Project FY22-FS-001: Diagnostic Testing Services for Deoxynivalenol in the Eastern U.S.

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1. **What are the major goals and objectives of the research 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 or objectives?** *(For each major goal/objective, address these three items below.)*

   What were the major activities?
   In FY24, DON data was generated for 4,719 wheat and barley samples during the performance period, with an additional 1,150 samples pending analysis from the following USWBSI investigators: Betts (230), Bowen (31), Boyles (510), Darby (96), Glover (1,075 total; 665 remaining), Higgins (172), Santantonio (1,717 total; 485 remaining), Schmale (250), Vaillancourt (366), and Wegulo (272). These counts exclude controls, checks, and re-runs. Most samples were 100g kernel lots collected from FHB field trials, though some smaller lots originated from laboratory experiments. Sample extraction, clean-up, and DON quantification followed standard protocols using gas chromatography-mass spectrometry (GC/MS). PI David Schmale, research associate Niki McMaster, and graduate student Lola McMullan attended the 2024 USWBSI meeting in Austin, Texas.

   What were the 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.

   List key outcomes or other achievements.
   The research has contributed to the development and release of new FHB-resistant wheat and barley varieties and has 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 research associate Niki McMaster.

3. **What opportunities for training and professional development has the project provided?**
   Research associate Niki McMaster continued to improve her analytical skills in mycotoxin detection and quantification. Lola McMullan successfully defended her M.S. in SPES on May 9, 2025, co-advised by Drs. Schmale and Bargmann. Lola's thesis addressed one of the major challenges of modern agriculture: the need for earlier, more reliable detection of plant pathogens in the face of climate change and globalized trade. Her work explored an unconventional but exciting possibility: using engineered Arabidopsis thaliana callus tissue (masses of undifferentiated plant cells) as compact, living biosensors. She assessed whether these calli could be infected with the fungus *Fusarium graminearum*, and if they could reflect pathogen presence through visual and chemical signals, including a striking red

pigmentation system controlled by the pathogen-responsive PR-1 promoter. Her research was both elegant and practical. She quantified fungal disease in callus and whole plants using a Fusarium-Arabidopsis Disease (FAD) rating system, measured mycotoxin and fungal biomass levels, and engineered a molecular detection system that is readable by the naked eye. Her work laid the groundwork for portable biosensor technologies that could provide farmers with early warning systems, tools with the potential to significantly enhance agricultural resilience and disease preparedness.

4. **How have the results been disseminated to communities of interest?**

   McMaster communicated with USWBSI stakeholders via phone and email to coordinate sample collection, processing, and testing. Results were disseminated to stakeholders at the 2024 USWBSI meeting in Austin, Texas.

5. **What do you plan to do during the next reporting period to accomplish the goals and objectives?**

   DON testing services continue to be coordinated, supported, and managed by research associate Niki McMaster. FY25 samples are in the process of being received, logged, and processed for analysis. Quality control data continue to be 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).

Annual DON Lab Report of Sample Numbers

Please complete the following required data points for your DON lab analysis that took place during May 1, 2024 – April 30, 2025.

Detailed DON Lab PI Sample Result Breakdown

(additional rows can be added as needed)

Principal Investigator	**Grain Class** (*Indicate sample type: BAR, HRSW, HWW, SWW, DUR)	**Institution**	**Allotment FY Budgeted #**	**# Samples Analyzed in FY**	**# Samples Ground by DON Lab**
Betts, Alyssa Kohler	SWW, BAR	U Vermont	135	230	0
Bowen, Kira		Auburn	150	31	0
Boyles, Richard	SWW	Clemson	633	510	0
Darby, Heather	BAR	U Delaware	150	96	0
Glover, Karl	HRSW	South Dakota State U	1,250	1075 665 remain	0
Higgins, Doug	SWW	VA Tech	132	172	0
Santantonio, Nicholas	SWW, HWW, BAR	VA Tech	2,750	1,717 485 remain	0
Schmale, David	SWW	VA Tech		250	250
Toomajian, Christopher		Kansas State U	450	0	0
Vaillancourt, Lisa	SWW	U Kentucky	300	366	0
Wegulo, Stephen		U Nebraska-Linc	150	272	0

*BAR=Barley, HRSW=Hard Red Spring Wheat, HWW=Hard Winter Wheat, SWW=Soft Winter Wheat, DUR= Durum

TOTALS	**# Analyzed**
Total # Overall of Samples Analyzed	4,719 1,150 remain
Total # of Regular Grain Samples (4-100g)	5,253
Total # of Small Grain Samples (<4g)	366
Total # of Specialty Samples, please specify: Arabidopsis	250
Total # of Specialty Samples, please specify:	
Total # of Specialty Samples, please specify:	

Target Toxins Analyzed

Check all analyzed:	**Toxin**	**# Analyzed**
☒	DON	4,719 1,150 remain
☐	DON3G	
☒	15-Acetyl-DON	596
☒	3-Acetyl-DON	366
☒	Nivalenol	366
☐	Zearalenone	
☐	NX3	
☐	Other, please specify:	

Annual DON Quality Control Data

Internal lab quality control data (separate QC from Trilogy)

	Check 1	**Check 2 (optional)**	**Check 3 (optional)**
N[a]	261	43	
Mean (ppm)	4.06 ppm	0.91	
SD[b]	0.408	0.12	
%CV[c]	0.100	0.13	

[a] Number of check samples. [b] Standard deviation. [c] Coefficient of variance

Return your completed report along with your annual Performance Report submission. Please direct any questions to the USWBSI Networking and Facilitation Office (NFO) at nfo@scabusa.org. Thank you!