USDA-ARS | U.S. Wheat and Barley Scab Initiative
FY21 Performance Progress Report
Due date: July 26, 2022

Cover Page

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Fiscal Year:	2021
USDA-ARS Agreement ID:	59-0206-0-150
USDA-ARS Agreement Title:	Collaborative Research to Improve evaluation of Advanced Wheat Germplasm for FHB Resistance in the Atlantic Coastal Plain
FY20 USDA-ARS Award Amount:	$87,110
Recipient Organization:	Clemson University Pee Dee Research and Education Center 2200 Pocket Rd, Florence, SC 29506
DUNS Number:	04-262-9816
EIN:	57-6000254
Recipient Identifying Number or Account Number, if any:	207-2013983
Project/Grant Period:	5/15/21 - 5/14/23
Reporting Period End Date:	5/14/2022

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
VDHR-SWW	Developing and Evaluating Wheat Lines for FHB Resistance in Atlantic Coastal Plain	$67,926
VDHR-SWW	Double Haploids to Expedite Development of FHB Resistant Soft Winter Wheat Varieties	$19,184
FY21 Total ARS Award Amount		$87,110

I am submitting this report as an: ☒ Annual Report   ☐ 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.

_____   07/26/2022
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Principal Investigator Signature   Date Report Submitted

Project 1: Developing and Evaluating Wheat Lines for FHB Resistance in Atlantic Coastal Plain

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1. **What are the major goals and objectives of the research project?**
   The **goal of this project** is to increase the capacity and rigor required to (1) collect robust and accurate FHB resistance field data on commercially available and recently released wheat cultivars and (2) develop advanced breeding lines with further enhanced and more durable *Fusarium* head blight (FHB) resistance. This work aims to accelerate variety development and screening efforts by incorporating advanced genomics and phenomics methods that recently became available (and understood) for applied use in breeding programs.

   Research Objectives:
   1. Continue intercrossing of moderately FHB resistant elite lines to stack FHB QTL.
   2. Expand the SC scab nursery to include SunGrains DH populations and Clemson $F_{4:6}$ breeding lines.
   3. Support advanced phenomics methods and genomic selection pipelines to increase predictability of FHB resistance across collaborative breeding programs.

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. Develop biparental and 3-way crosses among moderately FHB resistant lines.
   Advanced breeding lines (*n*=36), F_1 biparentals (*n*=20), and introgression lines (*n*=25) were vernalized by refrigerating imbibed seeds at 4°C for eight weeks. Vernalized seedlings were first transplanted into greenhouse pots on 18 November 2021, and seed of the same lines were again transplanted 6 d later to stagger plantings and extend the crossing window. The F_1 seed was shipped in early March to Esten Mason at Colorado State for growout and advancement in a summer nursery in Fort Collins in order to receive F_2 seed for planting in Fall 2022. Seed from three carefully selected F_1 crosses was shipped to Heartland Plant Innovations for development of doubled haploids (DHs). Seed of additional crosses will also be sent to the USDA-ARS Raleigh DH-focused research technician (under current supervision of Gina Brown-Guedira) to obtain more DHs by Fall 2023.

   Objective 2. Expand the FHB nursery to include Clemson breeding lines.
   Field screening for scab resistance was performed at the Pee Dee Research and Education Center (REC) in Florence, SC. The nursery was planted on 12 November 2021 along with a spreader border of 'SS8641', and samples were hand-harvested in May 2022. In addition to growing advanced breeding lines from the Uniform Southern and SunGrains nurseries, the following Clemson breeding stages were also incorporated in the misted nursery: Wheat Advanced Trial (72 entries), Wheat Preliminary Trial (593 entries), and $F_{3:5}$ selections (*n*=4,000). PhD Candidate AJ Ackerman and the research technician funded by this project developed corn spawn inoculum, utilizing *F. graminearum* isolates provided by Dr. Christina Cowger (ARS-Raleigh) that were received in January 2022. Field index ratings were not recorded for this specific nursery due to a late incidence of FHB pressure that most likely

