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

FY21 FINAL Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-1-202
USDA-ARS Agreement Title:	Harnessing the microbiome for protection from Fusarium Head Blight
Principle Investigator (PI):	Barney Geddes
Institution:	North Dakota State University
Institution UEI:	EZ4WPGRE1RD5
Fiscal Year:	2021
FY21 USDA-ARS Award Amount:	$41,062
PI Mailing Address:	North Dakota State University, Department of Microbiological Sciences Van Es Hall, 1523 Centennial Blvd. Fargo, ND 58102
PI E-mail:	barney.geddes@ndsu.edu
PI Phone:	701-231-7692
Period of Performance:	6/1/21 - 5/31/23
Reporting Period End Date:	5/31/2023

USWBSI Individual Project(s)

USWBSI Research Category[1*]	Project Title	ARS Award Amount
TSCI	Breeding Potential for Microbiome Protection against Fusarium Head Blight	$41,062
	FY21 Total ARS Award Amount	$41,062

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.

Principal Investigator Signature

DocuSigned by:
Barney Geddes
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Date Report Submitted

07/26/2023

[1] 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: Breeding Potential for Microbiome Protection against Fusarium Head Blight

PI: Geddes, Barney | Agreement #: 59-0206-1-202

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

The major goal is to identify FHB recruited and genotype-responsive microbes in barley.

This was to be accomplished by: 1) Identification of groups of microbes (taxa) that are recruited in response to FHB disease using diverse germplasm from a training population. and 2) Identification of groups of microbes with differential abundance across barley genotypes.

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?

In the summer of 2021, we sampled head tissue from four separate mist-inoculated, breeding nurseries. We collected head samples from 10 genotypes per site and scored visual symptoms of disease. We sampled from each location, but with the severe drought of 2021, the entire training population was only kept for St. Paul, MN.

Head samples for microbiome processing were brought back to NDSU, ground to fine powder, and sampled for both toxin and DNA extractions. DNA was extracted from all 400 samples (4 sites x 10 genotypes x 2 disease status [yes/no] x 5 replicates).

At NDSU, all steps for the bacterial microbiome prep were optimized and >90% of the samples successfully sequenced. A small number of samples are currently being resequenced due to low quality read contamination or low sequencing depth overall. Data analysis for the bacterial microbiome results has been refined and it was determined that a second year of data would be necessary.

In addition, Co-PI Baldwin's lab had optimized qPCR primers for amplifying barley host DNA as a paired comparison with known *Fusarium graminearum* qPCR primer sets. The results of the qPCR were used to obtain Fusarium biomass in all samples and a quantitative metric of disease.

Fungal microbiome library optimization and sequencing was performed at the . At the USDA-ARS in Peoria. Preliminary sequencing efforts have been performed to identify a set of fungal primers that will reduce non-target host genomic amplification and capture the greatest degree of inherent fungal diversity in samples. Preliminary analysis of fungal data had been performed.

b) What were the significant results?

i. Analysis of the bacterial microbiome

The bacterial microbiome was analyzed by sequencing the 16S region and assigning ASVs for taxonomic comparison. The experimental workflow for sample collection, material processing, and data analysis is shown in Figure 1. The materials processing pipeline was the same for both the bacterial and fungal microbiome analyses.

Bacterial community composition was measured and location was found to have a large impact on community composition (Fig. 2), therefore, analyses were split by location.

