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

FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

Principle Investigator (PI):	Barney Geddes
Institution:	North Dakota State University
E-mail:	barney.geddes@ndsu.edu
Phone:	701-231-7692
Fiscal Year:	2021
USDA-ARS Agreement ID:	59-0206-1-202
USDA-ARS Agreement Title:	Harnessing the microbiome for protection from Fusarium Head Blight
FY20 USDA-ARS Award Amount:	$41,062
Recipient Organization:	North Dakota State University Department of Microbiological Sciences Van Es Hall, 1523 Centennial Blvd. Fargo, ND 58102
DUNS Number:	80-388-2299
EIN:	45-6002439
Recipient Identifying Number or Account Number, if any:	
Project/Grant Period:	6/1/21 - 5/31/23
Reporting Period End Date:	5/31/2022

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 an: X 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.

DocuSigned by:
Barney Geddes
C437520869FF41B...
Principal Investigator Signature

07/25/2022
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: Breeding Potential for Microbiome Protection against Fusarium Head Blight

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

The major objective of this research project was to: ***identify FHB recruited and genotype-responsive microbes in barley***.

This will 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?

Objective 1: 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. Whole plot toxin samples are also being obtained from across all locations (ongoing).

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 will need to be resequenced due to low quality read contamination or low sequencing depth overall. Preliminary data analysis for the bacterial microbiome results has begun and is ongoing.

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

After extraction of DNA from all samples and quality testing, an aliquot of the normalized DNA extraction was shipped to the USDA-ARS in Peoria for fungal microbiome library optimization and sequencing. At the USDA-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.

b) What were the significant results?

During the 2021 field season, much of the upper midwest experienced severe drought conditions and as a result disease severity was exceptionally low at three of the four sites tested (Figure 1). The St. Paul Minnesota site was the only site with visible disease scores above 50% on average.

