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

# FY21 FINAL Performance Progress Report

**Due date:** July 26, 2023

## Cover Page

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| **USDA-ARS Agreement ID:** | 59-0206-0-187 |
| **USDA-ARS Agreement Title:** | Microbial Interactions with Wheat and Barley Pathogens. |
| **Principle Investigator (PI):** | David Haak |
| **Institution:** | Virginia Tech. |
| **Institution UEI:** | QDE5UHE5XD16 |
| **Fiscal Year:** | 2021 |
| **FY21 USDA-ARS Award Amount:** | $36,251 |
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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 |
|---|---|---|
| PBG | Managing the Phytomicrobiome for Increased Disease Resistance | $36,251 |
| | **FY21 Total ARS Award Amount** | $36,251 |

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.*

David C Haak

Principal Investigator Signature

25-JUL-2023

Date Report Submitted

‡ 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:** Managing the Phytomicrobiome for Increased Disease Resistance

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

Our goal in this two-year USWBSI project is to characterize the host genotype x microbiome x pathogen interactions among wheat varieties with varying resistance levels in a systems framework. The specific objectives are:

1. Describe the metagenomic network landscape for the interactions of host-microbiome-pathogen in susceptible, moderate resistant, and resistant wheat varieties across two locations.
2. Identify microbe and microbial community nodes associated with *Fusarium* load.
3. Identify microbial metabolism genes and correlate their abundance with *Fusarium load.*

**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. Describe the metagenomic network landscape for the interactions of host-microbiome-pathogen in susceptible, moderate resistant, and resistant wheat varieties.*

This objective was designed to generate information about the interactions between host resistance genotype and the microbial community under pathogen infection. We have generated substantial amplicon data describing these interactions across 18 genotypes and two growth stages and 3 locations. From these we have generated 32 metagenomes across 2 comparative locations. This resulted in a total of ca. 1.3Gbp of sequence data with an average of ca. 41M reads per sample, or about 260M reads per resistance genotype. Assembly of these metagenomic reads resulted in relatively complete metagenomes with an average of 88.9% of raw reads mapping back to these assemblies.

*Objective 2. Identify microbe and microbial community nodes associated with Fusarium load.*

This objective uses the metagenomic analyses data produced from sequencing samples in objective 1 with an aim to connect microbial taxa with the presence of *Fusarium*. Analysis of these data is underway. Already, we have identified patterns of community shifts associated with the presence of *Fusarium*. In addition, we have identified changes in the species of *Fusarium* present in the sample. We have developed taxonomy networks across the resistance genotypes and are developing multi-layer networks to identify nodes associated with changes in Fusarium Head Blight index and quantitative estimates of *Fusarium graminearum* abundance. To accomplish this we have developed a bioinformatic tool based on machine learning to integrate these datasets.

*Objective 3. Identify microbial metabolism genes and correlate their abundance with Fusarium load.*

Similar with Objective 2, this objective uses data generated in objective 1 to and assign function to microbial reads to identify functions and genes that change in abundance among community members in the presence of fusarium. While still in the early analytical stages we have generated metabolic pathway models from the metagenomic sequencing.

**b) What were the significant results?**

Analyses of the data under objective 1 have revealed some interesting findings. First, we have found that bacteria in this system comprise 50-85% of the most abundant reads. Among the fungal taxa present *Fusarium spp.* and *Parastagnospora spp.* are among the most abundant. Predictably, among the *Fusarium spp.* present *Fusarium graminearum* shifts in abundance from 54% to > 90% corresponding with visual FHB ratings, but that the dominant species also shifts according to host resistance genotype. Predictably, *F. graminearum* is the most abundant fungal taxon among susceptible isolates, however among lines with resistance or moderate resistance *Parastangospora nodorum* is the most prevalent.

Exploring the communities present under objective 2 we find that these communities are relatively simple, with a range from 289-656 bacteria identified across samples. Consistent with our amplicon data showing differences in communities among host resistance genotype, community size decreased with host resistance type, with averages of 586 for sensitive, 486 for moderate resistance, and 468 for resistance genotypes. The same pattern holds for bins, or metagenome assembled genomes, from the metagenomic sequence reads, with averages of 10.6, 7, and 6.6 respectively, without any difference in read depth. Of these notable shifts in these communities occurs among host resistance genotype (R, MR, S), even grown in the same field, with taxa like *Sphingomonas* exhibiting substantially reduced relative abundance in MR and R lines in the presence of *Fusarium.* Conversely members of the genus *Methylobacterium* do not show a change in relative abundance across R, MR, or S hosts. Finally, we find shifts in particular members of these genera, which have previously been identified with suppression of FHB, across host genotype. To identify important nodes that shift between R, MR, and S genotypes *and Fusarium* abundance, we have developed an AI-based classifier to define relationships among taxonomic networks. While this work is in progress, we have identified surprisingly few but highly confident groups. For instance, between R and S groups we have identified changes in *Sphingomonas spp.,* consistent with our amplicon data. The next step will be connecting these changes with differences in infection to elucidate how these bacteria interact with the pathogen.

While still early, investigating shifts in functional roles across these data under objective 3 we have identified over 2,000 unique functional categories in an average of 9400 Kegg functions. Within these categories functional modules include metabolic pathways, environmental metabolism, cofactor biosynthesis, and importantly, secondary metabolism. The highest Kegg function estimates of transcribed genes are predictably contained in standard metabolism (e.g., iron

complex receptor protein, methyl-accepting chemotaxis protein), but specialized metabolites such as, microsomal epoxide hydrolase, showed differences in copy number across samples. Preliminary networks from these functional annotations have 3000-5000 nodes with 20,000-30,000 edges. Once fully constructed we will again apply our multi-layer analysis tool, to connect functional shifts with changes in *Fusarium* abundance and FHB index.

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

This work has already uncovered some important interactions among the microbial members in the wheat grain head microbiome. In addition, preliminary results from this work have been used as preliminary data in a large federal proposal. The bioinformatic workflows that are being built for these data have been used in graduate training and education as part of a course "Introduction to Microbial Community Analysis".

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

While some of our work was delayed as a result of the COVID-19 pandemic and subsequent turnover in study personnel, we have been able to train a number of people across academic levels. This project has supported the training of an undergraduate student and 2 graduate students, as well as one postdoctoral research associate. In addition, while the initial personnel trained as part of this project have graduated, the data generated are currently being used by a computational graduate student as part of their dissertation work. Graduate student training has included cross-training in field, lab, and bioinformatic techniques and approaches. The results from work completed to date have been used to enhance training in a graduate level course in bioinformatics. Finally, results from an undergraduate project investigating endophytes in mature kernels, is being prepared for publication.

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

These results are being prepared for a presentation at the National Fusarium Head Blight Forum 2023. Unfortunately, attendance and planned presentation at the 2022 Forum was disrupted by jury duty. In addition, we have connected with growers about this project through collaborations with the small-grains breeding program at Virginia Tech.

# 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). |
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## 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.