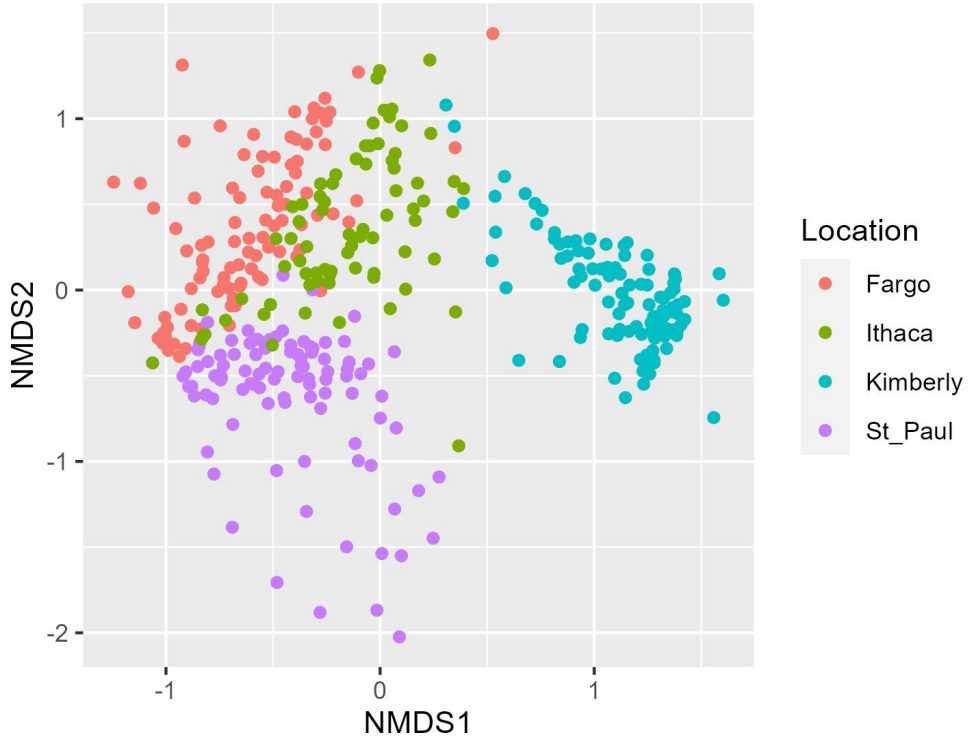

Figure 1. Beta diversity (Bray-NMDS) analysis of bacterial analysis across the study demonstrating significant impact of location on microbiome composition.

