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

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-2-146
USDA-ARS Agreement Title:	DON and Fusarium Head Blight (FHB) Resistance in North American Winter Barleys
Principle Investigator (PI):	Eric J. Stockinger
Institution:	The Ohio State University
Institution UEI:	DLWBSLWAJWR1
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$69,709
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Period of Performance:	May 1, 2022 – April 30, 2026
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Developing Fusarium Head Blight-resistant North American Winter Barleys	$69,709
	FY22 Total ARS Award Amount	$69,709

I am submitting this report as an: ☒ Annual 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.

Eric Stockinger
Principal Investigator Signature

July 23, 2023
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: Developing Fusarium Head Blight-resistant North American Winter Barleys

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1. **What are the major goals and objectives of the research project?**
   The overall goals of this project are to enhance and increase the number of winter barley varieties developed by U.S. public breeding programs that possess resistance to head blight disease caused by Fusarium graminarum. The specific objectives of this project are to: 1) coordinate a North American Barley Scab Evaluation Nursery (NABSEN) for winter barley, in which North American winter barley breeders submit their best lines for testing, 2) identify lines in the Ohio breeding program exhibiting Fusarium Head Blight (FHB) resistance in the forms of low Deoxynivalenol (DON) accumulation and low disease incidence, and 3) utilize modern breeding technologies to efficiently and rapidly introgress those resistances into elite lines for varietal release to farmers.

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?
   Objective 1, the winter NABSEN
   Six U.S. public breeders contributed lines for the 2021–22 season. A total of 37 lines from the contributors were tested. Eight checks vetted by the nursery participants were included. The nursery was planted at eight locations, which includes Wooster Ohio. Seven of the nurseries, including the Ohio nursery, screened lines using Fusarium inoculation combined with overhead mist irrigation. The eighth used natural infection only.

   For the Wooster location, changes were made to the nursery layout the 2021–22 season, including: (1) use of wheat as a spacer between rows of barley to reduce or altogether eliminate tangling of different barley lines as a consequence of lodging. The use of wheat also enabled us to move through the plots faster to harvest the material, i.e., it reduced the person-hours required for harvest from one week to a few days, which is expected to reduce variation in DON levels that might occur as a consequence of harvest date variation. A 2nd major change included: (2) the spatial separation of barley lines in which lodging susceptible lines were grouped together and separated spatially, from lodging-resistant lines. This change minimized lodging of lodging-resistant lines caused by a lodging-susceptible line falling into the plot of the lodging-resistant line. A 3rd key change included: (3) the timing we used for inoculating the nursery with corn spawn. This change was made possible through identifying early and late heading lines the previous season so that we could time placement of the corn spawn inoculum in conjunction with the overhead mist irrigation, which insured spore release (corn spawn was black) was occurring throughout anthesis, for all lines. A 4th major change included: (4) the use of taller irrigation risers that lessened the direct horizontal impact to standing plants and minimized the amount of water standing on the soil surface, which in turn reduced soil saturation. A 5th major change included: (5) the addition of border plots to eliminate edge effects. In this way we were better able to screen a wide and diverse collection of barley germplasm without compromising the data quality.

For the Wooster location each NABSEN line was planted in three reps. To obtain an index of disease severity, each rep of every line was independently rated by three different individuals for visual symptoms using a percentage scale, with 5% increments. The three independent disease severity scores were then compiled to obtain a mean index rating for each line. Mean indexes of the 2021–22 scab nursery were then compared to the mean indexes of the 2020–21 scab nursery. At maturity, the reps were hand-harvested. Each rep was then threshed in a stationary lab thresher to obtain clean seed for quantification of DON levels. Threshed material was then ground to a fine powder using a burr mill type coffee grinder. Each reps of the NABSEN set was individually ground. The target quantity of sample used for DON analysis was 100 g.

Objective 2, identifying lines in the Ohio breeding program exhibiting FHB resistance.
We assessed 378 lines the 2021–22 field season. Each line was planted in three reps, which were independently rated for disease index by two different individuals. Each rep was hand-harvested, and an equal portion of each rep was combined to create a composite sample that was then threshed in a stationary lab thresher to obtain clean seed for quantification of DON levels. The composite was then ground to a fine powder using a burr mill type coffee grinder. The target quantity of sample used for DON analysis was 100 g; just under 50% of the lines met this minimum. DON data for the 2021–22 samples was returned to PI Stockinger January 19, 2023.