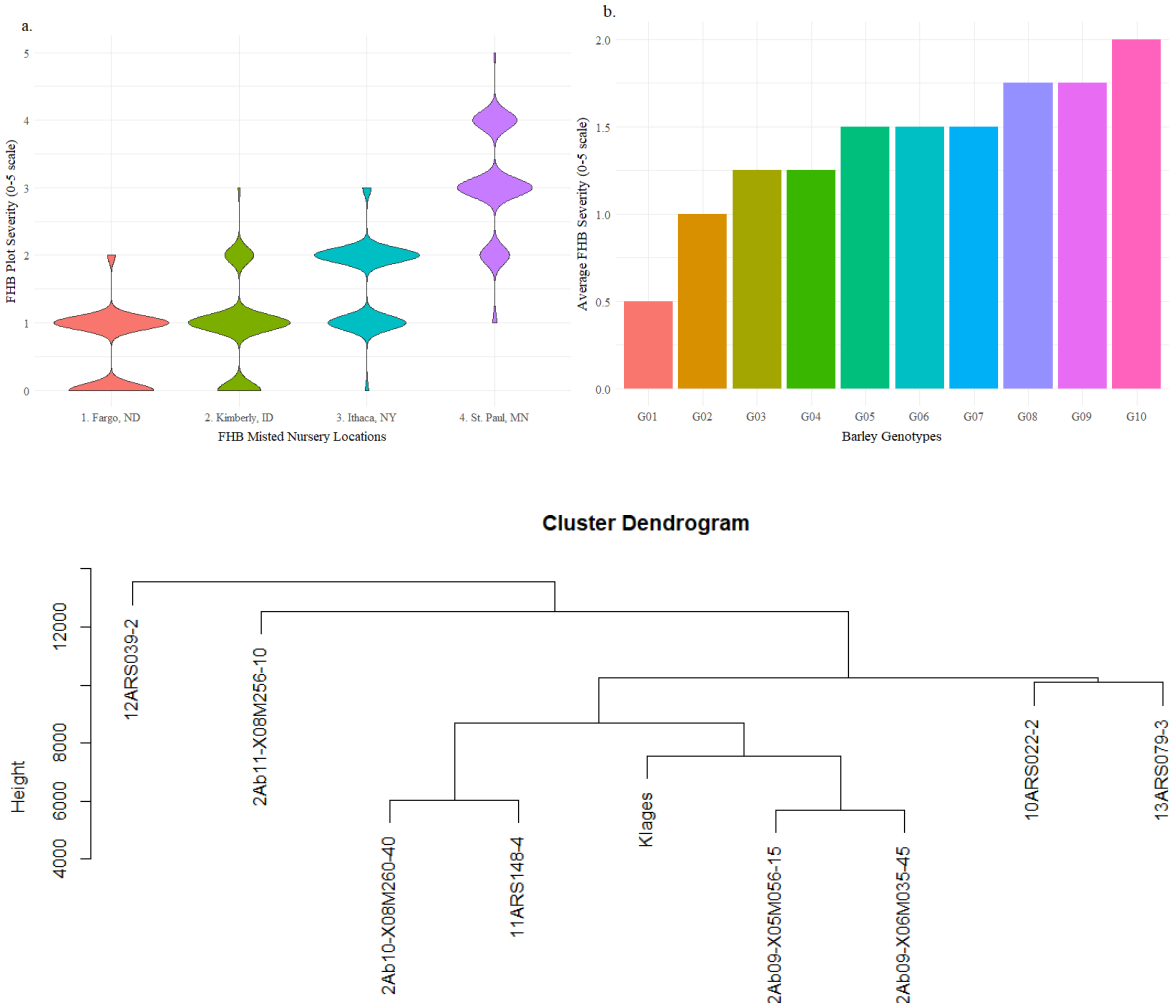

Figure 1: Average FHB visual severity measurements for the Aberdeen training population a) grown in 4 misted FHB evaluation nurseries and b) across 10 barley genotypes. The four misted nurseries spanned four States in the U.S. including North Dakota (ND), Idaho (ID), New York (NY), and Minnesota (MN). FHB Severity (0-5 scale) represents the visual severity of 248 barley genotypes planted in each location. Statistical significance was tested by ANOVA ($p < 0.05$). Cluster Dendrogram of the 9 of 10 barley lines c) genotyped by 50K illumina chip*. Tenth barley line has an error in the genotyping file.

The microbial community composition was heavily influenced by location (Figure 2b), and overall species richness was low across all locations (Figure 2a). In particular, Fargo and Ithaca showed exceptionally low species richness (<25 bacterial taxa on average), which is expected to reduce the power of the downstream analyses to detect genotype-responsive and disease-recruited taxa.

