USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY19 Final Performance Progress Report
Due date: August 31, 2021

Cover Page

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Fiscal Year:	2019
USDA-ARS Agreement ID:	58-6070-8-014
USDA-ARS Agreement Title:	Collaborative Research to Improve Evaluation of Advanced and Diverse Wheat Germplasm for FHB Resistance in the Atlantic
FY20 USDA-ARS Award Amount:	$ 41,891
Recipient Organization:	Clemson University Grants and Contracts Administration 230 Kappa Street, Suite 200 Clemson, SC 29634-5355
DUNS Number:	04-262-9816
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Recipient Identifying Number or Account Number:	20-207-xxxx-0185-207-2022776
Project/Grant Reporting Period:	8/1/19 - 7/31/21
Reporting Period End Date:	7/31/2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
VDHR-SWW	Identifying Sources of FHB Resistance in Diverse Wheat Germplasm for the Southeast	$ 16,697
VDHR-SWW	Development of an Accelerated Phenotyping Platform for Measuring FDK in Large Breeding Populations	$ 25,194
	FY19 Total ARS Award Amount	**$ 41,891**

Rick Boyles

Digitally signed by Richard Boyles
Date: 2021.08.31 11:08:51 -04'00'

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R – Research
S – Service (DON Testing Lab)
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: *Identifying Sources of FHB Resistance in Diverse Wheat Germplasm for the Southeast*

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

Goal: Evaluate wheat sources of Fusarium head blight resistance to identify new FHB resistant varieties adapted to South Carolina and other states in the southeastern US.

Objectives:

1) Conduct a coordinated field trial in Florence, SC to screen elite varieties, advanced breeding lines, and diverse germplasm for FHB resistance.
2) Implement greenhouse crossing to intercross lines that exhibit FHB resistance.

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?

1) Conduct a coordinated field trial in Florence, SC to screen elite varieties, advanced breeding lines, and diverse germplasm for FHB resistance.

The Florence scab nursery headrows were planted on 11/20/19, inoculated with scabby corn kernels twice in March, field rated for FHB on 5/1/19, and harvested in late May. Harvested samples were machine threshed without aspiration and then manually sieved to separate chaff from kernels. Cleaned grain samples (*n*=436) were visually rated for *Fusarium* damaged kernels (FDK) percentage. Cooperative nurseries evaluated include the USSN, SC OVT, USSRWWN, UBWT, GAWN, and SunWheat. FHB ratings were also taken on the Clemson Wheat Preliminary Trial entries (*n*=547) and early stage Clemson breeding lines ($F_{2:4}$ and $F_{3:4}$ generations), which were all grown in the mist-irrigated and inoculated scab nursery. All advanced nursery (USSN, USSRWWN, GAWN, SunWheat) samples were ground and sent to the VA Tech DON testing laboratory in two batches (10/13/20 and 11/2/20), and data were received back by early February. Range of DON levels were outstanding.

2) Implement greenhouse crossing to intercross lines that exhibit FHB resistance.

Wheat breeding lines were vernalized (9/20/19), transplanted (11/1/19), and cross-pollinated from 1/3/20 to 2/24/20. There were 36 elite lines selected to use as parents that collectively represented Clemson, LSU, NC State, UARK, UFL, UGA, and VA Tech, of which 12 lines contained *Fhb1*.

b) What were the significant results?

1) Conduct a coordinated field trial in Florence, SC to screen elite varieties, advanced breeding lines, and diverse germplasm for FHB resistance.

Visual ratings for FDK ranged from 0-95% across regional nurseries, but the USSN ranged only between 0-45% with a mean FDK of 11.7%, which is a testament to the VDHR-SWW focus of developing lines for FHB resistance. Each trial contained multiple lines with moderate resistance, with the earlier stage trials (USSN, GAWN, and SunWheat) demonstrating stronger mean levels of resistance. Again, this suggests that the latest emphasis on stacking multiple FHB resistance genes by breeders with the aid of molecular markers is having a positive effect. This was especially true for entries in the Uniform Southern Scab Nursery, which had a significantly lower mean DON of 14.1 ppm compared to other regional nurseries, which were all in the low 20s for the average. The USSN and SunWheat trials both included entries with <1 ppm DON levels from the misted nursery, which was exceptional given the high amount of Fusarium pressure in the 2019-2020 trial.

Trial	Entries	FHB Mean	FHB Range	FDK Mean	FDK Range	DON Mean	DON Range
USSN	48	1.9	0 - 8	11.7	5 - 45	14.1	0.4 - 42
SC OVT	71	2.1	0 - 8	21.5	5 - 70	NA	NA
USSRWWN	38	2.6	0 - 8	19.6	0 - 95	21.5	5.1 - 44
UBWT	45	1.9	0 - 6	33.4	5 - 95	NA	NA
GAWN	50	3.1	0 - 8	12.7	0 - 45	23.7	4.4 - 62
SunWheat	94	3.2	0 - 8	10.6	0 - 45	22.4	0.5 - 85
ALL	**346**	**2.5**	**0 - 8**	**17.4**	**0 - 95**	**0 - 95**	**0.4 - 85**

2) Implement greenhouse crossing to intercross lines that exhibit FHB resistance.