Objective 3, utilizing modern breeding technologies to breed for FHB resistance.
Each of the Ohio lines being phenotyped in the nursery was genotyped using the Barley 50k iSelect SNP Array, while the Bregitzer population recombinants were previously genotyped with the 9k Illumina Infinium iSelect Custom Genotyping BeadChip. The next step at the genetic level is to begin testing for association between markers and the DON level phenotypes. At the breeding level, crosses have been made with lines exhibiting low DON levels. Populations derived from these crosses are being advanced.

b) What were the significant results?

Objective 1, the winter NABSEN
A total of 498 different lines grown at the Wooster Ohio location the 2021–22 season were assessed for DON. Values ranged 26 ppm to 285 ppm. The mean was 116 ppm. The lowest value was observed for VA15H-73, one of the low DON accumulator checks used in the nursery. The highest value observed was for Hirondella, one of the high DON accumulator checks used. Six additional genotypes were used as checks. All of the checks were utilized eight independent times within the nursery. The three additional six-row check lines used as checks did not exhibit substantial variation from each other. Each of those was from the Virginia Tech breeding program, from which typically selects for a much earlier maturity date than is ideal for Ohio. Greater variation for DON levels was observed across the other three two-row genotypes used as the checks. Endeavor was at the low end, at 101 ppm, Calypso intermediate at 139 ppm, and Wintmalt at the upper end at 151 ppm. The row type, mean, and standard deviation for these eight checks is provided in the table below. None of these values is presented relative to heading date.

Table 1. DON levels of the check lines tested in Wooster Ohio 2021–22.

Genotype	Control #	Row type	DON ppm (mean) *	DON ppm (std dev)	Low value	High value
VA15H-73	Control #2	2	34	7	26	43
Endeavor	Control #1	2	111	7	98	121
Calypso	Control #3	2	139	20	100	163
Wintmalt	Control #4	2	151	20	132	188
Atlantic	Control #7	6	116	28	78	157
Thoroughbred	Control #5	6	117	26	87	173
Secretariat	Control #8	6	124	22	95	173
Hirondella	Control #6	6	222	30	195	285

* Averages of 24 plots per control, from all trials (8).

Disease index rating scores, DON levels, or both, from six of the winter NABSEN collaborating participants have returned data to PI Stockinger.

The greatest disease pressure was exhibited in the Ohio and Virginia environments. DON values for the Wooster Ohio location ranged from 26 ppm to 285 ppm. Don values for the Blacksburg Virginia location ranged from 6 ppm to 123 ppm. More detailed statistical analyses will be required, but preliminary analysis suggests a correlation between DON levels and disease index in the Ohio nursery, and a correlation between DON levels and disease index in the Virginia nursery. There also appears to be a correlation between the Ohio and Virginia nurseries in the DON levels exhibited by a given line.

Disease pressure at the other nursery locations was much lower. DON levels at the Lincoln Nebraska location ranged 0.1 to 7.2, at the Raleigh North Carolina location 0 to 1.11 ppm, and at the Beltsville Maryland location 0.25 to 10 ppm. The Montana and Idaho locations did not test for DON. Although DON levels were much lower at these other locations, some of the lines exhibiting DON levels at the upper end when grown at these locations also exhibit DON levels at the upper end for the Ohio and Virginia locations. Again, greater statistical analyses is required.

These findings will be reported to the breeders that submitted the lines prior to the 2023–24 winter NABSEN testing season.

Objective 2 and the Bregitzer population.
The Bregitzer population is a set of winter recombinants derived from a 95SR316A × Charles cross. Both parents and 83 recombinants were assessed. DON levels for this set ranged 54–222 ppm. Each recombinant was represented by three replicates in the scab nursery, while DON measurements were made using a composite of equal portions of each rep. The mean DON value was 135 ppm and the standard deviation for each line from that mean was 33 ppm. Charles was represented twice; the values exhibited by the two reps was 123 and 184. 95SR316A was represented once and exhibited 105 ppm. Twelve of the 84 95SR316A × Charles recombinants exhibited values less than 105 ppm, three of which were at least two

standard deviation units lower than 105. Twenty-nine of the recombinants exhibited values greater than 154, the mean of the two Charles entries, three of which were two standard deviation units greater than 154. Two of these three low-end DON accumulator recombinants in the 2021–22 nursery were amongst the 2020–21 nursery low-end DON accumulators, and two of the three high-end DON recombinants in the 2021–22 nursery were amongst the high-end DON recombinants in the 2020–21 nursery.

