Project FY22-BA-006: 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.)*

   What were the major activities?
   The Ohio scab nursery was planted in Wooster on October 11, 2022, in a field equipped with well-water access. Each line was planted into a 0.8 m long headrow in a field measuring 32 m × 72 m. Planting format consisted of four adjacent columns of barley, each separated by two columns of a wheat spacer. Each line was planted in three reps using complete randomized block design.

   On April 30, 2023, which was prior to heading and was early in canopy development, the nursery was inoculated with multiple race isolates of Ohio F. graminearum using corn cob "spawn" placed in nursery rows. An overhead irrigation system was put in place on May 1, 2023. Overhead sprinkler heads were gridded at 9 m intervals along X and Y axes with sprinkler heads sitting on a 5’ riser. Heading date of each line was recorded. At the first sign of head emergence (Zadoks 51-53) and prior to pollen dehiscence, 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 20 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. At maturity, the reps were hand-harvested, and then threshed in a stationary lab thresher. Following harvest and threshing, three different approaches were taken to prepare the reps for grinding and DON level assessment, which were done for the purpose of maximizing the number of technical reps assessed within our 1000 sample allotment by the University of Minnesota DON testing lab. The first approach was to process each individual rep. This was done for the NABSEN set. The second was to process rep 1 as its own sample and composite an equal portion of reps 2 and 3 together. This was done for most of the advanced Ohio breeding lines. The third approach was to composite an equal portion of all three reps together. This was done for the Bregitzer population and a subset of the less advanced Ohio breeding lines. Individual rep samples and the composites were 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. Ground samples were then sent to the laboratory of Dr. Yanhong Dong, at the University of Minnesota for DON analyses.

Objective 1, the winter NABSEN
Seven U.S. public breeders contributed lines to the winter NABSEN for the 2022–23 season. A total of 49 lines from the contributors were tested. Eight checks vetted by the nursery participants were included. Seed of all entries were sent to the Stockinger lab. The seed was treated with NipsIt™ SUITE Cereals OF Seed Protectant (Valent, https://www.valent.com/) following the manufacturer's recommendation and allowed to dry. Seed was then packaged and shipped to the nursery participants September 14, 2023. 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.

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.

What were the significant results?
Below I first provide a general overview of the results, which is then followed by sections that focus on results under each specific objective.

For the Ohio nursery, no physical symptoms of disease were apparent during heading. As such we did not score for disease index.

To circumvent the lack of disease index score we assessed DON levels from as many technical reps for our plots as permitted by our allotment, rather than from a composite in an effort to increase the ability to detect variation that occurs in DON levels for each line.

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.

DON data for the 2022–23 field season was returned to PI Stockinger January 2024. A total of 1000 samples were submitted for testing. Across all samples and the entire Ohio nursery, DON values ranged 0.5 ppm to 31 ppm. The mean was 9.2. The lowest DON value observed was for an Ohio

breeding line, GHRIL0201-088, and which was tested as part of the NABSEN. Notably, the three reps of GHRIL0201-088 exhibited the lowest DON values across the entire Ohio 2022–23 Nursery. The highest value, 30.7 ppm, was observed for 'Lightning', an Oregon State University NABSEN submission.

Amongst the eight checks used (for both the NABSEN and the entire Ohio nursery), the mean DON values ranged 3 ppm – 21 ppm (Table 1). VA15H-73 exhibited the lowest value, while Atlantic, Hirondella, and Secretariat all exhibited mean value of 21 ppm. Overall, DON values for the checks the 2022–23 season were about an order of magnitude lower than they were for the same lines tested 2021–22 (Table 1).

Table 1. DON levels of the check lines tested in Wooster Ohio 2021–22, and 2022–23.

		2021–22				2022–23			
Genotype	**Row type**	**DON ppm (mean)**	**DON ppm (std dev)**	**Low value**	**High value**	**DON ppm (mean)***	**DON ppm (std dev)**	**Low value**	**High value**
VA15H-73	2	34	7	26	43	3	2	2	5
Endeavor	2	111	7	98	121	10	4	7	18
Calypso	2	139	20	100	163	16	3	11	22
Wintmalt	2	151	20	132	188	14	3	10	17
Atlantic	6	116	28	78	157	21	4	16	25
Thoroughbred	6	117	26	87	173	14	3	9	18
Secretariat	6	124	22	95	173	21	3	17	27
Hirondella	6	222	30	195	285	21	4	16	26

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

Objective 1, the winter NABSENDON levels of the winter NABSEN lines tested in Wooster Ohio the 2022–23 season is provided in Table 2. How those levels compare to the eight check lines is shown in Figure 1.