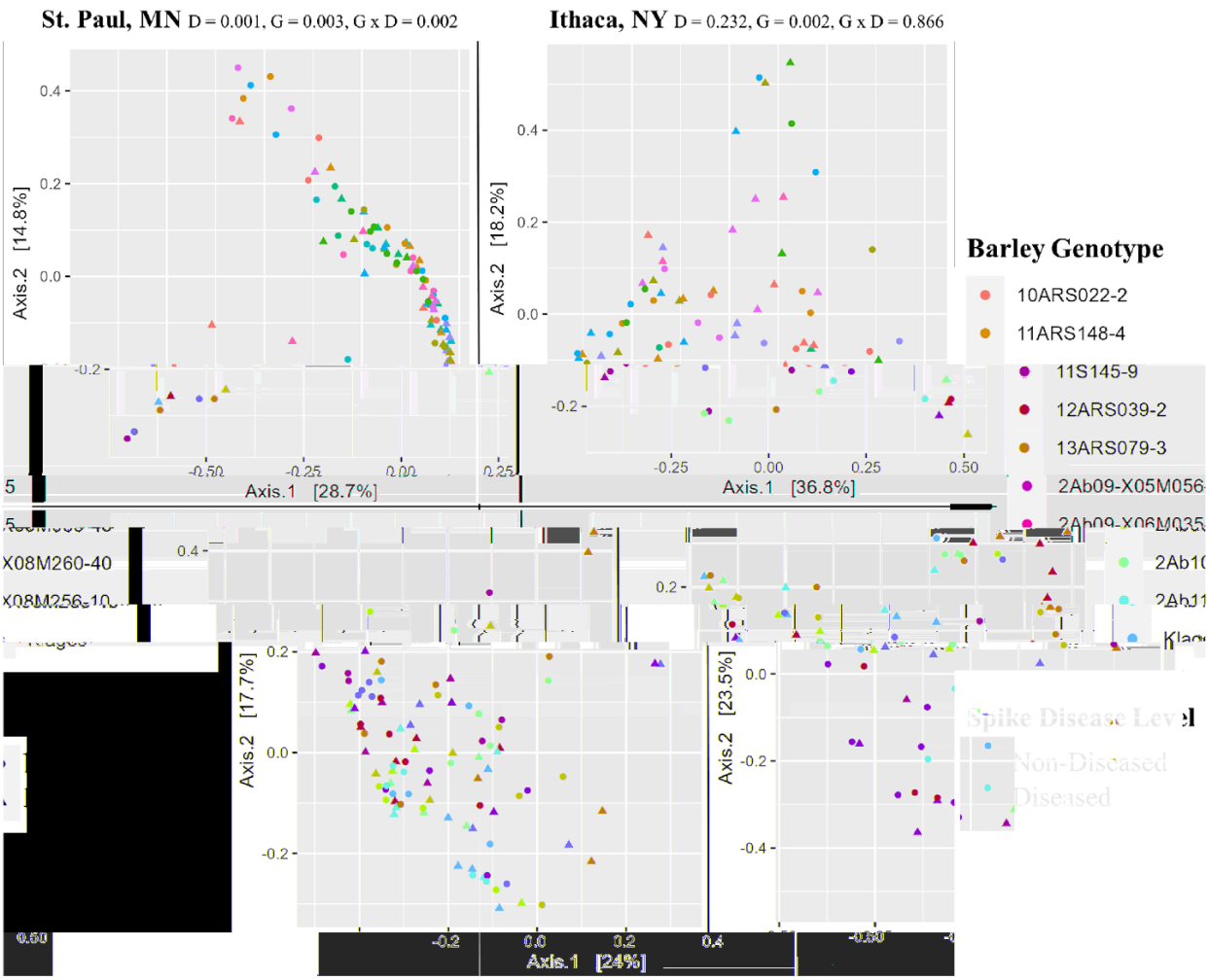

Figure 2. Beta diversity (Bray-NMDS) of bacterial microbiome by location. P-values are given. Disease (D), Genotype (G), Genotype by Disease (G x D).

Three of the four locations had significant genotype by disease effects and MN was the only location with a significant main effect of disease (Fig. 3). All locations had a significant effect by genotype (Fig. 3), suggesting a major role for barley genetics in the recruitment and retention of the microbiome in the phyllosphere. Locations had different genotypes showing a significant effect by disease (ND [1], MN [1], ID [3]) (Data not shown).

ii. Analysis of fungal microbiome

Fungal microbiome analysis was also completed by ITS sequencing and analysis, following evaluation of PNA blocker technology to limit barleyDNA amplification during ITS amplification (Figure 5 and 6). Similar to bacterial analysis, location was

found to be a major driver of the fungal microbiome. Location as a major driver is as expected with a large geographically diverse study such as this.

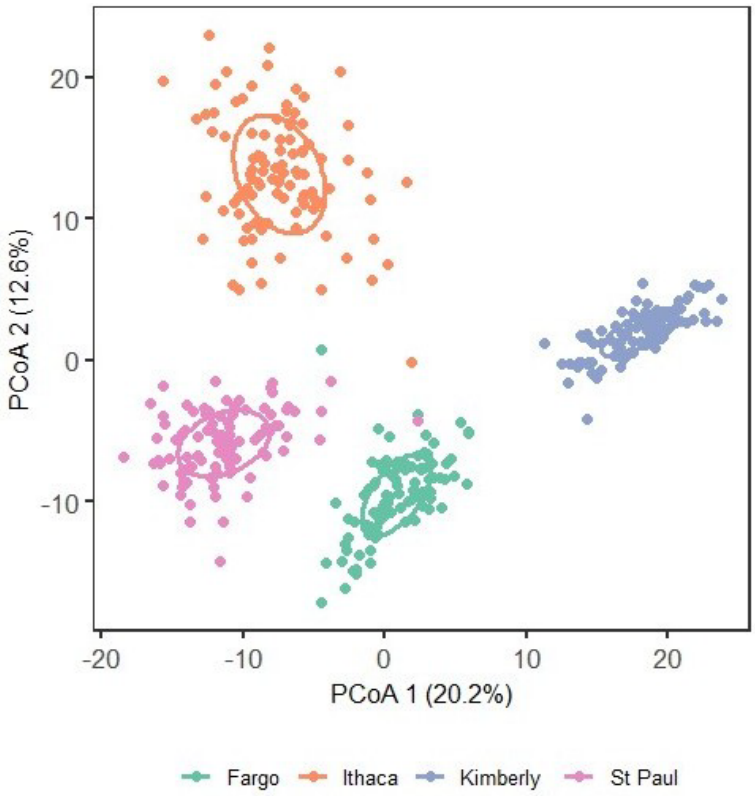

Figure 3. Beta Diversity (Principle Coordinate Analysis) of fungal ITS sequence diversity by location.

When subdivided by location and analyzed for effects of genotype and disease, disease showed a more profound effect on fungal community structure than disease community structure (Figure 3). Similar to bacterial microbiome composition, some effects were observed by genotype, although the specific genotypes responding varied by location (Figure 4).

