USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY20 Annual Performance Progress Report
Due date: July 29, 2021

Cover Page

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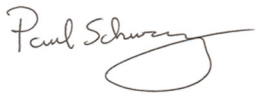
Fiscal Year:	2020
USDA-ARS Agreement ID:	59-0206-0-124
USDA-ARS Agreement Title:	Evaluation of Barley and Malt for DON and Deoxynivalenol-3-Glucoside
FY20 USDA-ARS Award Amount:	$ 287,218
Recipient Organization:	North Dakota State University Office of Grant & Contract Accouting NDSU Dept 3130, PO Box 6050 Fargo, ND 58108-0650
DUNS Number:	80-388-2299
EIN:	45-6002439
Recipient Identifying Number or Account Number:	FAR0031932
Project/Grant Reporting Period:	5/5/20 - 5/4/21
Reporting Period End Date:	5/4/2021

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
FST-S	Evaluation of Barley and Malt for Deoxynivalenol	$ 264,384
PBG	Localization of Fungi and Toxin Production within FHB Infected Grains	$ 22,834
	FY20 Total ARS Award Amount	**$ 287,218**



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Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R- Research
S – Service (DON Testing Labs)
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: *Evaluation of Barley and Malt for Deoxynivalenol*

1. **What are the major goals and objectives of the research project?**

   The goal of this project is to provide barley breeders, pathologists, and other researchers working on the development of Fusarium resistant barley, with affordable, accurate and timely DON analysis.

2. **What was accomplished under these goals or objectives?** *(For each major goal/objective, address these three items below.)*

   a) **What were the major activities?**

   Analysis of barley samples (n=13,606 samples plus 1,232 checks). Samples were submitted by nineteen scientists representing nine institutions/organizations. This was an increase in the number of cooperators over past years, and occurred because of reallocation of samples from the U of MN laboratory. There were some issues with samples from mult-researcher projects, not being submitted by the PI. This issue has largely been clarified . Total samples analyzed 14,838 was not much different then the estimate of the original grant (13,972)

   Horsley and Baldwin submitted significantly more samples than allotted. Klos was significantly under allotment, but this was due to some samples from this project being submitted by other project collaborators (e.g Sorrells). Although not shown here actuall sample numbers for Klos project was very close to the allotment

DON Analysis 2020-21

Collaborator	Allotted	samples	completed	Under/Over
Tom Baldwin-NDSU	1000	1725	1725	725
Venkata Chapara-NDSU	200	76	76	-124
Alyssa Collins-Penn St.	258	144	144	-114
Christina Cowger-USDA	182	182	182	0
Frankie Crutcher-Mont. St.	500	219	219	-281
Eastern Malting-NDSU (Horsley)	250	389	389	139
Shana Forster-NDSU	50	6	6	-44
Andrew Friskop-NDSU	72	237	237	165
Rich Horsley-NDSU	2000	3637	3637	1637
Gongshe Hu-USDA	500	270	270	-230

Collaborator	Allotted	samples	completed	Under/Over
Kathy Klos-USDA	3000	1950	1950	-1050
Joel Ransom-NDSU	100	0	0	-100
Jiajia Rao-NDSU	150	395	395	245
Kathy Satterfield-USDA		170	170	170
Blaine Schatz-NDSU Carrington	80	0	0	-80
Paul Schwarz-NDSU	500	189	189	-311
Jamie Sherman-Mont. St.		0	0	0
Kevin Smith-UofM	1500	1424	1424	-76
Mark Sorrells-Cornell	700	644	644	-56
Marie Timmerman-BARI	2000	1949	1949	-51
Total	13042	13606	13606	564
Standard Curves and Checks	930	1232	1232	302
Grand Total	**13972**	**14838**	**14838**	**866**

b) What were the significant results?

Completion of analyzing submitted samples largely within the reporting period.

c) List key outcomes or other achievements.

Improvement in intra-lab QC, as evidenced by lower check sample coeffecients of variation.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

The laboratory was open and processing samples during the entire reporting period. The main impacts of COVID-19 were reduced availability of student labor (for grinding of samples), and issues with supplies frequently being back-ordered. Another impact was the 4-5 month delayed install on the Agilent triple quadrapole mass spectrometer. Agilent has still not completed the on- site training.

4. What opportunities for training and professional development has the project provided?

NA

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

Results have been sent directly to investigators. Lab protocols and allotments are posted on the USWBSI website.

Project 2: *Localization of Fungi and Toxin Production within FHB Infected Grains*

1. **What are the major goals and objectives of the research project?**

   The overall goal of this project is to localize mucelium within grain kernels with advanced microscopy techniques and associate it with deoxynivalenol (DON) production of Fusarium Head Blight (FHB) infected barley and wheat. Specific objecties are to (1) evaluate the distribution of DON concentration on single kernels of grain and malt, (2) determine the physical localization of Fusarium within kernels, (3) investigate Fusarium viability and growth, and mycotoxin production during malting as influenced by infection parameters and grain storage.

