheat infection and DON contamination. Much larger replicated plots will be necessary to definitively assess the quantitative contribution of corn debris to local wheat infection and DON accumulation on an agricultural field scale. This is the approach being taken in the FY11 project by Bergstrom et al and being conducted in wheat fields in seven states.

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.

Berger, G., Khatibi, P., Brooks, W., Liu, S., Hall, M., Green, A., Griffey, C., and Schmale, D.G. 2010. 2010. Phenotypic characterization of Fusarium head blight resistance in hulled and hulless winter barley grown in the mid-atlantic region. Page 129 in Proc. 2010 National Head Blight Forum, Milwaukee, WI, December 7-9, 2010.

Bergstrom, G.C., K.D. Waxman, D.G. Schmale III, C.A. Bradley, L.E. Sweets, S.N. Wegulo, and M.D. Keller. 2010. Effects of within-field corn debris in microplots on FHB and DON in eleven U.S. wheat environments in 2010. Pages 69-70 in Proc. 2010 National Fusarium head Blight Forum, Hyatt Regency Milwaukee, Milwaukee, WI, Dec 7-9, 2010.

Bergstrom, G.C., K.D. Waxman, D.G. Schmale III, C.A. Bradley, L.E. Sweets, S.N. Wegulo, and M.D. Keller. 2009. Effects of within-field corn debris in microplots on FHB and DON in ten U.S. wheat environments in 2009. Page 22 in Proc. 2009 National Fusarium Head Blight Forum, Wyndham Orlando Resort, Orlando, FL, Dec7-9, 2009.

Keller, M.D, Waxman, K.D., Bergstrom, G.C., and Schmale, D.G. 2010. Local distance of wheat spike infection by released clones of *Gibberella zeae* disseminated from infested corn residue. Plant Disease 94: 1151-1155.

Keller, M.D., Thomason, W.E., and Schmale, D.G. 2010. The recovery of released clones of *Gibberella zeae* from winter wheat and barley is influenced by the amount of local corn stalk residue. Page 84 in Proc. 2010 National Head Blight Forum, Milwaukee, WI, December 7-9, 2010.

Keller, M.D., Schmale, D. G., Waxman, K.D., and Bergstrom, G.C. 2010. Tracking released clones of *Gibberella zeae* within wheat and barley fields. Page 51 in Proc. 2010 National Head Blight Forum, Milwaukee, WI, December 7-9, 2010.

Keller, M.D., Thomason, W.E., and Schmale, D.G. 2009. Influence of crop residues and disease resistance on FHB in Virginia wheat. Page 59 in Proc. 2009 National Head Blight Forum, Orlando, FL.

Keller, M.D, Thomason, W, and Schmale, D.G. 2011. The spread of a released clone of *Gibberella zeae* from different amounts of infested corn residue. Plant Disease, *In press.*

Khatibi, P. A., Newmister, S., Rayment, I., McCormick, S. P., Alexander, N. J., and Schmale, D.G. 2011. Bioprospecting for trichothecene 3-O-acetyltransferases in the fungal genus *Fusarium* yields functional enzymes that vary in their ability to modify the mycotoxin deoxynivalenol. Applied and Environmental Microbiology 77: 1162-1170.

Khatibi, P., Griffey, C.A., and Schmale, D.G. 2009. Mitigating deoxynivalenol contamination in hulless barley and fuel ethanol co-products. Phytopathology 99: S204.

Liu, S., Brooks, W., Chao, S., Griffey, C., Hall, M., Gundrum, P., Berger, G., Khatibi, P., and Schmale, D.G. 2009. Association analyses of SNP markers with scab resistance in winter feed barley. Page 133 in Proc. 2009 National Head Blight Forum, Orlando, FL.

Schmale, D. G., Ross, S.D., Fetters, T.L., Tallapragada, P., Wood-Jones, A.K., and Dingus, B. 2011. Isolates of *Fusarium graminearum* collected 40-320 meters above ground level cause Fusarium head blight in wheat and produce trichothecene mycotoxins. Aerobiologia, DOI 10.1007/s10453-011-9206-2.

Schmale, D. G., Wood-Jones, A.K., Cowger, C., Bergstrom, G.C., and Arrellano, C. 2011. Trichothecene genotypes of Gibberella zeae from winter wheat fields in the eastern United States. Plant Pathology, DOI: 10.1111/j.1365-3059.2011.02443.x.

Schmale, D. G. 2010. Linking field and atmospheric populations of toxigenic fungi in the genus *Fusarium*. 2010 Meeting of NCERA-213 Migration and Dispersal of Agriculturally-Important Biota Committee, Blacksburg, VA, October 26-27, 2010.

Schmale, D.G. and Munkvold, G.P. 2009. Mycotoxins in crops: A threat to human and domestic animal health. The Plant Health Instructor DOI: 10.1094/PHI-I-2009-0715-01.

