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

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-2-122
USDA-ARS Agreement Title:	Microbiome Antagonism of Fusarium Head Blight
Principle Investigator (PI):	Barney Geddes
Institution:	North Dakota State University
Institution UEI:	EZ4WPGRE1RD5
Fiscal Year:	2022
FY22 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:	May 1, 2022 – April 30, 2024
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category[1*]	Project Title	ARS Award Amount
TSCI	Metagenomics Informed Trait Development for Breeders	$41,062
	FY22 Total ARS Award Amount	$41,062

I am submitting this report as an: ☒ Annual 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
C437520869FF41B...

Date Report Submitted

07/26/2023

[1ǂ] 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: Metagenomics Informed Trait Development for Breeders

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

We are working to identify biocontrol taxa that are recruited from the environment under FHB biotic stress and also responsive to plant genotype
The **overall project goal** of developing an assay for disease-recruited and genotype-responsive microbial biocontrols that can be directly translated into trait screening pipelines for barley.

In FY21, we identified significant effect of environment and barley genotype. However, there was only a significant change in response to FHB on the bacterial microbiome was only identified in one location for 2021, which was St. Paul, Minnesota. In 2021 (although stronger affects were observed in the fungal microbiome)., St. Paul was the only one location that had sufficient rain and FHB, this is reflected in the 2021 data. With only one location year of data, there wasn't enough statistical power to make conclusions about the potential recruitment of microbes by barley genotypes to combate FHB. Anticipating this potential issue, all barley lines selected for analysis were planted again in 2022 across all four nurseries and are currently being harvested for a second year of data. Following the FY22 change request, the specific objectives for FY22 were adjusted to include the following:

Obj 1: Identify FHB recruited and genotype-responsive microbes, under non-drought conditions (2022)

Goal 1: Collect 10 Replanted Training Population (TP) genotypes for microbiome analysis
- 10 genotypes x 4 nurseries x 5 diseased-spikes x 5 nondiseased-spikes

Goal 2: DNA extraction/ Fusarium biomass measurements/ DON measurements
Goal 3: ITS and 16s Amplicon microbial community profiling from four disease nursery locations across the US (Year 2)
Goal 4: Combined data analysis of FY21 and FY22 data to identify disease- and genotype-responsive taxa

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?

Major activities of this project include the following:

1. Setting up and sampling the same 10 genotypes from 2021, in 2022 FHB nurseries in ID, ND, MN, and NY for the second year of microbiome data. Those samples were taken in 2022 and each location had higher disease prevalence than in 2021, except ID. As a result, ID was set up, planted, and sampled in 2023 with greater success.

2. 2022 material was ground and DNA was extracted and quantified. Currently, 2022 materials are being processed for sequencing, same as in 2021. The ID 2023 samples are currently being processed.
3. Further optimization of the 2021 analysis was performed with the aim of improving statistical power of our dataset. This included incorporating the SCRuB pipeline (https://doi.org/10.1038/s41587-023-01696-w) to remove contamination from the dataset as well as rarefaction which controls for over or undersampling bias prior to re-analysis of the data. These approaches improved the power of Principle Coordinate analysis such that the axes generally explained more of the variation in the data. However, they also demonstrated that the earliest samples sequenced from FY21 should be resequenced to improve results, since a greater proportion of reads were lost during rarefaction due to high levels of mitochondrial and chloroplast reads in the total data collected as PNA concentrations to inhibit those reads were optimized over the course of FY21 data collection.
4. Following the results in 3) we prioritized resequencing of specific samples from FY21 with higher PNA concentration to improve data quality. These sequencing runs have been performed but not analyzed, with the sequencing runs for FY22 now entering the que for sequencing and then analysis.

b) What were the significant results?

We collected and extracted DNA from 3 of four locations with improved disease prevalence. These are currently next in the pipeline for microbial community profiling of bacterial 16S and fungal 18S.

We also optimized our analysis pipeline to include contamination removal and rarefaction which improved the ability of Principle Coordinate Beta Diversity analysis to explain variance in the data and statistical support for genotype- and disease-dependant differences in community composition observed (Figure 1 and Figure 2). While this did not change the conclusions of the study, it is anticipated that these improvements will increase our ability to identify genotype- and disease-responsive taxa from the complete dataset once FY22 samples are sequenced.

