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

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Contribution of *Fusarium* diversity to variability of FHB resistance in barley
Principle Investigator (PI):	Hye-Seon Kim
Institution:	USDA-Agricultural Research Service
Institution UEI:	N/A
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$58,793
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Period of Performance:	May 1, 2022 - April 30, 2023
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Contribution of Fusarium diversity to variability of FHB resistance in barley	$15,000
PBG	Contribution of Fusarium diversity to variability of FHB resistance in barley	$43,793
	FY22 Total ARS Award Amount	$58,793

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 — 07/26/23 — Date Report Submitted

ǂ 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 and 2: Contribution of *Fusarium* diversity to variability of FHB resistance in barley (BAR-CP: BA-016 and PBG:PB-006)

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

The goal of this project is to determine the contribution of *Fusarium* genome diversity on variation in disease severity and mycotoxin contamination observed in barley genotype screening nurseries and facilitate incorporation of pathogen genotype data in variety screening programs to enhance the resilience of FHB resistance.

The specific objectives of this proposal are the following, **Objective 1**: Characterize genomic differences in FHB isolates within and among barley screening programs. **Objective 2**: Determine if standard susceptible and resistant barley cultivars exhibit the same level of disease and mycotoxin contamination in response to FHB isolates from different screening programs under controlled conditions. **Objective 3**: Determine if barley cultivars exhibit a differential metabolic response to genetically diverse FHB isolates.

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?

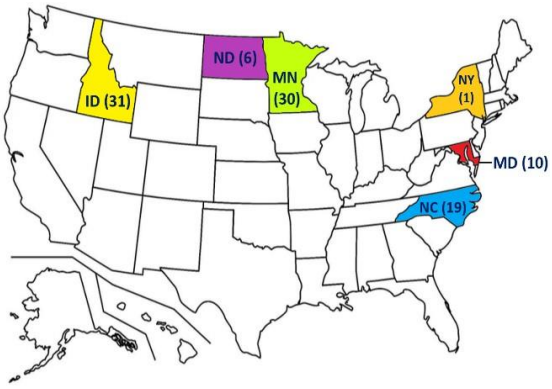

Fig. 1. Number of isolates received from 6 different states, Idaho (ID), North Dakota (ND), Minnesota (MN), New York (NY), Maryland (MD), North Carolina (NC)

This is the third report for this project since receipt of FY22 funding in May 2022. We have assembled a collection of 97 FHB isolates from barley screening programs in six states to evaluate viability of strains within and among screening programs (Fig.1).

To accomplish the **Objective 1**, each isolate has been reisolated from a single conidium and then all single-conidium isolates were subjected to trichothecene production analysis by growing them in laboratory cultures, extracting the cultures with solvents, and determining the content of trichothecenes by gas chromatography-mass spectroscopy. Of the 97 isolates tested, only 1 isolate failed to produce any trichothecenes in liquid media or on solid rice substrate. However, the 96 isolates produced either the 3ADON or 15ADON analog of trichothecenes in liquid culture (Table 1). We have selected a subset of 70 isolates of the 97 FHB isolates that represent different states, isolation years, and host origins for genomic DNA extraction using Qiagen DNeasy Plant Mini Kit. The 66 isolates that passed the DNA quality check were then submitted for whole genome sequencing with an Illumina MiSeq platform. To date, whole genome sequences of 52 isolates have been assembled using CLC Genomics Workbench 23.0.4 (Table 1). Adapter sequences were trimmed, the trimmed data was then referenced to 73 bacteria contaminants which were removed. The unmapped sequences were then used for de novo assembly and then, the consensus of each isolate was then extracted and annotated using Augustus gene prediction tool. To confirm species identity and evaluate phylogenetic diversity of isolates, we have retrieved full-length sequences of three housekeeping genes (*TEF1*, *RPB1* and *RPB2*) from a subset of 42 genome sequences. We aligned the gene sequences from these three marker loci using MEGA and performed maximum likelihood bootstrapping phylogenetic analyses using IQ-TREE (Fig. 2).

Table 1. Number of FHB isolates that we have conducted for DNA extraction, toxin analysis in vitro, whole genome sequencing, and infection assay.