A total of 509 crosses were made from 823 crosses of interest— 68 *Fhb1/Fhb1*, 302 *Fhb1* SG, and 495 with multiple Fhb QTL segregating. There were 263 (52%) successful three-way crosses and the rest biparental crosses. Of the 509 crosses, F_1 seed from 321 crosses were sent to Aberdeen, ID for growout/increase to screen F_2 populations in 2020-2021.

c) List key outcomes or other achievements.

1) Conduct a coordinated field trial in Florence, SC to screen elite varieties, advanced breeding lines, and diverse germplasm for FHB resistance.

There was low scab pressure early, likely caused by cooler than normal spring weather, but FHB incidence was evident in late April and spread quickly throughout the nursery. The grinding protocol using a FOSS Hammertec sample mill has been tested for proof-of-concept, and samples are currently being ground into flour to be shipped to VA Tech for DON testing.

2) Implement greenhouse crossing to intercross lines that exhibit FHB resistance.

Sources of *Fhb7* and *Fhb1+Fhb7* have been acquired to begin evaluating lines in the

2020-2021 scab nursery to find a suitable donor that is reasonably adapted for backcrossing Fhb7 into elite cultivars. The first backcross greenhouse crossing nursery will be transplanted on 5 October 2020 for initial crosses with multiple prospective *Fhb7* donor lines.

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.

Fortunately, no field-related research activities were limited as we were able to manage and harvest the entire scab nursery in Florence. However, grinding of the grain samples was delayed by the restricted ability to hire temporary labor. Quality of research was not affected, but timing to obtain and disseminate data, especially DON data, was delayed. These data turned out to be vital to calibration and testing of the Vibe QM3 Grain Analyzer.

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

PI Boyles was able to attend the Annual FHB Forum held in Milwaukee during 8-10 December 2019. At the forum, the PI learned valuable information on Breedbase data management, genomic prediction activities for FHB resistance, and effective FHB phenotyping strategies for improved selection accuracy. During separate breakout meetings, PI Boyles also was appointed to the VDHR-SWW steering committee and selected as chair of the Southern Small Grains Workers Association. There were a number of informal, one-on-one and small breakout discussions that were also valuable for professional development and network building.

There were no postdocs, graduate, or undergraduate students funded by this project. However, the primary field research technician for the Cereal Grains Breeding & Genetics Program partly funded by this NACA learned tractable skills in establishing a mist-irrigation system that included installation of a large sand media filter. The technician as well as a PhD student, AJ Ackerman, led the process of culturing inoculum and scaling up scabby corn for field deployment, which has provided hands-on pathology experience. To help learn these pathology skills, the Mr. Ackerman spent time in the lab of and discussion with Dr. Christina Cowger as well as time in the labs of Drs. Gina Brown-Guedira and Paul Murphy. Two technicians and an undergraduate who worked part-time on the project were trained to operate field equipment, seed processing machinery, and seed imaging software.

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

Data derived from the scab nursery was reported in Excel format to the VDHR-SWW breeders on 18 Aug 2020, which included FHB index rating, FDK visual observation, FDK manual separation and counting percentage, and FDK Vibe imaging data. FHB resistance data on entries in the SC Official Variety Trial were uploaded to ScabSmart once thoroughly vetted and then approved by the SC OVT Coordinator.

Project 2: *Development of an Accelerated Phenotyping Platform for Measuring FDK in Large Breeding Populations*

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

Goal: Evaluate wheat breeding lines for *Fusarium*-damaged kernels (FDK) using imaging and compositional approaches to identify a platform that is more accurate and higher throughput than the current method of sieving, visual inspection, and manual counting.

Objectives:

1) Collect scabby grain samples from regional scab nurseries.
2) Develop baseline FDK data using existing protocols (*i.e.* sieve and manual counting).
3) Image grain samples from each plot to establish image-based FDK predictions.
4) Send subset of grain samples across FDK spectrum for single kernel characterization.
5) Analyze whole grain samples for FDK using near-infrared (NIR) spectroscopy.
6) Perform ground truth analysis to determine accuracy and repeatability of each approach.

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?