Schmale, D.G. 2009. Linking field and atmospheric populations of toxigenic fusaria. Page 181 in Proc. 2009 National Head Blight Forum, Orlando, FL.

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FY10 FPR – USWBSI ADDENDUM
DON Service Labs – Quality Control Data

Insert below Quality Control Data/Results from the FY10 Award Period (May 10-May 11):

Quality control data was collected at Virginia Tech in the following ways: (1) the blind testing of samples with unknown DON levels (coordinated by J. Gillespie , NDSU), (2) the blind testing of samples for a barley cooperative project (coordinated by Andrea Stern, ASBC technical committee), and (3) the testing of subsamples of grain lots from the Mostrom Lab in each GC/MS run with low, medium, and high DON (to test for consistency among GC/MS runs). Known standards are run throughout the the GC/MS run to establish our standard curves.

(1) Blind testing of samples with unknown DON levels (coordinated by J. Gillespie , NDSU). We participated in the blind testing of two separate sets of blind samples during the funding period, but we have only received the results from J. Gillespie for one set. Comparitive data was provided by J. Gillespie via email (James.Gillespie@ndsu.edu). Lab ID 'S' is the Virginia Tech lab (highlighted in grey).

a. Barley 90 and Malt 90

Lab ID	Method	Sample	Results (ppm DON)
A	GC/ECD	Barley 90	3.95
		Malt 90	0.63
D	Diagnostix	Barley 90	5.36
		Malt 90	0.52
F	GC/MS #1	Barley 90	4.68
		Malt 90	0.40
F	GC/MS #2	Barley 90	4.66
		Malt 90	0.41
I	DON 2/3	Barley 90	4.80
		Malt 90	0.70
J		Barley 90	11.80
		Malt 90	1.70
M	EZ-TOX	Barley 90	6.00
		Malt 90	0.29
O	EZ-TOX	Barley 90	4.38
		Malt 90	0.50
Q	EZ-TOX	Barley 90	4.03
		Malt 90	0.37
S	GC/MS	Barley 90	4.32
		Malt 90	0.44

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b. Barley 91 and Malt 91

Lab ID	Method	Sample	Results (ppm DON)
A	GC/ECD	Barley 91	0.18
		Malt 91	8.19
D	Diagnostix	Barley 91	0.25
		Malt 91	10.40
F	GC/MS #1	Barley 91	0.28
		Malt 91	5.33
F	GC/MS #2	Barley 91	0.30
		Malt 91	5.54
I	DON 2/3	Barley 91	0.10
		Malt 91	8.10
J		Barley 91	0.60
		Malt 91	17.10
M	EZ-TOX	Barley 91	0.41
		Malt 91	3.93
O	EZ-TOX	Barley 91	0.42
		Malt 91	5.52
Q	EZ-TOX	Barley 91	0.17
		Malt 91	4.98
S		Barley 91	0.42
		Malt 91	5.24

(2) Blind testing of samples for a barley cooperative project (coordinated by Andrea Stern, ASBC technical committee). Comparitive data was provided by A. Stern via email (Andrea.Stern@malteurop.com). Lab ID 'G3' is the Virginia Tech lab (highlighted in grey).

	Sample Pair		**Sample Pair**		**Sample Pair**	
Collaborator	**1**	**2**	**3**	**4**	**5**	**6**
G1	2.55	1.8	0.39	0.43	0.84	0.82
G2	2.45	1.8	0.38	0.43	0.86	0.76
G3	2.86	2.8	0.29	0.5	0.98	0.96
G4	3.15	2.54	0.44	0.67	1.13	1.22
G5	3.5	2.8	0.4	0.59	0.98	1.03
Mean	2.902	2.347	0.379	0.522	0.956	0.957
Grand Mean	2.625		0.451		0.957	

GC Assay Method (Malt-13) for Detecting Deoxynivalenol (ppm) in Malt

	Sample Pair		**Sample Pair**		**Sample Pair**	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
G1	0.26	0.33	0.65	0.52	0.96	0.76
G2	0.25	0.32	0.59	0.54	0.97	0.75
G3	0.18	0.22	0.32	0.52	0.91	0.64
G4	0.15	0.22	0.4	0.36	0.93	0.64
G5	0.18	0.27	0.39	0.35	0.93	0.7
Mean	0.201	0.269	0.469	0.457	0.939	0.696
Grand Mean	0.385		0.709		1.704	

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(3) Testing of subsamples of grain lots from the Mostrom Lab in each GC/MS run with low, medium, and high DON (to test for consistency among GC/MS runs). Samples were wheat pool (WP), barley pool (BP), and corn pool (CP). The expected range determined by the Mostrom Lab was: 0.8 to 1.3ppm (mean 1.0 ppm) for WP, 2.5 to 3.2 ppm (mean 2.9 ppm) for BP, and 4.0 to 5.2 ppm (mean 4.6 ppm) for CP. Our data is reported as the mean with errors bars +/- the standard deviation of data from 68 runs.