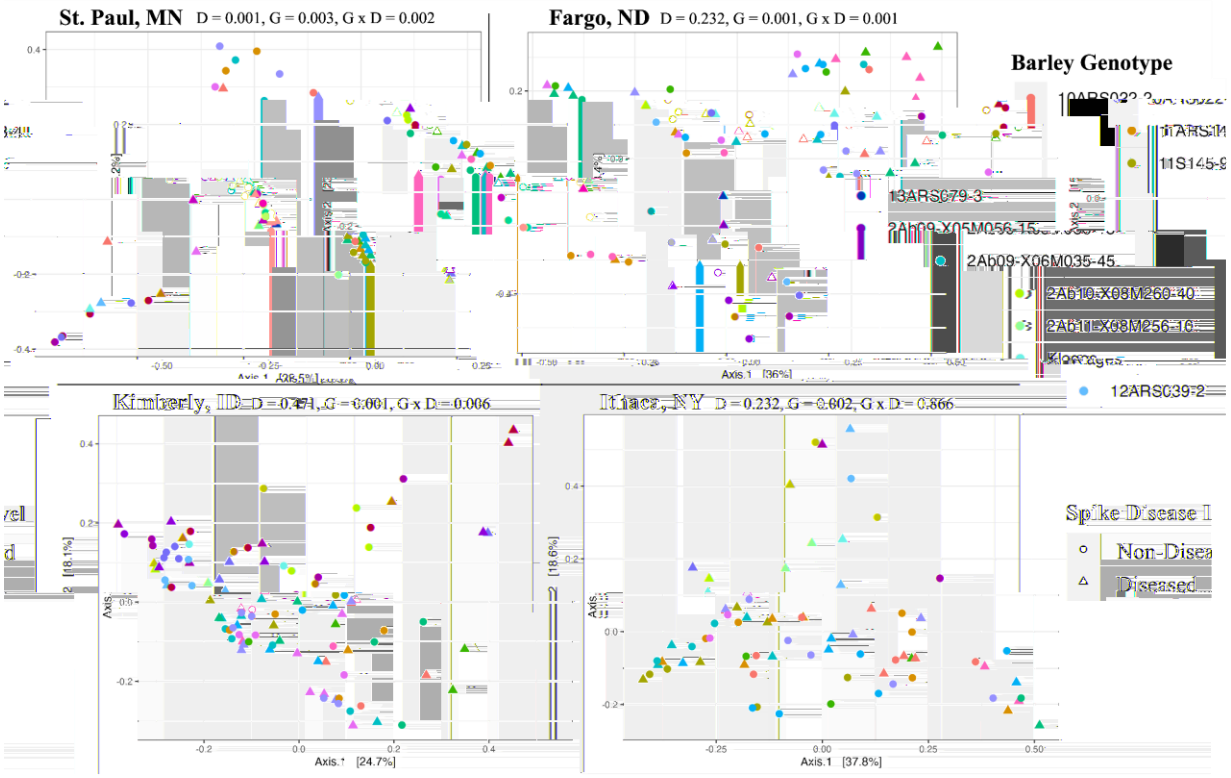


Figure 1. Community composition by location before SCRuB and Rarefaction

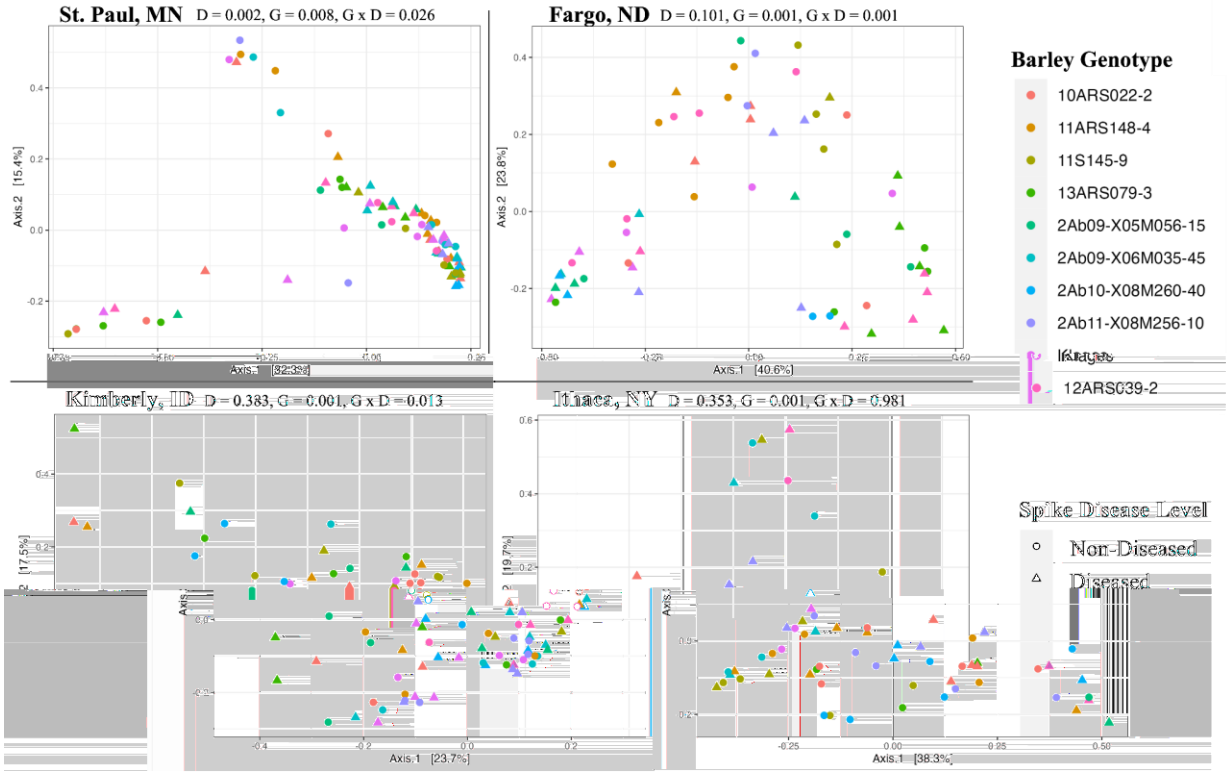
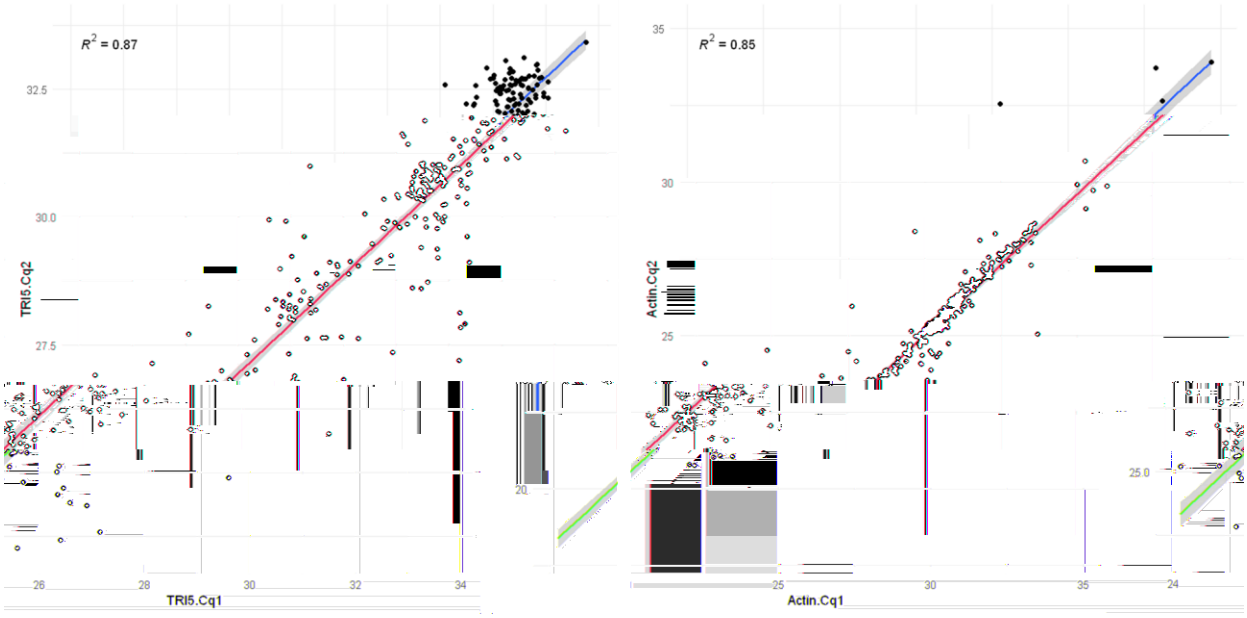


Figure 2. Community composition by location after SCRuB and Rarefaction

We have also resequenced samples from FY21 data that did not have sufficient depth to withstand contamination removal and rarefaction, this will improve the quality of FY21 data upon re-analysis. We are in the process of sequencing FY22 samples from St Paul, Ithaca and Fargo.