*****Related objective(s) listed in parentheses following activity.***

Grain samples were collected from misted scab nurseries in the following locations: Florence, SC; Winnsboro, LA; and Warsaw, VA (***Obj 1***). Genotypes from these locations represented the USSN (SC, LA, VA), USSRWWN (SC, LA), GAWN (SC, LA, VA), and SunWheat (SC, LA). Each grain sample was threshed without aspiration and manually sieved to remove glumes and debris from the grain. A total of 509 samples from 2018-2019 and 451 samples from 2019-2020 were analyzed for FDK. Analysis included visual FDK estimation as well as manually separating and counting *Fusarium*-damaged kernels in a 1,000-kernel subset to obtain a reliable FDK measure for comparison to visual ratings and other high-throughput methods (***Obj 2, 6***). We used digital smartphone images coupled with a publicly available computer processing software called SmartGrain (Tanabata et al. 2012) to predict FDK using a custom-built linear regression model developed with JMP software (***Obj 3***). In replace of the single kernel characterization system, we used a new grain imaging instrument called a Vibe QM3 grain analyzer to measure FDK nondestructively (***Obj 4***). NIR spectroscopic analysis of all samples has also been completed for the 509 samples from 2018-2019 (***Obj 5***). Grain samples analyzed using the range of different FDK phenotyping platforms were ground for DON testing at the Virginia Tech laboratory to compare the multiple FDK predictions with DON data (***Obj 6***). Once DON data were returned, PhD student AJ Ackerman led the correlation and ANOVA analyses to determine the optimal FDK method based on their relationship to DON levels.

b) What were the significant results?

As expected, significant variation for visual FDK percentage was observed in the 2018-2019 (0% - 95%) and 2019-2020 (0% - 75%) samples. When comparing 2018-2019 FDK predictions among platforms, the Vibe QM3 data had a stronger positive correlation with manual FDK values than other phenotypic platforms. Visual FDK estimates (5% increments) using developed standards had the strongest relationship with manual; however, it should be noted that there is potential bias given that an observation was made to discern healthy from damaged kernels in both manual and visual FDK data collection. Therefore, DON data will be used as a final comparative measure between FDK platforms to determine precision and accuracy of these methods.

	Analysis Time[a]		FDK (%)		Correlation with Manual (r^2)	Correlation with DON (r^2)
Method	**Mean**	**Range**	**Mean**	**Range**		
Manual	13:11	7:05 –	11.75	0 – 95.3	n/a	0.3781
Visual standard	0:53	0:36 –	19.72	0 – 75.0	0.7551	0.4625
SmartGrain[b]	n/a	n/a	-1.00	-85.0 –	0.5703	0.6096
NIR	2:00	1:18 –	55.97	12.0 –	0.4969	0.3381
Vibe QM3	1:49	1:42 –	25.37	0.55 –	0.6924	0.7700

[a]Includes all post-cleaning processes except instrument calibration (grain dispensing, imaging, counting, computation, analysis, etc.); derived from a random 10 samples.

[b]Only includes a subset of 100 samples due to its lack of accuracy.

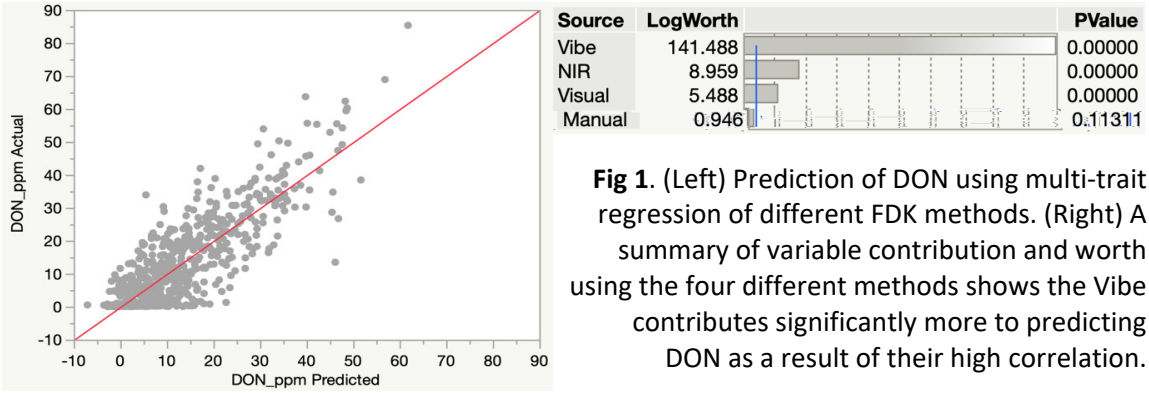

Fig 1. (Left) Prediction of DON using multi-trait regression of different FDK methods. (Right) A summary of variable contribution and worth using the four different methods shows the Vibe contributes significantly more to predicting DON as a result of their high correlation.

c) List key outcomes or other achievements.

