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

FY21 FINAL Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-0-174
USDA-ARS Agreement Title:	DON Accumulation and Fusarium Head Blight Resistance in Winter Barley
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Institution UEI:	DLWBSLWAJWR1
Fiscal Year:	2021
FY21 USDA-ARS Award Amount:	$59,961
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Period of Performance:	5/15/21 - 5/14/23
Reporting Period End Date:	5/14/2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Establishing a Winter Barley Fusarium Head Blight Screening Nursery in Ohio	$59,961
	FY21 Total ARS Award Amount	$59,961

I am submitting this report as a: ☒ 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.

____ July 23, 2023

Principal Investigator Signature Date Report Submitted

ǂ 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: Establishing a Winter Barley Fusarium Head Blight Screening Nursery in Ohio

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1. **What are the major goals and objectives of the research project?**
   The overall project goal was to:
   Identify winter barley lines possessing resistance to *Fusarium graminearum* toxin accumulation and incorporate that genetic resistance into modern elite two-row winter malting barley cultivars.

   The specific objectives for this project were to:
   1) Assess lines in the Ohio Winter Malting Barley Breeding Program for resistances to FHB and fungal toxin accumulation.
   2) Assess a mapping population derived from a FHB-resistant parent for resistances to FHB and fungal toxin accumulation.
   3) Establish a uniform winter barley scab nursery consisting of a core set of 20–30 lines, and test this set for resistances to Fusarium Head Blight (FHB) and fungal toxin accumulation.

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?**
   We planted the winter barley scab nursery in Ohio for the first time autumn 2020 and carried out activities for **Objectives 1 and 2**. We planted the Ohio winter barley scab nursery a second time October 12, 2021, expanding **Objectives 1 and 2** activities and carrying out activities to initiate accomplishing **Objective 3**.

   For **Objective 1** we assessed a set of highly-diverse two-row winter barley lines associated with the Ohio breeding program. For **Objective 2** we assessed a population of recombinant lines from the 95SR316A × Charles cross, a population we refer to as the "Bregitzer population" in recognition of its developer, who had preliminary data indicating the population is segregating for FHB resistance. For **Objective 3** we planted 45 lines that comprise the winter North American Barley Scab Evaluation Nursery (NABSEN), a set of lines being tested in scab nurseries across North America. Included in the latter are four two-row and four six-row controls, selected as being the best by the eight participants planting the NABSEN to represent the spectrum, susceptible to resistant, for both visual and DON level resistance level differences. Six North American winter barley breeders submitted their lines for testing in the 2021–22 NABSEN. We are also the coordinator of the NABSEN. Combined, we tested 263 different winter barley lines in Ohio the 2020–21 season, and 498 different winter barley lines the 2021–22 season.

   An overhead irrigation system was put in place prior to heading. Early in canopy development the nursery was inoculated with multiple race isolates of Ohio *F. graminearum* using corn cob "spawn" placed in nursery rows. Heading date of each line was recorded. At the first sign of head emergence and continuing through full head emergence (Zadoks 49-60), heads were spray-inoculated using a backpack sprayer at two-day intervals with asexual spores, also derived from multiple Ohio pathogenic races. The field was overhead irrigated intermittently for 5 minutes every 30 minutes between 5:00 AM and 10:00 AM, and again between 5:00 PM and 10:00 PM throughout anthesis, and for seven days following the latest line completing anthesis.

To obtain an index of disease severity, each rep of every line was independently rated by two individuals the 2020–21 season, and by three individuals the 2021–22 season, for visual symptoms using a percentage scale, with 5% increments. The 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 and bulked to create a composite. The composite was then threshed in a stationary lab thresher to obtain clean seed. Material was then ground to a fine powder using burr mill type coffee grinders and sent to the lab of Yanhong Dong, Department of Plant Pathology, University of Minnesota for DON analyses.

Following the receipt of the DON data in January 2022, crosses were made using low DON accumulating lines as parents.

The Pearson test was then used to test for correlations between DON levels and disease index, DON levels and heading date, and disease index and heading date.

A subset of the lines were genotyped using the Barley 50k iSelect SNP Array.

b) What were the significant results?

2021–22 Ohio scab nursery disease index ratings relative to the 2020–21 nursery

Disease index and DON levels for the set of four two-row and four six-row controls, for the 2020–21 and 2021–22 seasons is presented in Table 1.

Comparison of the mean disease index ratings of the 2021–22 nursery to the 2020–21 indicated a general trend of lower values for the 2021–22 season (Table 1). This trend was observed for the sets of Ohio lines entered into the Ohio scab nursery and tested both years, the check lines supplied by VT, and the check lines used in the NABSEN. The average rating of a subset of the Ohio lines indicated about a 16% lower incidence index overall. Nonetheless, some lines were given disease incidence index scores much lower than this 16%, while a few lines exhibited much higher disease index scores relative to the 2020–21 season.

Comparison of the DON levels of the 2021–22 nursery to the 2020–21 indicated a general trend of much higher values for the 2021–22 nursery over those of the 2020–21 nursery, i.e., DON values were higher for the 2021–22 nursery, despite much lower overall disease index scores. Mean values for four two-row and four six-row controls, for the 2020–21 and 2021–22 seasons is presented in Table 1, along the disease index scores for these two years.

Table 1. Disease index and DON levels of the check lines tested 2020–21 and 2021–22.