Table 2. DON levels of the winter NABSEN lines tested in Wooster Ohio the 2022–23 season.

Entry	Line	Rep 1	Rep 2	Rep 3	Mean	Std dev
NASBEN-001	2MW18_3372-008	10.4	6.1	10.9	9.1	2.6
NASBEN-002	2MW18_3373-001	5.1	3.3	6.3	4.9	1.5
NASBEN-003	2MW18_3374-015	4.2	6.6	7.5	6.1	1.7
NASBEN-004	2MW18_3374-019	3.8	6.0	9.4	6.4	2.8
NASBEN-005	2MW18_4462-011	7.6	5.9	7.9	7.1	1.1
NASBEN-006	MN-Equinox	7.7	6.1	7.1	7.0	0.8
NASBEN-007	MTWF6F2_50-1	6.6	4.0	7.1	5.9	1.7
NASBEN-008	MTWF6F2_50-7	7.2	8.2	7.4	7.6	0.5
NASBEN-009	MTWF6F2_51-5	9.9	14.4	13.2	12.5	2.3
NASBEN-010	MTWF6F2_51-9	7.3	7.9	7.3	7.5	0.3
NASBEN-011	MTWF6F3_05-01	10.0	9.9	7.9	9.3	1.2
NASBEN-012	MTWF6F3_24-01	9.4	7.1	8.1	8.2	1.2
NASBEN-013	MTWF6F4_13-01	2.1	3.9	4.1	3.4	1.1
NASBEN-014	NB17431	3.0	2.5	3.8	3.1	0.7
NASBEN-015	NB19420	6.8	10.9	8.7	8.8	2.1
NASBEN-016	NB19422	13.7	19.9	14.0	15.9	3.5
NASBEN-017	NB20419	10.9	18.8	20.1	16.6	5.0
NASBEN-018	NB21411	15.8	16.1	15.7	15.9	0.2
NASBEN-019	2011-725-02	8.6	22.1	13.2	14.6	6.9
NASBEN-020	DH0214-007	7.4	6.7	5.2	6.4	1.1
NASBEN-021	DH02FL-028	9.0	8.7	9.8	9.2	0.6
NASBEN-022	GHRIL0201-103	10.4	5.2	16.1	10.6	5.5
NASBEN-023	GHRIL0201-088	0.7	0.7	0.5	0.6	0.1
NASBEN-024	RIL0257-01-011	5.1	5.4	5.8	5.4	0.4
NASBEN-025	RIL02FL-029	4.6	6.7	4.4	5.2	1.3
NASBEN-026	RIL02SP-014	6.6	4.1	3.3	4.7	1.7
NASBEN-027	DH141917	9.0	16.2	17.8	14.3	4.7
NASBEN-028	DH150683	14.9	11.2	10.1	12.1	2.5
NASBEN-029	DH162310	5.8	9.6	4.0	6.5	2.9
NASBEN-030	DH170472	10.8	8.2	9.4	9.5	1.3
NASBEN-031	DH180676	8.9	12.3	7.2	9.5	2.6
NASBEN-032	Lightning	19.0	30.7	18.5	22.7	6.9
NASBEN-033	Thunder	5.8	7.8	8.8	7.5	1.5
NASBEN-034	10ARS839-2	5.2	4.0	4.0	4.4	0.7
NASBEN-035	12ARS777-1	16.1	11.8	8.5	12.1	3.8
NASBEN-036	13ARS514-6	4.4	6.0	8.3	6.2	2.0
NASBEN-037	13ARS534-8	7.7	7.5	7.9	7.7	0.2
NASBEN-038	13ARS537-13	12.1	14.2	11.8	12.7	1.3
NASBEN-039	13ARS537-19	6.6	9.7	9.9	8.7	1.9
NASBEN-040	13ARS537-25	8.6	12.9	8.7	10.1	2.5
NASBEN-041	14ARS733-4	2.2	9.8	1.3	4.4	4.7
NASBEN-042	Greg	2.1	1.0	1.6	1.6	0.6
NASBEN-043	VA16H-27	1.1	1.1	0.9	1.0	0.1
NASBEN-044	VA16M-84	4.1	2.9	3.3	3.4	0.6
NASBEN-045	VA20MFHB-18DH533	7.8	10.5	8.3	8.9	1.4
NASBEN-046	VA20MFHB-18DH535	6.1	11.0	10.9	9.3	2.8
NASBEN-047	VA21HFHB-19DH0152	3.8	2.9	4.1	3.6	0.6
NASBEN-048	VA21HFHB-19DH0301	3.5	3.2	3.1	3.3	0.2
NASBEN-049	VA21HFHB-19DH0714	2.1	3.2	2.6	2.6	0.6