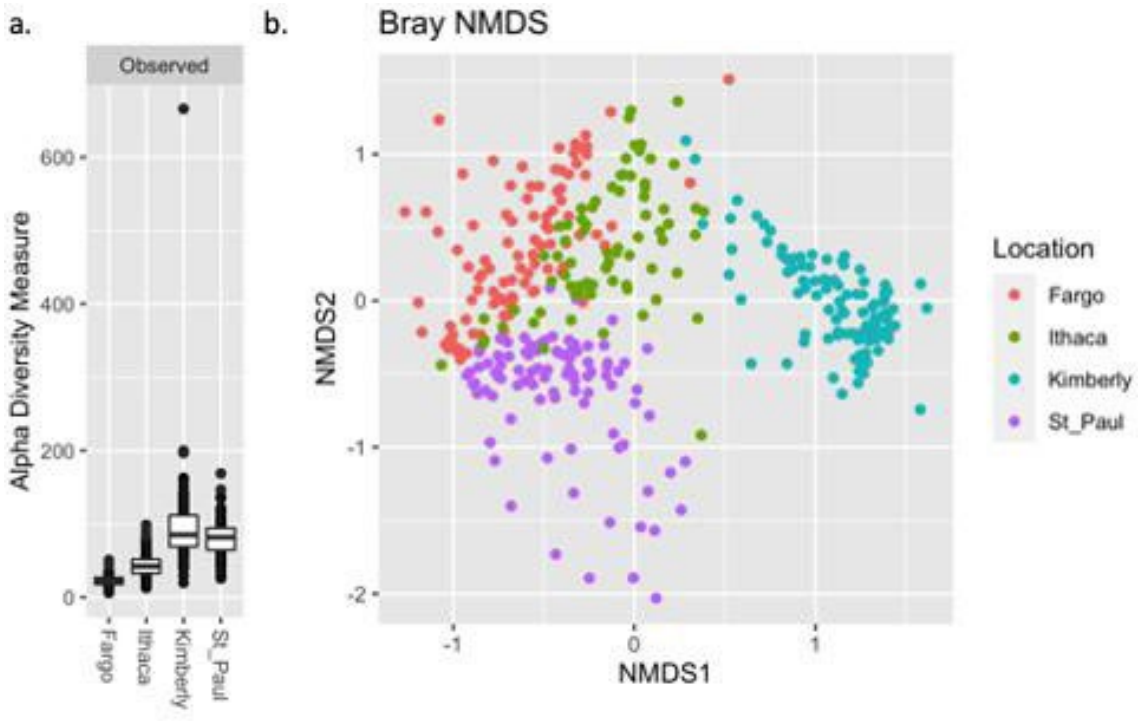

Figure 2: Bacterial community responses across the four nurseries. a) The observed richness of amplicon sequence variants (i.e., number of unique taxa) across locations. b) Differences in communities as assessed by nonmetric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity. The further apart points are in NMDS ordination, the more dissimilar the microbial communities of the two samples.

We detected differences in microbial community composition between genotypes and between diseased and non-diseased samples. In preliminary analysis, differences in bacterial community structure were analyzed by PERMANOVA (permutational multivariate analysis of variance) of a Bray-Curtis dissimilarity matrix for each location with genotype or disease as factors. At all four sites genotype was found to have a significant effect on microbial community composition (Fargo P=0.001, Kimberly P = 0.001, St Paul P = 0.005, Kimberly P = 0.002). Disease was only found to have a significant effect on community composition at St Paul (P = 0.001), where the disease severity was highest (Figure 1) and drought impacts were lowest. The relative abundance of the 30 most abundant taxa at St Paul is shown in Figure 3 as an example. Although disease was not found to be a major factor in microbial community composition at other locations, within specific genotypes we did observe evidence for

disease responsive taxa. For example in the genotype 12ARS039-2 at Fargo, a member of the genus Pantoea, a well known plant growth promoting taxa that has been shown to inhibit FHB was found to be enriched by Deseq analysis (log2 fold change = 5.15065, P= 1.54326e-04).

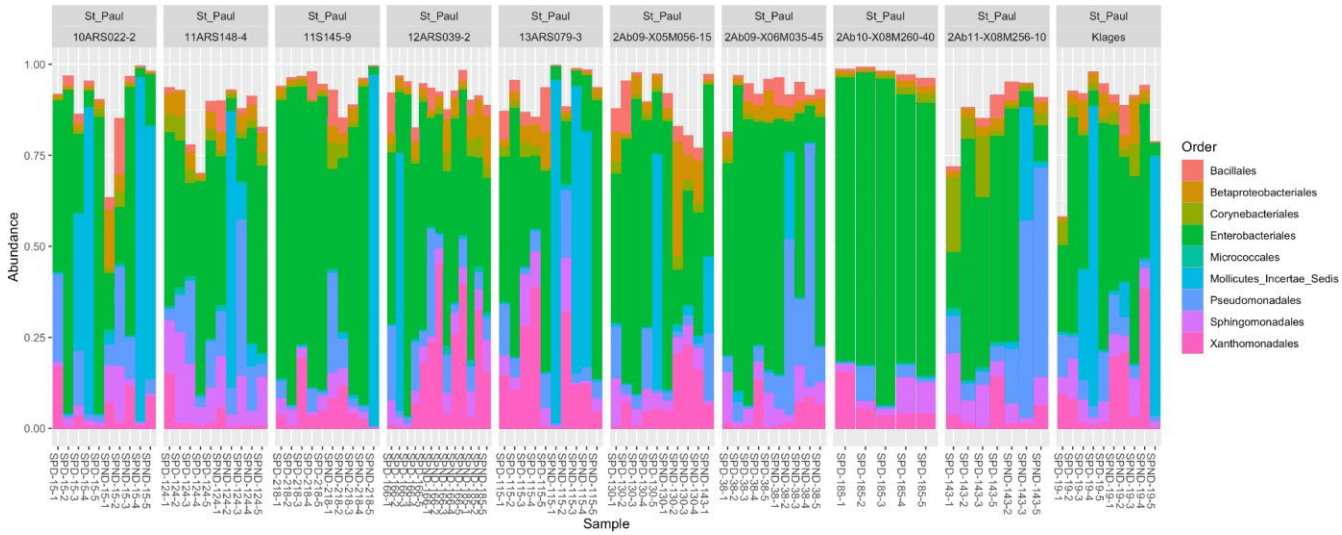

Figure 3: Relative abundance of the top 30 most common bacterial taxa in the St Paul location. Taxa are coloured by the taxonomic rank Order. Within each genotype, diseased samples are arranged on the left and non-disease samples on the right.