2. **What was accomplished under these goals or objectives?** *(For each major goal/objective, address these three items below.)*

   a) **What were the major activities?**

   To achieve the major goal, the project has been conducted in phases. In the first phase, barley and wheat samples with different infection timings were prepared by simulating early and late infection in nurseries, and harvesting at early and late dates (before and after a heavy rain). In the second phase, grain samples were malted following different storage time periods. In the third phase, the production of DON was measured in barley and wheat grain and malt. In the forth phase, the distribution of DON concentration was investigated on single kernels of grain and malt, and associated with the localization of Fusarium within the same kernels.

   For the objective (1), the following activities were conducted. First, the kernel DON content was investigated on single kernels of barley and wheat grains (46 kernels for each sample). Then, the grains were malted immediately after harvest and also after another six months storage at 20 ℃ and 4 ℃. Third, DON levels were measured in the bulk malt samples and in the malt kernels. For the objective (2), the physical localization of Fusarium and DON levels were investigated on the same kernels of grain and malt samples.

   For the objective (3), we are preparing for the Tri5 DNA levels using qPCR to reflect Fusarium viability and growth during malting.

b) What were the significant results?

On the major goal, Fusarium infection timing and grain harvest timing were fount to have significant effects on the DON production in bulk grain and malt. Early infection and and late harvest result in dramatically high malt DON levels. For objective (1), wide distribution of kernel DON levels were observed in each grain and malt sample. For objective (2), the localization of fungal hyphae was found in the external tissues such as husk, pericarp, and testa of barley in low DON kernels of barley (<1.0 μg/g). Infection in was presentinternal tissues such as pericarp cavities, endosperm, and embryo with the high DON kernels (>100 μg/g). Fungal hyphae were found in the interior of wheat kernels even in low kernel DON levels.

For objective (3), *Fusarium* growth during malting would be investigated by qPCR, instead of RNA Scope because because technical problems in the sample preparation.

c) List key outcomes or other achievements.
No outcomes for now, but we are achieving the goal with the conduction of the research work and gain outcomes

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

With COVID-19 restrictions, the researcher was not able get access to the laboratory for PCR and alsothe microscopy lab for several months. This was due to the temporary entry restrictions and to the lack of support technicians.

4. What opportunities for training and professional development has the project provided?

A post-doctoral research fellow has been provided with opportunities to initiate the proposal, prepare samples, conduct experiments, write papers, deliver presentations to the barley and malt growers, plant pathologists, end users (malters and brewers).

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

The results have been disseminated by delivering posters and presentations at conferences and webinars, and publishing research articles on professional journals.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (5/5/20 - 5/4/21). 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 FY20 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY20 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

3. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

4. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

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 FY20 award period (5/5/20 - 5/4/21). All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

NOTE: 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	FHB Rating (0-9)	Year Released
N/A	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
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Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year

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

Publications, Conference Papers, and Presentations

Instructions: Refer to the PR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY20 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (5/5/20 - 5/4/21**) should be included. If you did not publish/submit or present anything, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each citation, you **must** indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in the publication/ presentation. See example below for a poster presentation with an abstract:

> Winn, Z.J., Acharya, R., Lyerly, J., Brown-Guedira, G., Cowger, C., Griffey, C., Fitzgerald, J., Mason R.E., and Murphy, J.P. (2020, Dec 7-11). Mapping of Fusarium Head Blight Resistance in NC13-20076 Soft Red Winter Wheat (p. 12). In: Canty, S., Hoffstetter, A. and Dill-Macky, R. (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum*. https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
> Status: Abstract Published and Poster Presented
> Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

Z. Jin, S. Solanki, G. Ameen, T. Gross, R.s. Poudel, P. Borowicz, R.S. Brueggeman, and P. Schwarz. "Expansion of internal hyphal growth in Fusarium Head Blight infected grains contributes to the elevated mycotoxin production during the malting process." Molecular Plant – Microbe Interactions. 2021, Online: https://apsjournals.apsnet.org/doi/10.1094/MPMI-01-21-0024-R
Status: in proof
Achnowledgement of Federal Support: Yes

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

None.

Other publications, conference papers and presentations.

