USDA-ARS | U.S. Wheat and Barley Scab Initiative

FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

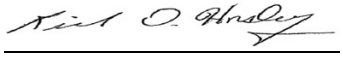
Principle Investigator (PI):	Richard Horsley
Institution:	North Dakota State University
E-mail:	richard.horsley@ndsu.edu
Phone:	701-231-8142
Fiscal Year:	2021
USDA-ARS Agreement ID:	59-0206-0-124
USDA-ARS Agreement Title:	Evaluation of Barley and Malt for DON and Deoxynivalenol-3-Glucoside
FY20 USDA-ARS Award Amount:	$274,063
Recipient Organization:	North Dakota State University Department of Plant Sciences NDSU Dept # 7670, PO Box 6050 Fargo, ND 58108-6050
DUNS Number:	80-388-2299
EIN:	45-6002439
Recipient Identifying Number or Account Number, if any:	FAR0031932
Project/Grant Period:	5/5/21 - 5/4/23
Reporting Period End Date:	5/4/2022

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
FST-S	Evaluation of Barley and Malt for Deoxynivalenol	$251,229
PBG	Localization of Fungi and Toxin Production within FHB Infected Grains	$22,834
	FY21 Total ARS Award Amount	$274,063

I am submitting this report as an: ☒ Annual Report ☐ Final Report

I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.



Principal Investigator Signature

7/22/2022

Date Report Submitted

‡ BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
EC-HQ – Executive Committee-Headquarters
FST-R – Food Safety & Toxicology (Research)
FST-S – Food Safety & Toxicology (Service)
GDER – Gene Discovery & Engineering Resistance
HWW-CP – Hard Winter Wheat Coordinated Project
MGMT – FHB Management
MGMT-IM – FHB Management – Integrated Management Coordinated Project
PBG – Pathogen Biology & Genetics
TSCI – Transformational Science
VDHR – Variety Development & Uniform Nurseries
NWW –Northern Soft Winter Wheat Region
SPR – Spring 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=10,026 samples plus 937 checks). Samples were submitted by 28 scientists representing 11 institutions/organizations. This was an increase in the number of cooperators over past years. The total number of samples analyzed 10,063 was less than the estimate of the original grant (16,006). The reduced number of samples analyzed occurred because disease levels in the FHB nurseries were either low or non-existent due to the severe drought in the northern Great Plains.

   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 the intra-lab QC, as evidenced by lower check sample coefficients of variation.

3. **What opportunities for training and professional development has the project provided?**
   One undergraduate assisted in the laboratory with the testing. The undergraduate student has learned basic laboratory skills and laboratory quality control.

4. **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

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

The overall goal of the project is to address causes of deoxynivalenol (DON) increase during the malting of barley and wheat, which occurs occasionally, but of considerable importance in both economic and food safety terms. Practical experience has been shown that Fusarium Head Blight (FHB) infected barley with low DON levels (e. g. <0.5 mg/kg) can be used in malting as DON declines during steeping and remains low on malt. However, a dilemma is that germination of low DON grains sometimes results in malt with higher DON levels. This behavior was last extensively observed with commercial grains, including barley, wheat, rye, and triticale from the upper Midwestern USA and sometimes from the Prairie provinces of Canada.

Objectives of the project are to investigate the causes of the differential production of DON and other mycotoxins that are observed during the malting of FHB infected barley and wheat grains. Factors to be evaluated include:
(1) The distribution of DON concentration on single kernels of grain and malt;
(2) The physical localization of *Fusarium* within the kernel;
(3) 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?

Wheat grains infected with *Fusarium graminearum* were obtained from North Dakota State University research nurseries. The *Fusarium* Damaged Kernels (FDKs) and healthy kernels were separated before malting. With barley, the *F. graminearum* infection at early and late timing (i.e. before and after anthesis, respectively) were simulated by using a grain spawn method. The control referred to uninoculated barley spikes protected from the exposure of *Fusarium* spores by bagging when growing in the field. The infected barley and wheat grain samples were malted twice after the storage for three and nine months. To evaluate each factor of the objective, the specific activities were carried out as below.

(1) The distribution of DON concentration on single kernels of grain and malt;
Forty-six single kernels were randomly taken out from each grain and malt sample and measured for DON content using GC-MS.

(2) The physical localization of *Fusarium* within the kernel;
Ten to twenty single kernels of each sample were examined under confocal laser scanning microscope and scanning electronic microscope to observe the hyphae

localization. DON concentration was detected in the same kernel that was laterally sectioned into three parts.
(3) *Fusarium* viability, and growth, and mycotoxin production during malting as influenced by infection parameters and grain storage.

The wheat (i.e. FDKs, healthy kernels, and the unseparated grain sample) and barley samples (i.e. barley grains with early and late infection and the control) were malted after two storage time lengths (i.e. three and nine months). DON production was measured on the subsamples taken at the ends of steeping, germination and kilning. ANOVA was used to analyze the DON content changes during malting.

*Note: **The unfinished part of research**, including Fusarium viability and growth and effects of the storage time lengths (at the interval of six months), is in the implementation in a barley plant pathology laboratory at NDSU. The measurement of DON contents in the single kernels of wheat grain and malt samples is in the progress.*

(b) What were the significant results?

In terms of the localization of hyphae and DON production during the malting of ***F.graminearum*** **infected barley**, the significant results include:

(1) Effects of infection timing on the production of DON during the malting of barley

DON contents were lowest in the control (0.30±0.28 μg/g) and late infection samples (3.26±2.34 μg/g). The contents were significantly (p<0.05) higher in both early infection sample (26.71±4.86 μg/g) and that with late harvest sample (27.20±1.57 μg/g). Following the malting of barley at a three-months storage, the malt DON content of control sample was below 0.20 μg/g. The malt DON content of late infection sample was 39% on average lower than that of the unmalted samples significantly (p<0.05). However, the malt DON of early infection and that with late harvest increased by 7% insignificantly (p>0.05) and 53% significantly (p<0.05) on average of that observed in the unmalted barley, respectively.