State	# of FHB isolates received	# of isolates of toxin produced in vitro	DNA extraction	Genome Sequencing	Infection Assay
Idaho	31	20 (15ADON), 11 (3ADON)	24	22	2
Maryland	10	10 (15ADON)	10	10	2
Minnesota	30	25 (15ADON), 4 (3ADON), 1 (No toxin)	20	20	2
New York	1	1 (15ADON)	1	1	1
North Carolina	19	19 (15ADON)	10	10	3
North Dakota	6	6 (15ADON)	5	3	2

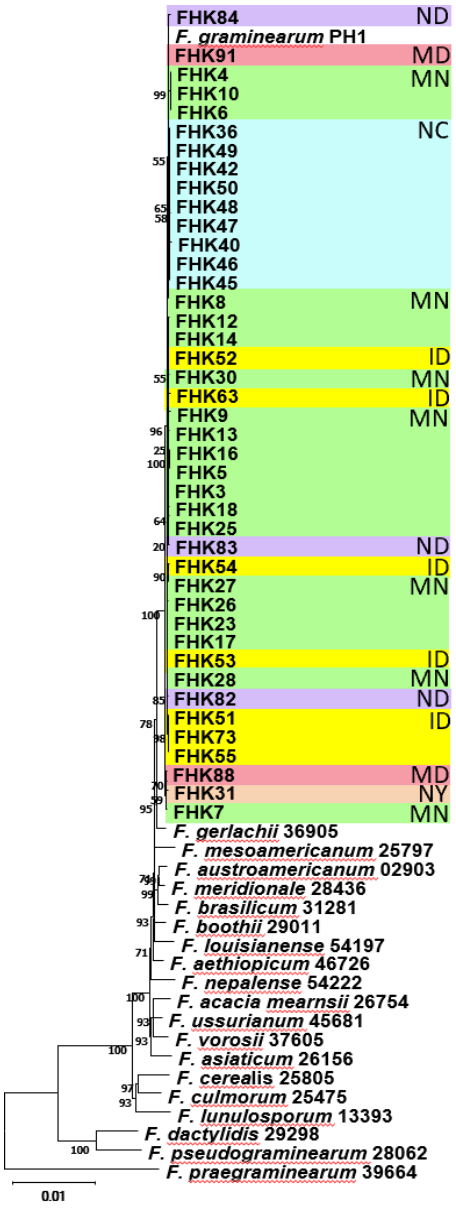

Fig. 2. Phylogeny inferred from 3 HK genes in genomes of 42 isolates

To address the **Objective 2**, we planted the check barley varieties, AAC Synergy (susceptible) and Pinnacle (resistant) for seed propagation. A total of 12 isolates representing 6 different states were tested for their ability to cause FHB on two barley varieties (Table 1). Depending on the availability of plant growth rooms in space, we have conducted infection assays with different number of isolates, respectively (1st infection: 4 isolates, 2nd infection: 3 isolates and control with tween, 3rd infection: 5 isolates). Spores of each isolate were obtained from 4-day old mung bean liquid cultures. The macroconidia were collected and suspended in a 0.04% Tween solution with 10^5 conidia per milliliter. About sixty days after emergence, five barley heads from 3-4 pots (a total of 15 heads) were each inoculated with one isolate by dipping the heads into a spore suspension of the isolate (1×10^5 spores/ml in 0.04% tween solution). Inoculated barley heads were covered with plastic bags for high humidity, bags were removed after three days. FHB disease was scored at 4-, 7-, and 10-days post-inoculation. Disease severity scoring has been completed and calculated for the twelve isolates used (Figs. 3A-C).

The barley heads, immediately after excision from plants, were weighed, frozen in liquid nitrogen, lyophilized and pulverized (GenoGrinder 2010). Five biological replicates were used, each containing 0.5g of pulverized tissue. The tissue was extracted with acetonitrile water (86:14). Extracts were purified with Romer columns. Deoxynivalenol (DON) concentrations in ground tissue were determined using GC-MS. DON was detected in all twelve isolates that were used for virulence assays (Table 2). We also estimated pathogen biomass in the barley head by using Fluidigm, a nanofluidic automated real-time PCR system. Quantification of fungal DNA in the barley heads at 10 days post inoculation were submitted, using DNA extracted from 40-60mg of pulverized tissue. *Fusarium* specific primers and barley GAPDH primers were used to obtain relative biomass of *Fusarium* in infected tissue by qPCR.