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resulted from low humidity and cool temperatures during anthesis, which slowed disease progression. However, FHB pressure was still evident prior to physiological maturity. Because blighted heads were observed, albeit at a lower frequency than in years past, replicated FHB samples were collected for entries in the Uniform Southern Scab Nursery, Uniform Southern Soft Red Winter Wheat Nursery, Gulf Atlantic Wheat Nursery, and the SunWheat trial. Harvested grain samples are currently being threshed without air and subsequently sieved to removed debris prior to FHB analysis. Fusarium-damaged kernels (FDK) will be measured using the previously-calibrated (see publication Ackerman *et al.* 2022) Vibe QM3 Grain Analyzer. After FDK analysis, samples will be ground into flour and shipped to the Schmale Lab at VA Tech for DON testing. Data collected for FDK and then DON will be disseminated to all VDHR-SWW breeders to aid in line selection and support making decisions for lines to include (or exclude) as parents for crossing to develop new doubled haploids or conventional segregating populations.

Objective 3. Support advanced techniques to increase predictability of FHB resistance.

Comparative analysis among phenotypic methods to measure FDK was completed, and results were published in the journal Agronomy in February 2022. Methods included the Vibe QM3, Perten DA7250 NIR Analyzer, SmartGrain imaging (Tanabata *et al.* 2012), and manual sorting followed by automated counting.

Postdoctoral researcher, Dr. Carolina Ballen-Taborda (not funded by project), analyzed historical phenotype data and PCR-based marker data to examine relationships between line performance for four agronomic traits (yield, test weight, heading date, and plant height) and presence/absence of major effect QTL (based on PCR-marker calls). This study dissected these relationships within the context of mega-environment across the southeastern US. This effort was performed to determine whether tradeoffs exist between the presence of FHB resistance alleles and grain yield (or test weight). In other words, is linkage drag observed to suggest FHB resistance alleles (or major effect alleles for other traits) significantly alter agronomic performance to lower yield potential or grain quality?

b) What were the significant results?

Objective 1. Develop biparental and 3-way crosses among moderately FHB resistant lines.

A total of 508 unique crosses (660 crosses including duplicates for future DH production) were made between 10 January 2022 to 7 March 2022. Of these unique crosses, 270 (53%) were 3-way crosses to stack FHB resistance QTL into segregating populations and enable selection of future lines with more durable resistance from multiple contributing QTL/sources. There were 400 crosses that had a parent carrying the Fhb1 resistance allele, 425 that contained Fhb_1B Jamestown, 435 that contained Fhb_1A Neuse, and 110 crosses that contained at least one resistance QTL from Bess (2B or 3B). Also, 61 crosses were made between elite lines with moderate resistance to FHB and introgression lines developed by Paul Murphy at NC State that possess strong Hessian fly resistance genes (H25, H33, and H25+H33) that have yet to reach the commercial soft red winter (SRW) wheat market. The SRW wheat industry primarily relies on a single locus, H13, for Hessian fly resistance, which makes this gene vulnerable to virulence as seen with other prior effective sources.

Objective 2. Expand the FHB nursery to include Clemson breeding lines.
Results are still in progress as seed from FHB samples is being processed (threshed and sieved) for measuring FDK and subsequently ground into flour to be shipped for DON analysis. The significance was effectively incorporating all Clemson breeding lines at the F_5 generation or later into the misted, inoculated nursery to screen and eliminate genotypes that were found to be highly susceptible to *F. graminearum* infection. This included planting more than 4,600 genotypes in single-row headrows for screening. To accommodate these accessions, we doubled the amount of grain spawn inoculum developed. For $F_{3:5}$ lines, we only applied scabby corn inoculum at a rate of 20 g m^{-2} instead of the 40 g m^{-2} used in headrow plots that were evaluated solely for FHB resistance. This allowed adequate seed quality of $F_{3:5}$ lines that will be needed for yield testing in the next season. Of the 4,000 F3:5 headrows evaluated, we harvested 630 (16% selection intensity) for 2022-2023 multi-location yield evaluation in the WPT. Another four introgression lines were harvested from a novel spring wheat FHB resistance source UX2060 (GP80) that was backcrossed with multiple adapted cultivars by Dr. Gina Brown-Guedira at the USDA-ARS ERSGGL. These introgression lines will be used as parents for crossing and making segregating populations with additional FHB resistance QTL.