A Vibe QM3 was purchased (from USWBSI VDHR-SWW FY20 funds) and was received on July 13, 2020. Calibration on the instrument was performed using manually sorted FDK samples (5% increments), which led to an accurate and robust ability to measure FDK in subsequent samples. Based on data collected and analyzed, the Vibe QM3 imaging platform showed the best promise for replacing visual-based FDK estimates out of the FDK platforms.

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.

Fortunately, no field-related research activities were limited as we were able to manage and harvest the entire scab nursery in Florence. However, grinding of the grain samples was delayed by the restricted ability to hire temporary labor. Quality of research was not affected, but timing to obtain and disseminate data, especially DON data, was delayed. These data turned out to be vital to calibration and testing of the Vibe QM3 Grain Analyzer.

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

A virtual workshop was held in July 2020 to train research personnel on how to operate and calibrate the Vibe QM3 grain analyzer. Included in the training were two research associates, a PhD student, and a high school student from the SC Governor's School of Science and Mathematics who was in the lab as part of a summer internship program. PhD student AJ Ackerman had the opportunity to also receive training on operating the Perten DA7250 NIR analyzer. Both PhD student Ackerman and the GSSM student (Zeke Gaskins) learned valuable phenotyping skills revolving around FDK platforms as well as how to properly analyze and compare data among them.

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

A formal presentation was provided by the PI on August 11, 2020 to give an overview of the final results. Final preparations of a manuscript to be submitted in Crop Science, Agronomy, or Plant Methods are being completed by PhD student AJ Ackerman and the PI. Publication is expected by the end of the 2021 calendar year. PhD student Ackerman will also virtually present a poster at the 2012 Annual FHB Forum to ensure these results reach all VDHR members, pathologists, and other scientists that may be interested in adopting objective FDK methods for improved accuracy of FHB resistance in wheat.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (8/1/19 - 7/31/21). 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 FY19 award period?**
   ☐Yes ☐No ☒ Not Applicable
   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 FY19 award period?**
   ☐Yes ☐No ☒ Not Applicable
   If yes, how many? Click to enter number here.

3. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   ☐Yes ☐No ☒ Not Applicable
   If yes, how many? Click to enter number here.

4. **Have any post docs who worked for you during the FY19 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 ☒ Not Applicable
   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 **FY19 award period (8/1/19 - 7/31/21)**. 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
AR01040-4-1	SRW - Soft Red Winter	MS - Moderately Susceptible	6.5	2019
GA071518-16E39 (Dyna-Gro Blanton)	SRW - Soft Red Winter	S - Susceptible	8.0	2019
GA09129-16E55 (AGS 3015)	SRW - Soft Red Winter	MR-MS	4.5	2019
GA09377-16LE18	SRW - Soft Red Winter	MS - Moderately Susceptible	6.0	2019
GA09436-16LE12	SRW - Soft Red Winter	MR-MS	5.0	2019
ARLA06146 (Delta Grow 1800)	SRW - Soft Red Winter	MR - Moderately Resistant	2.3	2020
GA10268-17LE16 (PGX 20-15)	SRW - Soft Red Winter	MR-MS	5.0	2020
TX-EL2 (Go Wheat 6000)	SRW - Soft Red Winter	MR-MS	4.0	2020

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 (8/1/19 - 7/31/21**) 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.

Ackerman AJ, Gaskins E, Jordan K, Hicks D, Fitzgerald J, Mason RE, Harrison SA, Murphy JP, Griffey CA, Boyles RE. Improved methods for measuring *Fusarium*-damaged kernels in wheat.
Status: In preparation
Acknowledgement of Federal Support: YES

Mohamed M, JW Johnson, J Buck, S Sutton, B Lopez, D Bland, Z Chen, G Buntin, D Mailhot, MA Babar, E Mason, S Harrison, JP Murphy, A Ibrahim, R Sutton, G Brown-Guedira, B Simoneaux, H Bockelman, B-K Baik, D Marshall, C Cowger, J Kolmer, Y Jin, X Chen, S Cambron, R Boyles. 2021. Soft Red Winter Wheat, 'GA 051207-14E53': Adapted Cultivar to Georgia and the USA Southeast Region. *Journal of Plant Registrations* 15(1):132-139.
Status: Published
Acknowledgement of Federal Support: YES

Mergoum M, JW Johnson, JW Buck, S Sutton, B Lopez, D Bland, Z Chen, GD Buntin, DJ Mailhot, MA Babar, RE Mason, SA Harrison, JP Murphy, AMH Ibrahim, R Sutton, B Simoneaux, CA Griffey, HE Bockelman, B-K Baik, D Marshall, C Cowger, G Brown-Guedira, J Kolmer, Y Jin, X Chen, R Boyles, S Cambron. 2021. A new soft red winter

wheat cultivar,'GA 07353-14E19', adapted to Georgia and the US Southeast environments. *Journal of Plant Registrations,* 15(2):337-344.
Status: Published
Acknowledgement of Federal Support: YES

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

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.