(2) Distribution of DON levels between kernels of barley grain and malt samples

In the control and late infection samples, more than 90% of barley kernels had DON < 1.0 μg/g. However, in the early infection samples, only 72% of kernels had DON <1.0 μg/g, and 6% of kernels had DON levels > 100 μg/g. The ratio of control kernels with DON < 1.0 μg/g didn't change following malting, but the late infection kernels with DON > 1.0 μg/g increased from 10% to 22%. With early infection samples, the ratio of kernels with >1.0 μg DON/g increased from 28% to 72% following malting.

(3) Localization of fungal hyphae within barley grain and malt kernels

Fungal hyphae were mostly observed in the husk tissues of kernels with DON < 1.0 μg/g, which was named as "external infection". Hyphae were also observed in the aleurone layer, endosperm, and embryo tissues of kernels with DON > 10 μg/g, which

was named as “internal infection”. The “internal infection” was observed in the interior tissues of steeping-out kernels. The hyphae grew much heavier in the interior tissues of the high DON malt kernels (e.g. >100 µg/g).

In terms of ***F.graminearum* infected wheat**, the significant results include:

(4) The production of DON in the malting of wheat FDKs and healthy kernels

The contents of DON in the *F. graminearum* infected wheat grain and malt were 4.22±0.55 µg/g and 18.55±4.07 µg/g, respectively. With the FDKs, the contents of DON were 8.26±1.48 µg/g and 41.11±6.31 µg/g in the grain and malt, respectively. With the healthy kernels, the contents of DON were 0.64±0.26 µg/g and 0.96±0.28 µg/g in the grain and malt, respectively. The results indicated that FDKs were responsible for the growth of *Fusarium* and mycotoxin production during malting.

(5) Localization of fungal hyphae within wheat grain and malt kernels

FDKs of wheat and malt were infected in the interior tissues, such as aleurone layer, endosperm and embryo (i.e. “internal infection”), with DON contents as high as above 300 µg/g. In contrast, the hyphae mainly observed in the out layers including pericarp and testa (i.e. “external infection”) of healthy kernels, where *Fusarium* didn’t grow considerably neither produce mycotoxins during malting.

(c) List key outcomes or other achievements.

This piece of research achieved two key outcomes. (i) It clarified the early infection of *Fusarium* on barley when growing in the field, i.e. during anthesis, caused the higher percentage of kernels with the “internal infection” and higher DON contents in the barley grain and malt than that in the later infection, i.e. after anthesis and filling. (ii) FDKs of wheat were characterized with the “internal infection” and responsible for the production of malt DON during malting. The effects of *Fusarium* infection timing on the hyphal localization and mycotoxin production during the malting of grains were not reported previously.

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

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

The results have been disseminated to **barley and wheat breeders and pathologists** by Dr. Zhao Jin delivering a poster presentation at National Fusarium Head Blight Forum (virtual), with the title of “Effects of *Fusarium* infection timing on the production of deoxynivalenol in barley grain and malt”. Dec. 7-11, 2020.

The results have been disseminated to **malting and brewing communities** by Dr. Zhao Jin delivering an invited presentation, with the title of “Characterization of Trichothecene Mycotoxin Development during the Malting of Fusarium Infected Barley and Other Grains” at a webinar of American Society of Brewing Chemists. March 4, 2021.

Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your FHB work that were a result of funding from your FY21 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period** should be included.

Did you publish/submit or present anything during this award period?

☒ Yes, I've included the citation reference in listing(s) below.
☐ No, I have nothing to report.

Journal publications as a result of FY21 grant award

List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like.

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published [include DOI#]; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Jin, Z., Solanki, S., Ameen, G., Gross, T., Poudel, R.S., Borowicz, P., Brueggeman, R.S. and Schwarz, P., 2021. 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*, 34(7), pp.793-802. https://doi.org/10.1094/MPMI-01-21-0024-R, acknowledgment of federal support - yes.

Books or other non-periodical, one-time publications as a result of FY21 grant award

Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (book, thesis or dissertation, other); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Other publications, conference papers and presentations as a result of FY21 grant award

Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication.

[1] Oral presentation: Zhao Jin. "Characterization of Trichothecene Mycotoxin Development during the Malting of Fusarium Infected Barley and Other Grains." American Society of Brewing Chemists Webinar, March 4, 2021. (*invited*)
[2] Poster and Flash/Dash presentation: Zhao Jin. "Effects of Fusarium infection timing on the production of deoxynivalenol in barley grain and malt". At the Virtual Forum of National Fusarium Head Blight Forum. Dec. 7-11, 2020.
[3] Oral Presentation: "Fungal localization associated with mycotoxin production during the malting of Fusarium infected grains." at the Virtual Conference of Institute of Food Technologists, July 12-15, 2020.

FY21– USWBSI ADDENDUM

DON Service Labs – Quality Control (QC) Data

Note: What is being requested is the across lab quality control data (separate QC from Trilogy).

Project 1: Evaluation of Barley and Malt for Deoxynivalenol

Insert below Quality Control Data/Results from the FY21 Award Period (5/5/21 - 5/4/22):

QC SAMPLES	LOW PPM	HIGH PPM
AVERAGE	0.21	25.13
STD DEV	0.07	3.77
CV	18.50	14.89
LOW	0.00	22.82
HIGH	0.60	31.50
NUMBER	130	155