Objective 3. Support advanced techniques to increase predictability of FHB resistance.
In this study that include more than 1,000 FHB samples, the Vibe QM3 FDK measurement had the strongest pairwise correlation with DON value (ppm) among the high-throughput, analytical platforms tested. Further, FDK results from the Vibe QM3 were used as the phenotype in a GWAS to effectively map the SNP nearest *Fhb1*, the most effective resistance QTL present in adapted SRW wheat advanced breeding lines. Finally, and importantly, FDK results using this platform was effectively utilized to build a genomic prediction model (using FDK as training data) that demonstrated high predictability of DON (*r*=0.59). All FDK values that were generated using the Vibe QM3 during the project period were reported to VDHR-SWW breeders to support breeding decisions.

Results, which were summarized in a manuscript that was submitted to Frontiers in Plant Science in June 2022, provide evidence that some linkage drag may exist for the FHB resistance QTL Fhb1, Fhb_1B Jamestown, and Fhb_1AN. However, evidence also showed that exceptional lines existed in the material evaluated that contained one or more of these FHB resistance QTL and that only in specific environments did a tradeoff emerge significant. Collectively, results suggest that tremendous progress has been made toward introgressing effective FHB resistance alleles into adapted SRW wheat material, but there is still room for improvement by further breaking up regional linkage between FHB QTL and potential deleterious genes that reduce grain yield or quality.

c) List key outcomes or other achievements.

Multiple peer-reviewed publications were developed from this project that provide in-depth analysis and reporting of (1) advanced phenotyping methods that more objectively assess FHB resistance to improve selection accuracy and breeding efficiency, (2) genomic prediction accuracy of new models that were tailored for Clemson wheat breeding material, and (3) QTL-by-environment interaction among major effect QTLs, including ones for FHB resistance such as Fhb1 and Fhb_1B Jamestown. This project also facilitated the development of and

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selection within segregating populations that really forms the foundation of a breeding program to ultimately release improved lines for producers that possess increased FHB resistance, favorable agronomic traits, and better yield. FHB nursery phenotypic data are imperative to disseminate to breeders of advanced lines for future selection as well as to seedsmen/producers of commercially available (or lines nearing release) cultivars to increase wheat acreage grown to resistant varieties year-over-year. As more growers heavily weight the importance of FHB resistance, researchers must get the information out in clear format to assist growers in making variety selection decisions.

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

Postdoctoral training. Although not funded directly by the project, Dr. Carolina Ballen-Taborda has learned the practical steps of managing a misted, inoculated FHB nursery for germplasm screening. She now has a firm working knowledge of relevant FHB resistance QTL present in SRW wheat breeding material. She has utilized existing computational skills to adapt and learn how to build genomic prediction models to estimate breeding values of genotyped Clemson lines, with receiving support and training from Jeanette Lyerly at NC State and guidance from Dr. Gina Brown-Guedira at the ARS ERSGGL. Starting in June 2021, Dr. Ballen-Taborda has learned a tremendous amount during her first year in the program, which is in-part a result of being involved in the VDHR-SWW project and interacting with this cohort of scientists.

Graduate student training. PhD Candidate (graduating in August 2022) AJ Ackerman has worked closely with the VDHR-SWW project over his graduate tenure with the program. Prior pathology experience that he gained in industry was put to good use in helping develop grain spawn inoculum from FHB isolates received from the Cowger Lab. AJ was also able to build on his management and training skills by mentoring technical staff on how to develop, manage, increase, and deploy fungal inoculum cultures for disease evaluation. In 2021, Mr. Ackerman was recognized as a prestigious Borlaug Scholar by the National Association of Plant Breeders. Another PhD student as well as an MS student helped apply scabby corn to the FHB nursery to provide some practical, hands-on learning experiences.