Line	Control #	Row type	Disease index 2021	Disease index 2022*	DON 2021†	DON 2022*
VA15H-73	2	2	28	24	18	34
Endeavor	1	2	71	35	58	111
Calypso	3	2	52	40	79	139
Wintmalt	4	2	81	45	90	151
Atlantic	7	6	90	85	31	116
Thoroughbred	5	6	92	68	48	117
Secretariat	8	6	83	85	28	124
Hirondella	6	6	-	57	-	222

* Averages of 24 plots per control, from all trials (8).
† The two-row DON values for 2021 are from a single rep from the first planting date set. The six-row are from a two-rep composite from the two different planting date sets (9/28/2020 and 10/15/2020).

Objective 1, the Ohio breeding program germplasm set.
The 2020–21 season DON values for the six-row lines in the OH set tested in the 2020–21 Ohio scab nursery ranged 21.1 (Wong) to 124.7 ppm ('Mercer'). DON values for the two-row lines in the same set ranged 26.0 (MO B2613) to 106.3 ppm ('Antelope'). The Ohio set was planted September 28, 2020. Within the Ohio set, 'Carstens', a key two-row line that figures prominently in the pedigree of many modern two-row barley lines, exhibited values of 36.8. Ohio selection 2011-725-02, which is the offspring of 'Endeavor' × MO B2549, exhibited intermediate values (43.9 ppm), while 'Endeavor' was substantially higher (58.3 ppm), which was just under the mean of 60.5 ppm for the ~170 highly-diverse two-row winter barley lines tested in the Ohio set.

The 2021–22 season, 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.

Objective 2, the Bregitzer population set.
The 2020–21 season, DON values for the Bregitzer population ranged from 46.5 ppm (95SR316A) to 123.0 ppm DON (12ID60). Within this population set, 95SR316A exhibited the lowest value (46.5 ppm), 12ID60 the highest value (123.0 ppm), and 'Charles' an intermediate value (64.4 ppm).

The 2021–22 season, DON values for the Bregitzer population ranged 54–222 ppm. 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 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.

Using Pearson's test to test for correlation between the different traits measured the two trial years for all lines indicted no correlation between disease index and DON levels (Table 2). A negative association was detected between disease index and heading date the 2020–21 and 2021–22 seasons (-0.41, 2020–21; -0.53, 2021–22), and a positive correlation between DON levels and heading date the 2020–21 season (0.53), but no correlation between DON levels and heading date the 2021–22 season.

Table 2. Pearson correlation coefficients between traits assessed for all lines tested the 2020–21 and 2021–22 seasons.

	2020–21	2021–22
Index vs DON	-0.0884	0.2155
Index vs HD	-0.4140	-0.5344
HD vs DON	0.5315	0.1121

c) List key outcomes or other achievements.

A winter barley scab nursery was established in Ohio the 2020–21 season.

Our experience the first season guided substantial improvements in the design of the scab nursery we used the second season, 2021–22. These improvements were expected to maximize data quality and minimize experimental error.

DON levels detected in the Ohio scab nursery were an order of magnitude greater than the values recorded by others in their nurseries (Cowger, C., Arellano, C., Marshall, D. and Fitzgerald, J.; Managing Fusarium head blight in winter barley with cultivar resistance and fungicide; Plant Disease, 103: 2019; 1858-1864; published [10.1094/PDIS-09-18-1582-RE]).

Many of the MO B lines that form the winter-hardy founding parental lines for the Ohio breeding program and many of the offspring we have generated from crosses between those lines and elite modern two-row malting barley lines, exhibited DON values at the lower end of the spectrum, both the 2020–21 season and the 2021–22 season. This finding supports the notion that low DON accumulating lines already exist within the Ohio breeding germplasm set, and that it should be possible to incorporate that genetic resistance into modern elite two-row winter malting barley cultivars.

An unexpected results was the finding that the two-row malting barley cultivar 'Endeavor' exhibited relatively high levels of DON. This result was unexpected because it was selected by participants of the winter NABSEN for its high levels of resistance to *F. graminearum*, both in terms of visual disease indexes and DON accumulation levels.

The low DON levels observed for 95SR316A the 2020–21 season was consistent with it being the hypothesized source of resistance in the population. However, its much higher DON levels the 2021–22 season, combined with the finding that numerous lines in the population exhibited far lower levels than 95SR316A the 2021–22 season suggest a more complex interplay between the fungus and the plant.

The variability in DON levels between environments and between individual reps of the same line in a single year, particularly for the 83 lines in the Bregitzer population, indicate that additional testing years and testing environments will be required to test for (and ideally identify) associations between DON toxin levels produced in the plant and regions of the barley genome. This variability also suggests it may be better to test each individual rep in the nursery rather than to assess DON toxin levels from a composite sample. Nonetheless, the stronger correlation detected for DON toxin levels in the larger Ohio breeding germplasm set between the 2020–21 and 2021–22 seasons indicate that testing a greater number of lines in the nursery each year are more likely to enable identification of genetic factors associated with toxin levels.

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 after the first season. These changes minimize errors and maximize data quality. All changes were initiated by the technician. The changes include: (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. 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.

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.

1. **How have the results been disseminated to communities of interest?**
   The results of the 2020–21 scab nursery were presented at the 2021 National Fusarium Head Blight Forum, Virtual Meeting. December 6–7, 2021. 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.

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 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).

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

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

Eggers, B, and Stockinger, E.J. (2021). DON accumulation and Fusarium Head Blight resistance in winter barley. Proceedings of the 2021 National Fusarium Head Blight Forum; Virtual. December 6–7, 2021. Retrieved from: https://scabusa.org/forum/2021/2021NFHBForumProceedings.pdf
Acknowledgment of federal support: YES