Objective 2 and the Ohio breeding program germplasm set.
DON values for the Ohio breeding germplasm set ranged 33–225 ppm, with an Ohio selection, B66N74A026, at the low end, and Violetta at the upper end. An independent headrow of VA15H-73 was included once amongst this set. It exhibited 53 ppm DON. Endeavor was included twice amongst this set and exhibited DON values of 136 and 141 ppm. The mean and standard deviation from the mean for this set were 106, and 33 ppm, respectively. Approximately 65 genotypes were two standard deviation units less than the mean of 106 ppm, 76 ppm. Many of the genotypes exhibiting less than 76 ppm DON were also at the low end of the DON spectrum for the 2020–21 nursery and were either the MO B lines that formed the winter-hardy founding parental lines for the Ohio breeding program or were offspring from those lines. Of the 153 lines tested both the 2020–21 and 2021–22 seasons, 29 lines exhibited DON values less than the mean both years. The Pearson's correlation coefficient for DON levels for this set of 153 lines is 0.39.

c) List key outcomes or other achievements.

The high disease pressure, high DON levels, and large range in DON values exhibited by the lines in the Ohio and Virginia nursery environments suggest these environments are an excellent testing ground to screen winter barley lines being considered for cultivation across North America.

Infection and disease pressure in the 2021–22 Ohio scab nursery was very robust.
The low and high DON accumulator check genotypes defined the extremes of DON values for the Ohio nursery, and for the most part, the three reps of each of these two genotypes parsed together. This data indicates that Hirondella clearly provides a robust susceptible check line. Similarly, VA15H-73 provides a robust susceptible check line. However the low DON values for VA15H-73 must be considered in the context that it is a hulless genotype.

Nonetheless, many of the hulled MO B lines and their offspring that are being utilized in the Ohio breeding program exhibited DON values that approached VA15H-73, and thus were substantially lower than the two-row check Endeavor. Similarly, many of the recombinant lines in the Bregitzer population, which are also hulled, exhibited DON values that were substantially lower than Endeavor. Many of these low DON accumulator lines were also at the low end of the DON spectrum for the 2020–21 nursery. Taken together, these data suggest it is possible to develop winter barley lines that possess resistance to FHB in the form of low DON accumulation. And that to do so will require replicated testing across multiple high-pressure environments.

3. What opportunities for training and professional development has the project provided?
This project provided tremendous opportunities for learning and professional development for a technician and three undergraduate summer student researchers. The technician in particular made significant advances in redesigning the scab nursery following the first season. These changes minimized errors and maximized data quality. All changes were initiated by the technician. These changes are described above under “major activities.”

The project also provided the lead technician with the opportunity to lead and manage a team, and to directly interact with the public. This latter opportunity was impromptu and happened because the derecho storm that caused widespread destruction and left roads impassable in the PI’s residential area the night before the small grains field day. For the undergraduate summer student research helpers this project has provided their first employment opportunity. The project has allowed them to experience firsthand what it is like to work as a team in an academic research and teaching environment.

4. How have the results been disseminated to communities of interest?
The results of the 2021–22 scab nursery were presented at the 2022 National Fusarium Head Blight Forum, Tampa FL. December 4–6, 2022. Workings of the Ohio scab nursery were presented to the Ohio wheat, corn, and small grains farmer group attending the Wooster small grains field day, June 14, 2022.
Once the results of the 2021–22 winter NABSEN are more thoroughly analyzed statistically, they will be provided to the breeders who submitted lines to the nursery.

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 FY22 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 May 1, 2022 – April 30, 2023?

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

Journal publications as a result of FY22 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).

None

Books or other non-periodical, one-time publications as a result of FY22 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).

None

Other publications, conference papers and presentations as a result of FY22 award

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

Eggers, Ben and Stockinger, Eric J. (2022). DON and FHB resistance in North American winter barleys. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa FL. December 4–6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf
Work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-0-174, and award number AWD-112740.