Posters and Presentations

Z. Jin and P. Schwarz. 2020. "Effects of Fusarium infection timing on the production of deoxynivalenol in barley grain and malt" Processing of the 2020 National Fusarium Head

Blight Forum (p. 58.), virtual; December 7-11. Online: https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf
Status: Abstract published and Poster presented
Achnlowledgment of Federal Support: Abstract (Yes), Poster (Yes)

Z. Jin and P. Schwarz. 2021. "Characterization of trichothecene mycotoxin development during the malting of Fusarium infected barley and other grains." American Society of Brewing Chemists Webinar. Invited speaking, Virtual; March 4. Online: https://www.asbcnet.org/lab/webinars/webinars/Pages/trichothecene-mycotoxin.aspx
Status: Webinar summary published and presentation delivered
Acknowledgment of Federal Support: Webinar summary (No), Presentation (Yes)

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FY20 PR – USWBSI ADDENDUM
DON Service Labs – Quality Control (QC) Data

Note: What is being requested is the lab's quality control (i.e. check) data.

Insert below Lab's Quality Control Data/Results from the FY20 Award Period (5/5/20 - 5/4/21):

Internal Lab Checks (Intra-Lab)

A total of 1232 check samples were run over the award period. These included twelve samples of either malt, barley, or corn. The check samples were selected to represent low, medium, and high DON ranges, as well as various matrices. Data is shown in Tables 1 and II and is from a total of 4 detectors (4 ECD and 1 MS).

It is notable that overall coefficients of variance (cv) observed during this period were considerably lower than for 2019-20. For example, the cv for malt check #7 was 10.3%, compared to 16.1% in the previous reporting period. The cv for barley check #17 was 5.2%, compared to 31% in the previous reporting period The reduction in cv's, in part, reflects improvements in general quality control practices.

Table I. Quality Control Data on Malt DON Internal Checks

	DON results					
DON level	Low	Middle	Middle	High	High	High
Sample	malt	malt	malt	malt	malt	malt
ID#	20	7	31	15	29	32
average	**1.93**	**3.99**	**10.32**	**17.36**	**16.80**	**26.06**
Std dev	0.21	0.41	0.97	2.13	1.83	3.00
cv	**10.94**	**10.31**	**9.40**	**12.29**	**10.91**	**11.50**
Min	1.71	3.58	9.35	15.22	14.97	23.07
Max	2.14	4.40	11.29	19.49	18.63	29.06
N	84	129	108	117	105	107

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Table II. Quality Control Data on Barley and Corn DON Internal Checks

	DON Results					
DON level	Clean	Low	Low	High	High	Middle
Sample	barley	barley	barley	barley	barley	corn
ID#	22	12	17	44/45	mix	corn
average	**0.02**	**2.12**	**0.55**	**20.26**	**23.48**	**4.29**
Std dev	0.03	0.22	0.03	2.75	3.22	0.64
cv		**10.56**	**5.21**	**13.56**	**13.71**	**14.96**
Min	-0.02	1.90	0.52	17.52	20.26	3.65
Max	0.05	2.35	0.58	23.01	26.70	4.94
N	70	126	22	122	148	94

Trilogy Inter-Lab Checks

Trilogy check samples are sent to each of the four labs each month from August to May. The monthly checks include a low, medium, and high DON sample. These serve as a means of assessing between lab variation. Comparative plots of each labs' results for the low, medium, and high samples are shown in Figures 1 to 3. For the low DON checks (Figure 1) the standard deviation across the labs was generally quite low (0.05 - 0.29). Months with higher variation were not consistently due to any specific laboratory.

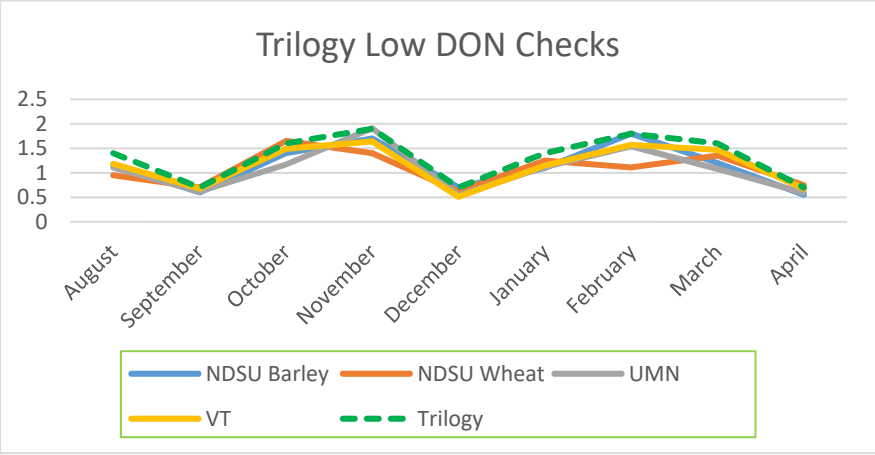

Figure 1. Trilogy low DON checks across the four laboratories (August 20202 – May 2021)

The standard deviation for the medium DON checks (across labs) (Figure 2) ranged from 0.07 to 1.03. Again, no trend was observed as to a specific lab, consistently running high or low.