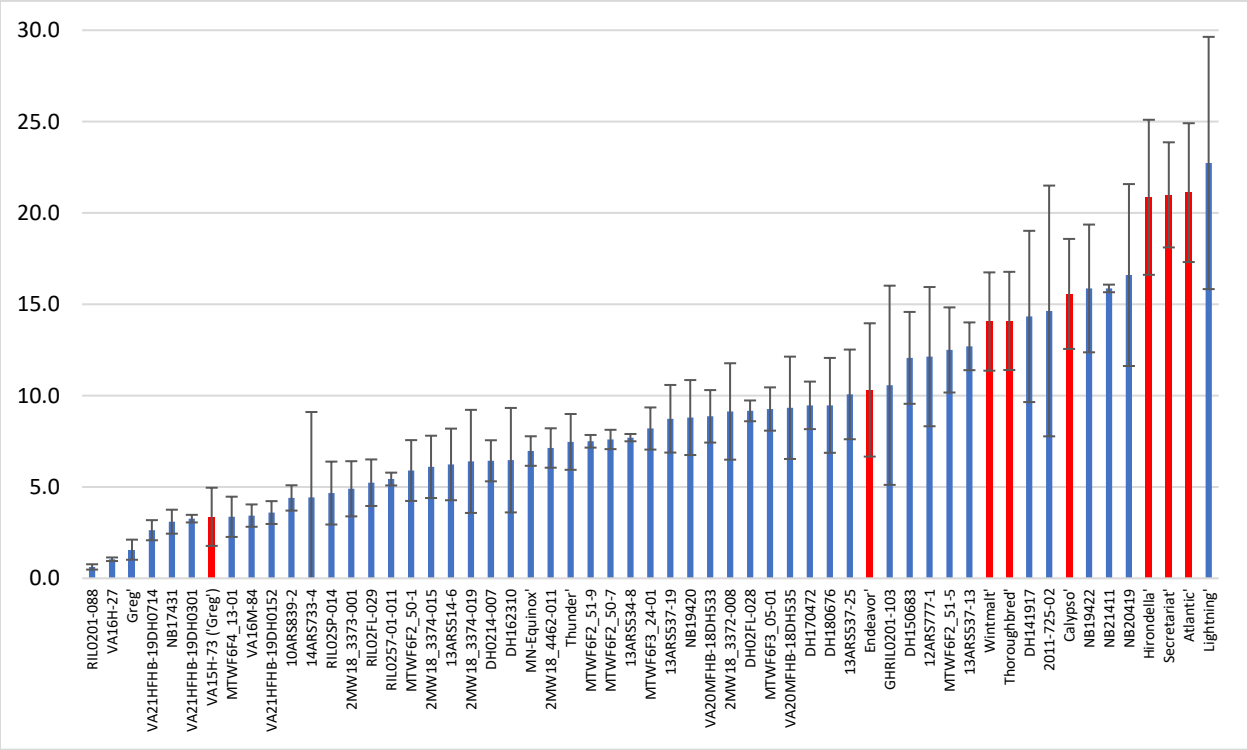

Figure 1. DON (ppm) for the winter NABSEN set of lines tested in Wooster Ohio 2022-23. NABSEN lines are indicated by blue bars, the eight checks are indicated by red bars.