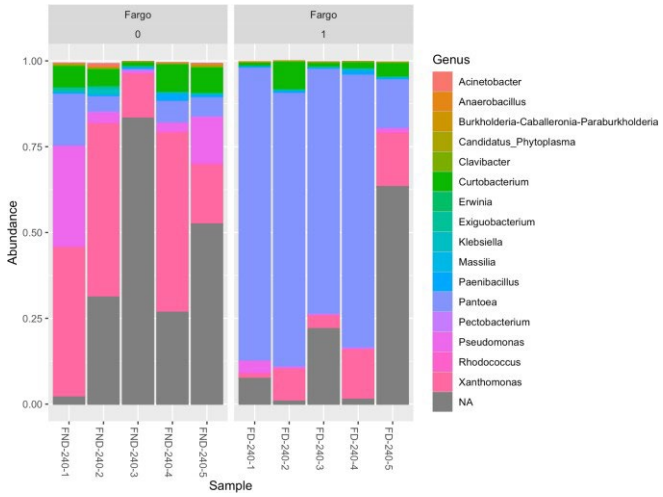

Figure 4: Relative abundance of the top 30 most common bacterial taxa of genotype n. Taxa are coloured by the taxonomic rank Genus. Within one genotype (12ARS039-2) at Fargo location, diseased samples are arranged on the left (0) and non-disease samples on the right (1).

For the fungal microbiome, work at USDA Peoria is ongoing to optimize fungal library preparation and identify fungal primer sets that preferentially amplify fungal taxa over plant ITS sequences (see Briana Whitaker report for same project).

c) List key outcomes or other achievements.

A major effort at the beginning of this work involved optimizing the DNA extraction from barley heads, the library preparation protocols for 16S and ITS amplicon sequencing and bioinformatic analysis pipelines.

Preliminary results from the fungal amplicon library prep have identified a primer set with reduced plant amplification and highest capture rate of fungal diversity (Primer set #2). Ongoing tests will work to optimize sequencing parameters to achieve highest sequencing read depth, capture the highest fungal diversity, and least plant genomic ITS amplification per sample.

Using optimized protocols, we have now successfully sequenced the bacterial microbiome of >90% of samples using our optimized methodology. Preliminary data analysis of these samples shows encouraging support for the presence of genotype-responsive microbes. However, we note that lack of disease severity associated with drought conditions may limit our power to identify disease-recruited microbes from this data-set in three of the four locations.

Although we identified strong support for genotype-responsive taxa, a key outcome from this work indicates that higher disease severity across more sites would improve our statistical power to identify disease-recruited taxa. Early disease reports indicate much higher disease severity across the four breeding nurseries in 2022. **Therefore, we plan to seek permission to refocus our funded Year 2 proposal towards this goal. We believe this can be accomplished with the currently approved budget.**

A second year of funding, in a higher disease year, would provide us with the necessary statistical power to identify microbial taxa that are both disease-recruited and genotype-responsive, a current limitation to the 2021 data. These data would establish the potential for utilizing breeding to manipulate the microbiome for improving barley resilience to FHB disease.

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

During the first year of this project, a graduate student Brooke Benz, was partially funded by this grant. Brooke's research activities included gaining experience performing field research and FHB scoring, molecular techniques such as DNA extractions and library preparations, and statistical analyses. In addition, Brooke has presented this research at one local and one national research forum (see below for details).

A post doctoral researcher, Eglantina Lopez gained experience mentoring Brooke Benz in next-generation sequencing and microbial community data analysis.

Two undergrads, Amber Kalvoda and Ashley Potter, gained research training as part of this project in: planting, sorting seed, preparing for harvest, processing samples, and performing DNA extractions.

Four early career scientists (Geddes, Baldwin, Whitaker, Banerjee) performed research during the first year of this project.

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

The graduate student, Brooke Benz, presented this research at the Asilomar Fungal Genetics Conference (FY21), as well as at the graduate student research seminar series for the Department of Plant Pathology at NDSU.

Co-PI Whitaker presented preliminary research and directions for this grant to the Gene Discovery and Engineering Resistance (GDER) CP in May 2021.

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?

X 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).

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).

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.

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.