Technical support staff training. Research technicians, Ryan Holmes and William Caughman, who are partly supported by this project, have developed a firm working knowledge of small grains breeding and genetics. Tasks that have been educational include learning how to (1) make hand emasculations and pollinations in wheat, (2) implement an effective FHB nursery that includes setting up an elaborate misting irrigation system, (3) developing and maintaining fungal inoculum, and (4) collect pertinent data on multiple FHB resistance traits such as FHB index rating, FDK, and DON. Both technicians have learned how to operate the Vibe QM3 grain analyzer for FDK analysis.

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

Results from the advanced FDK phenotyping effort were drafted into an original research article that was published in the journal Agronomy in February 2022. Results from the work on understanding linkage drag between FHB resistance (and other trait) QTLs across sets of environments in the southeastern US were reported in a manuscript submitted to Frontiers in Plant Science in June 2022 by postdoctoral fellow Dr. Carolina Ballen-Taborda. Data from the misted FHB nursery collected on advanced breeding lines will be circulated to VDHR-SWW breeders via email (in the form of standard Excel spreadsheets) as soon as complete. South Carolina growers and other producers in neighboring states can access an updated list of recommended SRW wheat cultivars that have moderate resistance to FHB through the ScabSmart website.

Project 2: Double Haploids to Expedite Development of FHB Resistant Soft Winter Wheat Varieties

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

   Goal: Expedite wheat cultivar development for improving FHB resistance using established doubled haploid (DH) technology.

   Objective: For this proposal, each program (VA, GA, AR, LA, **SC**, TX, MD) will use one or more crosses to develop 350+ double haploid lines per year. These lines will be genotyped in collaboration with the Eastern Regional Genotyping Center and collaboratively phenotyped through exchange after initial selection for basic adaptation.

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?**

   Development of new DH populations. Of the 644 crosses successfully made in the SC 2021 greenhouse nursery, three biparental F_1s were carefully selected for DH development. Seeds from these three F_1 crosses were shipped to Heartland Plant Innovations (HPI) on 28 July 2021 to produce approximately 150 DHs per population. A report back from HPI on 29 June 2022 stated DH plants were either in the greenhouse maturing for seed increase or in the process of receiving colchicine treatment to undergo chromosome doubling. This puts HPI on pace to send DH seed back to Clemson by the November 2022 planting deadline, where seed will be increased in the field in headrows and backed up (if needed) in the greenhouse for evaluation and increase.

   Selection and advancement of existing DH lines. The most promising wheat DH line currently in advanced testing from the Clemson program is the shared VDHR-SWW line SCLANC11558-33, which was initially selected by NC State, picked up by LSU, and then passed to Clemson for additional testing. This line has multiple resistance QTLs, Fhb_1B Jamestown and Fhb_1A Neuse, that act together to provide equal the level of FHB resistance of Fhb1 alone. Thus, this line has above average FHB resistance.

   Previously developed DH selections from the VDHR-SWW project were evaluated in the Clemson WPT (single replication yield trial planted in two SC locations— Florence and Clemson). Sixty-one of the 558 (11%) entries in the WPT were DH lines from the VDHR-SWW project. Of these 61 DHs, 51 were genotyped using genotyping-by-sequencing. Using genotype data only, existing SunGrains training populations were leveraged in genomic prediction to generate genomic estimated breeding values (GEBVs) for the DH lines. Using this GBS data, NC State also used a machine learning approach (see Winn _et al._ 2022, TAG) to predict allele presence at major effect QTLs.

First-year shared VDHR-SWW DH lines were planted in headrows in 2021-2022. Of the 298 shared DHs planted, 20 were selected for placement into the 2022-2023 WPT for yield evaluation. Seed for selected DHs will be sent to VDHR-SWW breeders to plant a headrow and get a final chance to select and advance a given DH line for further testing or use in breeding (*e.g.*, crossing parent).

b) What were the significant results?