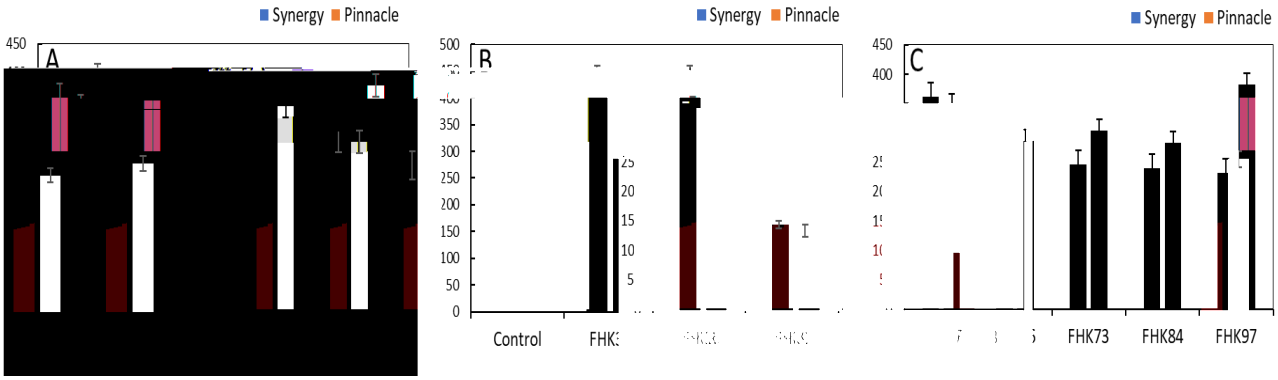

Fig. 3. (A) Area Under the Disease Progress Curve (AUDPC) of four isolates used in 1st infection assay. (B) AUDPC of three isolates and a control used in 2nd infection assay (C) AUDPC of five isolates used in 3rd infection assay.

Table 2. FHB isolates that conducted for infection assay and level of DON production in infected barley tissue.

	Synergy		Pinnacle	
State	Isolate	average ugDON/g	Isolate	average ugDON/g
MN	FHK16	94.77210584	FHK16	133.0191974
NY	FHK31	43.83135651	FHK31	53.91921183
NC	FHK42	66.71260323	FHK42	119.1531548
ID	FHK63	25.60103323	FHK63	39.78880219
NC	FHK36	198.2771736	FHK36	99.36634968
ND	FHK82	144.8816819	FHK82	82.41898102
MD	FHK91	100.9505822	FHK91	83.47212118
MN	FHK27	132.5427521	FHK27	162.1445971
NC	FHK46	116.8659996	FHK46	113.5595526
ID	FHK73	128.6876268	FHK73	138.7425799
ND	FHK84	70.2249453	FHK84	94.70210614
MD	FHK97	78.03007555	FHK97	139.8158767

To achieve the **Objective 3**, subsamples of pulverized tissues (45-55mg) from barley plant mentioned above were subjected to metabolomics analysis using HPLC-MS. Interpretation of raw metabolomic data was aided by processing with metabolomics software, Compound Discoverer 3.3. The metabolomic data analysis for the 1st infection assay with strains FHK16 (MN), FHK31 (NY), FHK42 (NC), FHK63 (ID) was completed (Figs. 4A and 4B).

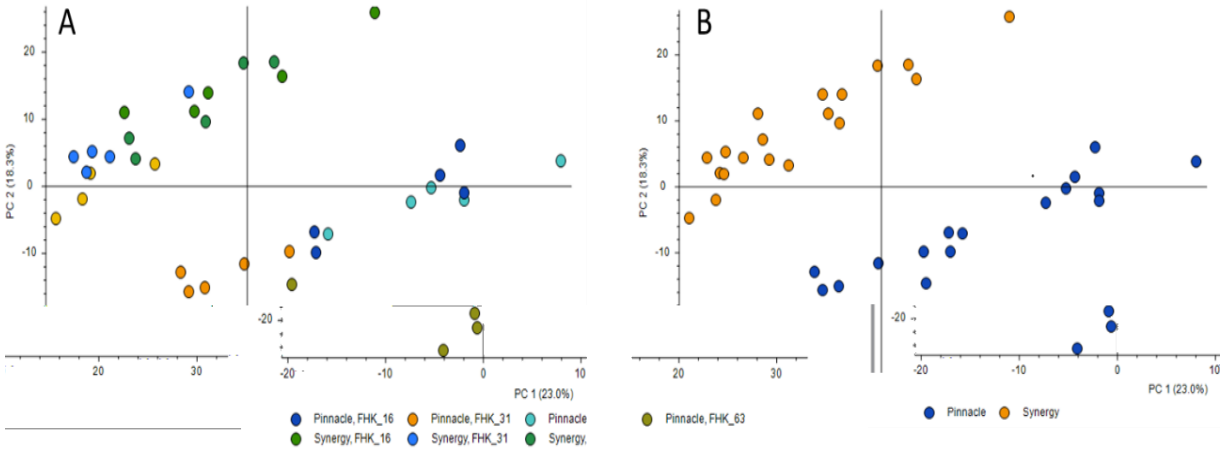

Fig. 4. (A) The Principal Component Analysis (PCA) scores plot for 1st infection assay with 4 isolates (FHK16, FHK31, FHK42, FHK63) produced by metabolomic studies. (B) PCA scores plot grouped by cultivars (Synergy and Pinnacle).

b) What were the significant results?