Biomass analysis:

Each sample was run twice for Fusarium biomass analysis by testing the Cq values of TRI5 (Fusarium) and comparing that to Actin (Barley). An R^2 of 0.87 and 0.85, respectively, indicates the replicates have comparable measurements (figure 1).



Despite purposefully selecting barley spikes exhibiting high levels of symptoms and no visible symptoms, The two groups had inconsistent differences in Fusarium biomass measured by qPCR in different locations (Fig. 2) and only 2 lines had significant differences in Fusarium biomass (Fig. 3). The relationship between disease symptoms and Fusarium biomass is complex and the impact on the microbiome is currently being explored. Based on these first year preliminary results, it seems the Fusarium biomass has less impact on the microbiome than the expression of disease symptoms (data not shown).

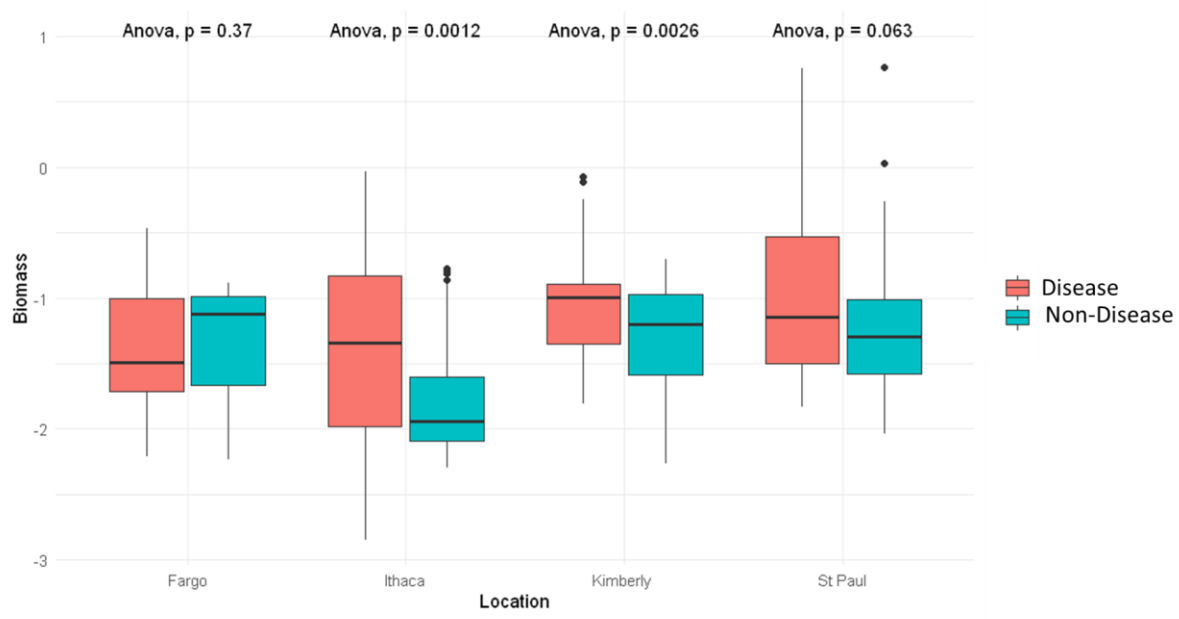


Figure 2: Fusarium biomass in disease and non-diseased samples by location

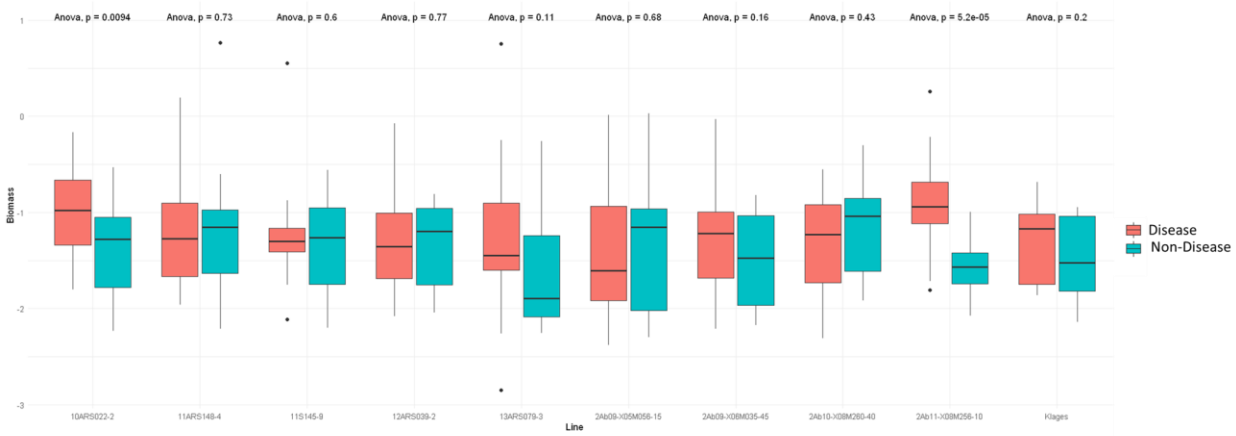


Figure 3: Fusarium biomass in disease and non-diseased samples by line

Deoxynivalenol (DON) measurements were taken for each sample and analyzed by location (Fig 4) and Line (Data not shown). Only two locations, ID and NY, had significant differences between diseased and non-diseased samples. For some samples, particularly in St. Paul, MN, there was no sufficient sample to run both DNA extraction and DON analysis. Of the 10 lines, only 5 of the lines had significant differences in DON accumulation by diseased or non-diseased sample type. Most samples tested had no detectable levels of DON and there were higher detectable concentrations of 3A and 15A-DON then is typically seen in harvested grain samples. Unexpectedly, some samples from Idaho had detectable levels of NIV. These metabolites are likely present on green tissue and metabolites by the barley before final harvest. The impact of these metabolites on the microbiome are being investigated and will be better understood with the second year of analysis.

