USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY19 Performance Report
Due date: September 30, 2020

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
FY19 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
Agency PI:	Gina Brown-Guedira
Project/Grant Reporting Period:	8/1/19 - 7/31/20
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 09/30/20

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
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

FY19 Performance Report
CPI: Boyles, Richard
NACA Agreement #: 58-6070-8-014
Reporting Period: 8/1/19 - 7/31/20

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 items a-b) below.)*

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

a) What were the major activities?
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 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.

b) What were the significant results?
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.

Trial	Entries	FHB Mean	FHB Range	FDK Mean	FDK Range
USSN	48	1.9	0 - 8	11.7	5 - 45
SC OVT	71	2.1	0 - 8	21.5	5 - 70
USSRWWN	38	2.6	0 - 8	19.6	0 - 95
UBWT	45	1.9	0 - 6	33.4	5 - 95

Trial	Entries	FHB Mean	FHB Range	FDK Mean	FDK Range
GAWN	50	3.1	0 - 8	12.7	0 - 45
SunWheat	94	3.2	0 - 8	10.6	0 - 45
ALL	**346**	**2.5**	**0 - 8**	**17.4**	**0 - 95**

c) List key outcomes or other achievements.
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.

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

a) What were the major activities?
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?
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.
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, 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 has been delayed by the restricted ability to hire temporary labor. Quality of research should not be affected, but timing to obtain and disseminate data, especially DON data, may be delayed.

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

Although a graduate student and an undergraduate are not directly funded by the project, they are involved in research and are learning important breeding strategies for disease resistance. A graduate student, Mr. AJ Ackerman, with technical support, 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 student spent time in the lab of and discussion with Dr. Christina Cowger. Two research technicians have added tractable skills as a result from managing this project. Both technicians and an undergraduate who works part-time on the project have been trained to operate field management 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 will be uploaded to ScabSmart once thoroughly vetted and then approved by the SC OVT Coordinator. Journal articles are in preparation to inform the scientific community of significant results.

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 items a-b) below.)*

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

a) What were the major activities?
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 are in process of being ground (10% completed) for DON testing at the Virginia Tech laboratory to compare the multiple FDK predictions with DON data (***Obj 6***).

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)**
Method	**Mean**	**Range**	**Mean**	**Range**	
Manual	13:11	7:05 – 18:58	11.75	0 – 95.3	n/a
Visual standard	0:53	0:36 – 1:15	19.72	0 – 75.0	0.7551
SmartGrain[b]	n/a	n/a	-1.00	-85.0 – 99.6	0.5703
NIR Spectroscopy	2:00	1:18 – 2:35	55.97	12.0 – 103.0	0.4969
Vibe QM3	1:49	1:42 – 1:58	25.37	0.55 – 85.9	0.6924

[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 to date.

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. While initial calibration on the instrument has been performed, using additional samples to add to the calibration and improve its accuracy and robustness will likely improve the instrument's ability to measure FDK in future samples. Based on data collected and analyzed to date, the Vibe QM3 imaging platform and NIR analyzer (Perten DA7250) show the best promise for replacing visual-based FDK estimates to tandemly improve accuracy and throughput.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

Grinding of the grain samples for DON analysis has been hindered by the restricted ability to hire temporary labor. Quality of research should not be affected, but timing to obtain and disseminate data may be delayed.

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 graduate 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. The graduate student had the opportunity to also receive training on operating the Perten DA7250 NIR analyzer.

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

Preliminary results have been circulated throughout the VDHR-SWW research group, including a presentation provided by the PI on August 11, 2020. This study will be published in Plant Methods or a similar reputable journal once final DON data are received.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (8/1/19 - 7/31/20). 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?**
   No.

   If yes, how many?

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?**
   No.

   If yes, how many?

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?**
   No.

   If yes, how many?

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?**
   No.

   If yes, how many?

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. 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** (S, MS, MR, R, where R represents your most resistant check)	**FHB Rating** (0-9)	**Year Released**
GA 051207-14E53	SRW	MR	3.1	2019
ARLA06146 (Delta Grow 1800)	SRW	R	2.3	2020
GA10268-17LE16 (PGX 20-15)	SRW	MS	4.1	2020

Add rows if needed.

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Abbreviations for Grain Classes

Barley - BAR
Durum - DUR
Hard Red Winter - HRW
Hard White Winter - HWW
Hard Red Spring - HRS
Soft Red Winter - SRW
Soft White Winter - SWW

Publications, Conference Papers, and Presentations

Instructions: Refer to the FY19-FPR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY19 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (8/1/19 - 7/31/20**) 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:

De Wolf, E., D. Shah, P. Paul, L. Madden, S. Crawford, D. Hane, S. Canty, R. Dill-Macky, D. Van Sanford, K. Imhoff and D. Miller. 2019. "Impact of Prediction Tools for Fusarium Head Blight in the US, 2009-2019." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 12), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

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. Soft Red Winter Wheat, 'GA 051207-14E53': Adapted Cultivar to Georgia and the USA Southeast Region. Journal of Plant Registrations. *Accepted.*
Status: Accepted but not yet in press
Acknowledgement of Federal Support: YES

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

Other publications, conference papers and presentations.