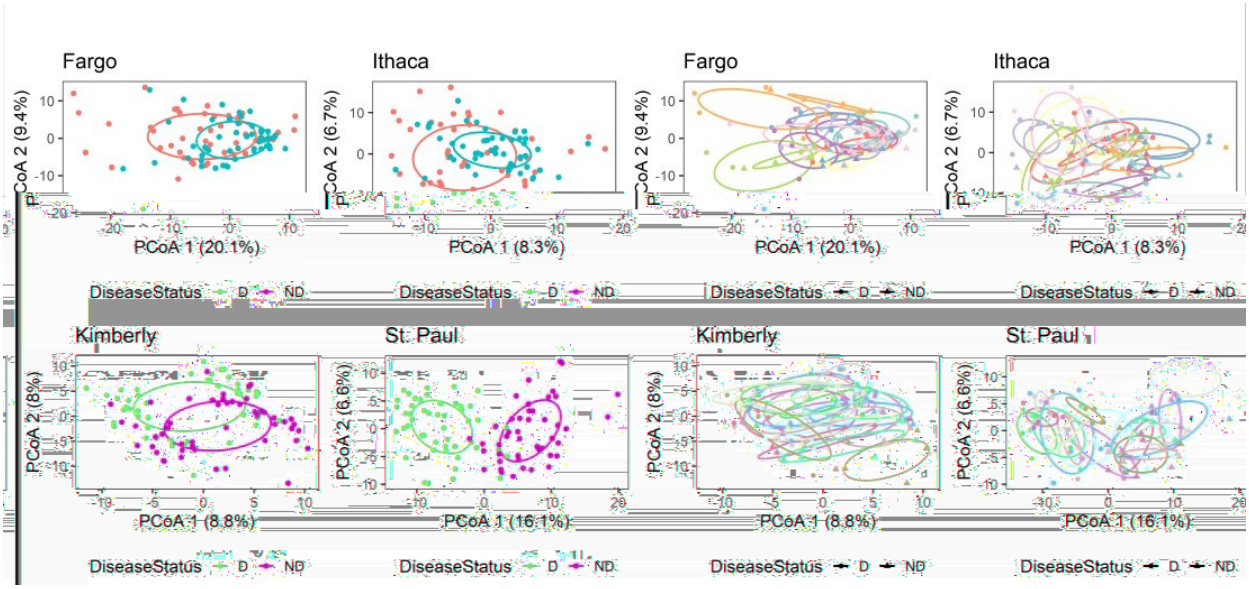

Figure 4. Beta diversity (Principle Coordinate Analysis) of fungal community structure at each location by disease (left two panels) and location (right two panels)

c) List key outcomes or other achievements.

Key outcomes include evidence for community response to disease and to genotype. In addition the data will allow the identification of genotype and disease responsive taxa. Due to low disease pressure, it is believed that a second site year of data is required before conclusively identifying disease and genotype-responsive taxa which will be done following the completion of a second year of sample collection and microbiome analysis in Year 2. (Currently ongoing with FY22 support).

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

Because of the success of this project we were able to send a graduate student to the following conferences to present these results

1. 31st Fungal Genetics Conference, Pacific Grove, CA (Poster)
2. Scabforum 2022, Tampa FL (Poster)
3. IS-MPMI 2023, Providence, RI (Poster)
4. Plant Health 2023, Denver, CO (Travel Award for Microbiome workshop)

In addition, the graduate student received hands on training in the laboratory

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

We have presented the results at scientific conferences across the US (see 3). We plan to combine the results with Year 2 data in 2 manuscripts published in peer-reviewed journals.

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).

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.

Benz, Brooke R., Navasca, A., Velasco, D., Banerjee, S., Whitaker, B., Geddes, B. A., and Baldwin, T. Genotypes and Fusarium head blight selection for microbiomes across barley spikes. Poster and abstract presented at 31st Fungal Genetics Conference, Pacific Grove, California, USA., March 17, 2021.

Benz, N. R., Navasca, A. R., Velasco, D. D., Lopez-Echartea, E., Banerjee, S., Whitaker, B., Baldwin, T., Geddes, B. A. (2022). Genotype and Fusarium headblight selection for microbiomes across barley spikes. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa Bay, Florida. December 5, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf

Benz, N. R., Navasca, A. R., Velasco, D. D., Lopez-Echartea, E., Banerjee, S., Whitaker, B., Baldwin, T., Geddes, B. A. Genotype and Fusarium Head Blight Selection for Microbiomes Across Barley Spikes While Incorporating High-Throughput Bacterial Culturing. Poster and abstract presented at 2023 International Society of Plant-Microbe Interactions Conference. Providence, Rhode Island, USA., July 17,2023.