Most lines tested in the winter NABSEN exhibited DON values lower than seven of the eight checks. The seven checks were above the mean of 9.2 for the entire Ohio nursery and at the high end of the spectrum (Figure 1). VA15H-73 (released as 'Greg') was the one exception amongst the checks and was the benchmark lowest DON accumulating line the previous testing season, 2021–22 (Table 1). Six 2022–23 NABSEN entries exhibited values lower than VA15H-73, including GHRIL0201-088, VA16H-27, 'Greg', 'VA21HFHB-19DH0714', 'NB17431', 'and VA21HFHB-19DH0301'. GHRIL0201-088 is an Ohio entry, 'NB17431' is a Nebraska entry, and the four others are Virginia Tech entries.

Objective 2, identifying lines in the Ohio breeding program exhibiting FHB resistance.
Across all lines tested in the 2022–23 Ohio nursery, most of those exhibiting the lowest DON levels were from the Ohio or Virginia Tech program, while most of the lines exhibiting the highest DON levels tended to be from European programs (Tables 3 and 4).

The absence of physical disease symptoms is attributed to weather conditions throughout most of the heading process not being conducive for development of fusarium head blight disease symptoms, i.e., Ohio experienced a prolonged period of extremely dry conditions and mild temperatures during May and June. Absence of physical disease symptoms was also noted by many of the NABSEN nursery participants. It was only at maturity when the humidity increased and temperatures warmed when disease symptoms started to appear.

The greatly diminished DON levels detected for lines the 2022–23 season relative to the 2021–22 season is also attributed to the extremely dry conditions and mild temperatures. It is hypothesized that those weather conditions were highly unfavorable for fungal growth and colonization of the barley heads. Nonetheless, despite the significantly reduced DON levels, i.e., by an order of magnitude, the

low and high DON accumulating checks VA15H-73 ('Greg') and 'Hirondella', defined low and high values for both seasons. Thus while the levels of resistance displayed by newly tested lines (e.g., first time NABSEN entries), can be inferred relative to the checks, those resistance levels should be considered preliminary until multiple year-location data are in hand.

Table 3. Lines having the lowest DON across all 1000 samples.

Lowest DON	**DON (ppm)***	**Program†**
GHRIL0201-088	0.7	OH
MO B2549	0.8	MO
Admire-Pallas #02-05	1.1	OH
GHRIL0201-009	1.1	OH
VA16H-27	1.1	VA
GHRIL0201-002	1.4	OH
MO B2549-Halcyon-221	1.4	OH
Admire-G. Promise #01-04	1.8	OH
GHRIL0201-088	1.9	OH
MO B2414	1.9	MO
12ID69	2.0	ID
FLDRIL02OPL-082	2.0	OH
MO B2190-Sublette-11	2.0	OH
VA15H-73 (check #6, AKA Greg)	2.0	VA
Greg	2.1	VA
12ID40	2.2	ID
Puffin-(MO B2169-NT 074-060)-36	2.2	OH

*Only lines in which all three reps were ranked in the lowest 5%, or rep 1 AND the rep 2-3 composite, or the 3-rep composite are listed.
†U.S. state where lines were developed.

Table 4. Lines having the highest DON across all 1000 samples.

Highest DON	DON (ppm)*	Program†
Lyberac	26.8	EU††
2011-725-02-Flagon (2274)-19	26.2	OH
DH130910	25.2	OR
Joy	25.1	EU
AC11/341/28	25.0	EU
Regina	24.7	EU
06ARS617-25	24.5	ID
Opal	23.9	EU
Mombasa	23.7	EU
Hickory	23.6	EU
Flavia	23.1	EU
Atlantic (check #3)	22.3	VA
Malwinta	21.9	EU
Hirondella (check #2)	21.7	EU
Wintmalt (check #8)	20.6	EU
05ARS749-8	20.3	ID
DH10.1044	20.3	OR
Secretariat (check #4)	20.3	VA

*Only lines in which all three reps were ranked in the highest 5%, or rep 1 AND the rep 2-3 composite, or the 3-rep composite are listed.
†U.S. state where lines were developed.
††EU, developed in Europe

Objective 2, 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 the 2022–23 season ranged 2.0–15.0 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 7.6 ppm and the standard deviation for each line from that mean was 2.5 ppm. 'Charles' was represented twice; the values exhibited by the two reps was 9.0 and 14.0. 95SR316A was represented once and exhibited 4.2 ppm. Five of the 84 95SR316A × 'Charles' recombinants exhibited values less than 4.2 ppm. Seven of the recombinants exhibited values greater than 11.5, the mean of the two 'Charles' entries. Three of these three low-end DON accumulator recombinants in the 2022–23 nursery were amongst the 2021–22 nursery low-end DON accumulators, and four of the seven high-end DON recombinants in the 2022–23 nursery were amongst the high-end DON recombinants in both the 2020–21 and 2021–22 nurseries.