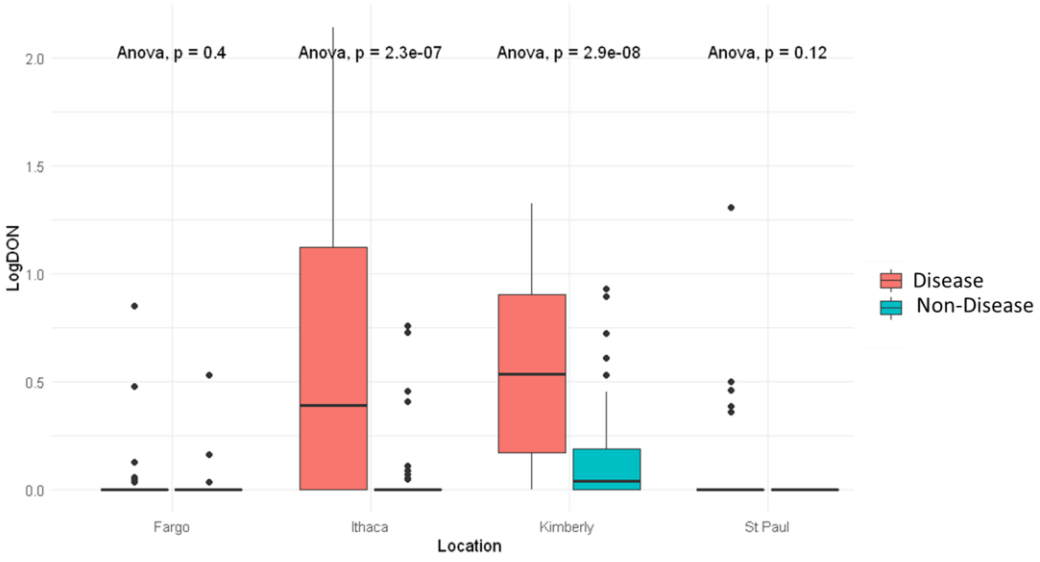


Figure 4: Deoxynivalenol measurements in samples by location

c) List key outcomes or other achievements.

FY22 samples were planted, harvested and the DNA has been extracted. Microbiome sequencing workflows have been optimized. Following completion of FY22 microbiome sequencing and data analysis, we anticipate identifying disease- and genotype-responsive taxa that may have potential to be utilized as a breeding target for microbiome-based suppression of FHB.

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. National Fusarium Head Blight Forum 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 FY22 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 May 1, 2022 – April 30, 2023?

X Yes, I've included the citation reference in listing(s) below.

☐ No, I have nothing to report.

Journal publications as a result of FY22 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 FY22 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 FY22 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. (2021). 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. Acknowledged federal support: Yes.

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 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf. Acknowledged federal support: Yes.

Benz, N. R., Navasca, A. R., Velasco, D. D., Lopez-Echartea, E., Banerjee, S., Whitaker, B., Baldwin, T., Geddes, B. A. (2023). 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. Acknowledged federal support: Yes.