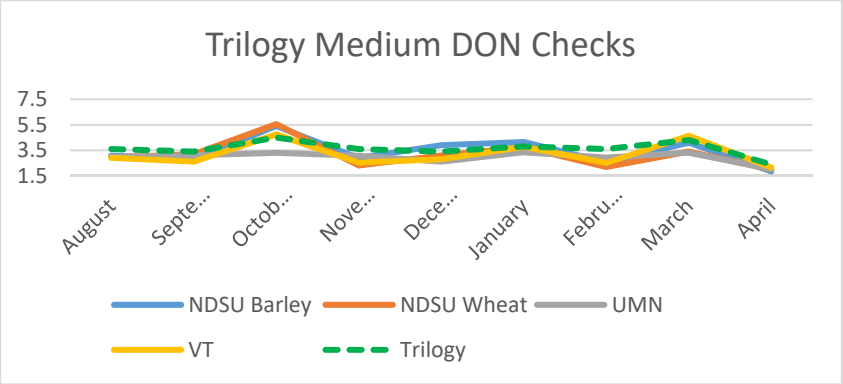

Figure 2. Trilogy medium DON checks across the four laboratories (August 20202 – May 2021)

The two NDSU labs had the highest results for seven of the nine months, suggesting these labs trend slightly higher on these samples. This is perhaps due to construction of the standard curve, as both labs use the same protocol.

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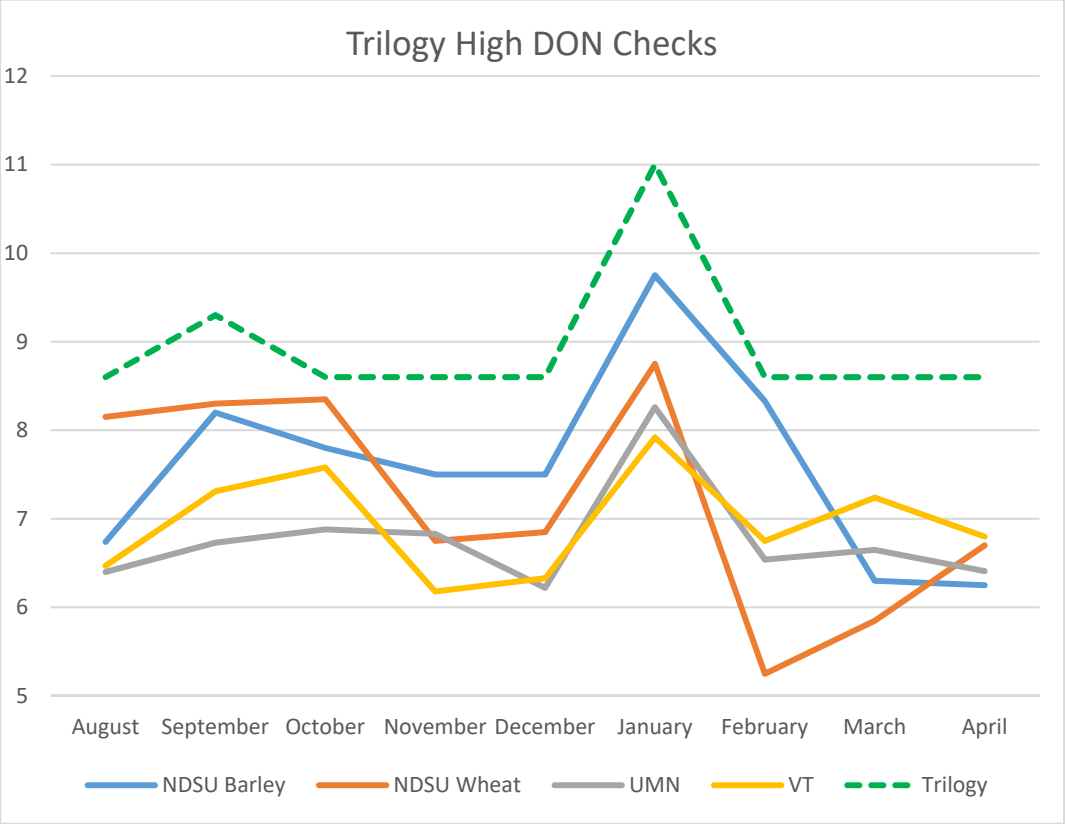

Figure 3. Trilogy high DON checks across the four laboratories (August 20202 – May 2021)

The Trilogy check samples show that while there are differences between laboratories, these are not greatly different than normal intra-lab standard deviations. However, results emphasize the important of having all samples from a particular experiment run within the same lab and same timeframe.