Objective 3, utilizing modern breeding technologies to breed for FHB resistance.
The Ohio selection GHRIL0201-088 exhibited the lowest DON values across the entire Ohio 2022–23 Nursery. In addition to being the lowest DON accumulator, GHRIL0201-088 also exhibits good malting quality and high levels of resistance to lodging and stem breakage. These favorable qualities have been observed over multiple years and multiple locations. Because of the multiple highly desirable qualities we have used GHRIL0201-088 extensively as a parent in crosses, including as a recurrent parent in a backcrossing strategy. One of the crosses, to Ohio selection FL-029/WI-013-08, is now in the pipeline for a

doubled haploid population development at the U.S. Wheat and Barley Scab Initiative-supported genomics facility at Oregon State University.

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 excellent testing grounds to screen winter barley lines being considered for cultivation across North America.

While the infection and disease pressure in the 2022–23 Ohio scab nursery was not as robust as that of the 2021–22 Ohio scab nursery, DON levels of lines defining the extreme low and extreme high ends of DON accumulation in the 2021–22 nursery also defined the low and high extremes of the 2022–23 nursery. Lines that were intermediate in DON levels between the two extremes were less well stratified in the 2022–23 nursery than they were in the 2021–22 nursery. Some of this reduced stratification may be the result of a higher level of variation relative to the absolute DON values, i.e., absolute DON values were an order of magnitude lower in the 2022–23 nursery relative to the 2021–22 nursery, yet the variation was proportionally much greater in the 2022–23 nursery than it was in the 2021–22 nursery. Nonetheless many of the recombinants in the Bregitzer population that exhibited DON values below the presumptive resistant parent 95SR316A, or above the presumptive susceptible Charles, parsed similarly in the 2022–23 nursery as they did in the 2021–22 nursery. Taken together these findings support the notion that incorporating genetic resistance to DON accumulation through genetics and breeding is doable when multiple environments and multiple years of testing are a component of the breeding program.

That many of the lines in the Ohio and Virginia program exhibit DON accumulation values at the low end of the spectrum also suggests that these programs may also be selecting for lines that do well under high disease pressure.

3. **What opportunities for training and professional development has the project provided?**
This project provided tremendous opportunities for learning and professional development for a graduate student, Madison Dahn, and two undergraduate summer student researchers the 2022–23 season. The graduate student was trained by the technician Ben Eggers, who had been with the project since its inception. Madison presented our findings at the 2023 National Fusarium Head Blight Forum and used the meeting as an opportunity to network with other members of the wheat and barley scab community.

The project also provided the graduate student with the opportunity to lead and manage a team. 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 and 222–23 scab nurseries were presented at the 2023 National Fusarium Head Blight Forum, Cincinnati OH, December 3–5, 2023.
We are currently carrying out statistical analyses of the results from the 2021–22 and 2022–23 winter NABSEN and will summarize key findings alongside presentation of the results from each individual nursery in a report for the Uniform Nursery Reports at USWBSI. These findings will also be provided to the breeders who submitted lines to the nursery.

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

We will continue to oversee and coordinate the winter NABSEN and we will continue to put all advanced Ohio breeding lines into the winter scab nursery. For the 2024–25 season we will rotate into the scab nursery a new population of approximately 250 lines. The lines in the population share a common ancestor, 'Wong' (as a parent or grandparent) and are the products developed by breeders across North America mid-20th Century. 'Wong' exhibited high levels of resistance in the 2020–21 nursery. The genetic relatedness of this population is anticipated to enhance our ability to genetically interrogate DON accumulation. The lines were obtained from the U.S. National Plant Germplasm System and are currently being increased via single seed decent in the greenhouse. A new person, Frolence Paul Fidelis, expressed interest in taking on this project, and will start as a Masters Student with me Autumn 2024.