Development of new DH populations. The following three F_1 pedigrees were selected for DH production:

Population 1: **15VDH-FHB-MAS38-01** / LA12275DH-56
Population 2: **GA15VDH-FHB-MAS23-18LE43F** / <u>14VDH-SRW14-150</u>
Population 3: **SCGA17VADH-FHB-MAS1702-179-6** / TX16DDH579
   Lines in **bold possess the favorable Fhb1 allele for FHB resistance
   **Lines in <u>underline</u> possess the favorable H13 allele for Hessian fly resistance

This will enable approximately 150 DH lines per population to be evaluated in 2022-2023 in headrows. Selected lines from these populations that all segregate for Fhb1 and H13 will be advanced into yield testing in 2023-2024. This established, crucial DH pipeline accelerates the time from cross made to yield testing to just 2.5 years, which is significantly down from five years using conventional breeding methods of year-over-year selection and yield testing at the F_6 generation. Reducing the time from cross to yield evaluation by 50% has substantial, positive effects on genetic gain.

Selection and advancement of existing DH lines. In the 2021-2022 season, the shared VDHR DH line SCLANC11558-33 ranked 2nd in statewide yield average in Georgia. This line was increased on five acres of land by the SC Crop Improvement Association to generate a large, pure source of Foundation seed. A final decision on whether to release this DH line will be made at the upcoming SunGrains summer meeting that will be held from 26-28 August 2022.

Two VDHR-SWW SC DHs were among the top 15 highest yielding in the 2021-2022 WPT, which is exceptional given that the WPT contained more than 550 entries. Out of the 61 DHs that were included in this yield trial, 10-12 will be advanced into multistate testing in 2022-2023. Based on machine learning predictions, 10 DHs carried the resistance Fhb1 allele while 90% (46 of 51) of lines were predicted to carry a resistance allele at a minimum of one QTL conferring FHB resistance. Several DHs had either 3+ FHB resistance QTLs or 2+ FHB QTLs in addition to also possessing H13, which is the leading source of Hessian fly resistance in adapted SRW wheat germplasm.

Of the 298 shared DHs planted in headrows in the Florence 2021-2022 field nursery, 20 were selected for placement into the 2022-2023 WPT for yield evaluation. Seed of these 20 selected DHs will be sent to VDHR-SWW breeders to plant a headrow and get a final chance to select and advance a given DH line for further testing or use in breeding (*e.g.*, crossing parent).

c) **List key outcomes or other achievements.**

Four of the top seven, and seven of the top 15, highest yielding lines in the 2021-2022 Gulf Atlantic Wheat Nursery (GAWN) were DH lines. Further, seven of the top 13 yielding lines in the 2021-2022 SunWheat (SunGrains advanced Wheat) nursery were also DHs. Most of the lines that possess the Fhb1 resistance allele that were evaluated in advanced nurseries are also DHs, simply due to the reduced breeding cycle time facilitated by DH technology. On average, DH entries in the GAWN (26 of 57 total) demonstrated greater FHB resistance than conventional lines (21.2% FDK for DH vs. 25.1% FDK for conventional), excluding standard checks. The fact that so many DH lines generated by VDHR-SWW funded projects are reaching advanced testing and even cultivar release is a testament to the impact this project has on developing cultivars with improved FHB resistance.

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

Nothing to report.

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

The list of 20 shared DH lines that were selected and harvested by the Clemson program in spring 2022 was circulated to all VDHR-SWW CP members via email on 20 July 2022. The list of pedigrees from the crosses selected for DH production in 2021 were also circulated to the VDHR-SWW breeders to avoid duplicating efforts. Yield data and associated trait data from advanced nurseries that include DH entries are circulated periodically via email among SunGrains breeders as well as stored on the SunGrains website (www.sungrains.lsu.edu) that is maintained by LSU.

Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your <u>FHB work</u> that were a result of funding from your FY21 grant award. Only citations for publications <u>published</u> (submitted or accepted) or presentations <u>presented</u> 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 grant 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).

Ackerman AJ, Holmes R, Gaskins E, Jordan K, Hicks D, Fitzgerald J, Griffey C, Mason RE, Harrison S, Murphy JP, Cowger C, **Boyles R***(2022) Evaluation of methods for measuring *Fusarium*-damaged kernels of wheat. Agronomy 12(*2*):532. doi:10.3390/agronomy12020532
<u>Status:</u> Published
<u>Acknowledgment of Federal Support:</u> Yes

Mergoum M, Johnson J, Buck J, Buntin D, Sutton S, Lopez B, Mailhot D, Chen Z, Bland D, Harrison S, Murphy JP, Mason RE, Sutton R, Babar A, Brown-Guedira, Ibrahim A, **Boyles R**, Baik B-K, Marshall D, Griffey C, Cambron S, Chen X, Cowger C (2022) A new soft red winter wheat cultivar 'GA 08535-15LE29' adapted to Georgia and the U.S. southeast region. J Plant Registr. doi: 10.1002/plr2.20235
<u>Status:</u> Published – Early View
<u>Acknowledgment of Federal Support:</u> Yes

Winn Z, Lyerly J, Ward B, Brown-Guedira G, **Boyles R**, Mergoum M, Johnson J, Harrison S, Babar A, Mason RE, Sutton R, Murphy JP. Profiling of Fusarium head blight resistance QTL haplotypes through molecular markers, genotyping-by-sequencing, and machine learning. Theor Appl Genet.
<u>Status:</u> Published – Early View
<u>Acknowledgment of Federal Support:</u> Yes

Ballén-Taborda C, Lyerly J, Smith J, Howell K, Brown-Guedira G, Babar A, Harrison S, Mason RE, Mergoum M, Murphy JP, Sutton R, Griffey C, **Boyles R***. Utilizing genomics and historical data to optimize gene pools for new breeding programs: A case study in winter wheat. Front Plant Sci.
<u>Status:</u> Under review
<u>Acknowledgment of Federal Support:</u> Yes

Books or other non-periodical, one-time publications as a result of FY21 grant 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).

Mason E, Roberts T, **Boyles R**, Acuna A, Arguello M, Ballesteros D, Subramanian N (2021) Chapter 6: Molecular breeding for improving waterlogging resistance in wheat*. In: Alam M, Hossain M, Henry R, Seneweera S, Rakshit S (Eds.) Molecular breeding in wheat, maize, and sorghum: Strategies for improving abiotic stress tolerance and yield. Boston, MA: CAB International Publishing.
Status: Published
Acknowledgment of Federal Support: No

Other publications, conference papers and presentations as a result of FY21 grant award

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

Boyles R. Overview of Clemson Wheat Breeding Program. USDA OREI Organic Plant Breeding Institute, Clemson University, Clemson, SC (June 9, 2021) — *virtual oral presentation*

Boyles R. Genomic prediction models and implementation. USDA OREI Organic Plant Breeding Institute, Clemson University, Clemson, SC (June 9, 2021) — *virtual oral presentation*

DeWitt N, Guedira M, Lyerly J, Ward B, Murphy JP, Marshall D, Santantonio N, Griffey C, **Boyles R**, Mergoum M, Johnson J, Harrison S, Babar A, Mason RE, Ibrahim A, Van Sanford D, Tiwari V, Kuraparthy V, Kolb F, Sneller C, McKendry A, Mohammadi M, Maltecca C, Tiezzi F, Brown-Guedira G. Unpacking the yield effects of major heading date alleles in wheat through joint analysis of historic breeding panels and their climates. ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT (November 10, 2021) — *poster presentation*

Boyles R. Small grains breeding in the Southeast, New Crops for the Southeast, Tennessee State University, Nashville, TN (March 17, 2022) — *virtual oral presentation*