Genome assembly, phylogenetic analysis, and toxin analysis (Objective 1)

We generated whole-genome sequence data for 66 of the 97 FHB isolates that we acquired for this project. Data for 52 of the isolates have been fully assembled and annotated. Phylogenetic analysis of three housekeeping genes retrieved from a subset of 42 of the genome sequences confirmed that all 42 of the corresponding isolates are *F. graminearum* (Fig. 2). That is, in the phylogenetic tree inferred from the gene sequences, all 42 isolates and the reference *F. graminearum* strain (PH-1) formed a well-supported clade (bootstrap value 96) that excluded closely related species, such as *F. gerlachii* and *F. louisianense*, from the *Graminearum* clade of the *Fusarium sambucinum* species complex (FSAMC). Interestingly, even though it did not produce trichothecenes in liquid or solid rice culture, isolate FHK28 (MN) was nested within the *F. graminearum* clade along with reference strain PH-1 and other FHK isolates from this project.

Infection assay (Objective 2)

In the 1st and 3rd assays, barley varieties AAC Synergy and Pinnacle had similar levels of disease when inoculated with three isolates, but Pinnacle was more resistant when inoculated with six other isolates. In the 2nd assay, by contrast, Synergy was more resistant than Pinnacle to all three isolates examined. This is opposite of what we expected. In Fig. 3B, AAC synergy was more susceptible to FHB isolates, FHK36 (NC), FHK82 (ID), FHK91(MD) than Pinnacle for all three strains that we used in 2nd infection assay. The inoculated barley heads were then harvested and processed for toxin analysis, fungal biomass, and metabolomics. We are currently working on DNA extractions to submit for fungal biomass quantification and processing metabolomics data for 8 of the twelve isolates that were used in 2nd and 3rd infection assay.

Toxin analysis from infection assay (Objective 2)

As noted above, DON was detected in all twelve strains that were used for 1st – 3rd infection assay. A strain from Idaho (FHK63) produced the lowest amounts of DON in both varieties of barley (Table 2). While two strains from North Carolina (FHK36) and Minnesota (FHK27) produced the highest amounts of DON in AAC synergy and Pinnacle respectively (Table 2). The data showing the lowest amounts of DON produced in strain FHK63 (ID) is consistent with disease severity scoring data. The two strains producing the highest amounts of DON in both varieties do not align with the strains exhibiting the most FHB, meaning the severity of disease cannot be correlated with amount of DON production.

Fungal biomass results only from 1st infection assay (Objective 2)

Quantification of fungal biomass data for 1st infection assay with strains FHK16 (MN), FHK31 (NY), FHK42 (NC), FHK63 (ID) was generated. Higher fungal biomass was seen in FHK16 for both varieties, which also had higher disease severity scoring and higher production of DON. The lowest fungal biomass was seen in FHK63 of the four strains in both AAC synergy and Pinnacle. This strain was also the lowest producer of DON and caused minimal disease compared to other strains.

Metabolomic results only from 1st infection assay (Objective 3)

Although more metabolomic data from 2nd and 3rd infection assay needs to be gathered before we can consider the importance of the different compounds found in this analysis, we see some promising data (Figs. 4A and B) from the 1st infection assay with strains FHK16 (MN), FHK31 (NY), FHK42 (NC), FHK63 (ID), This analysis detected 778 total compounds, although we show only the top 50 compound list that may have some metabolomic significance (Tables 3 and 4).

Table 3. The first part of top 50 metabolomic compound list (1-25)

	Ta	Compound of interest Synergy Up + Pinnacle Up	Name	Formula	Annot. Sou	Annot.	Calc. MW	m/z	RT [min]	Area (Max	MS2	Group Areas							Log2 Fold Change				Adj. P-value			
					Predicted Compositions+ Lipid Search Metabolite Search BioCyc Search Chemspider Search MassList Search							Pinnacle, FHK_16+	Pinnacle_FHK_31	Pinnacle_FHK_42	Pinnacle_FHK_63	Synergy_FHK_16	Synergy_FHK_31	Synergy_FHK_42	Synergy_FHK_63	(Pinnacle, FHK_16) / (Synergy, FHK_16) +	(Pinnacle, FHK_31) / (Synergy, FHK_31)	(Pinnacle, FHK_42) / (Synergy, FHK_42)	(Pinnacle, FHK_63) / (Synergy, FHK_63)	(Pinnacle, FHK_16) / (Synergy, FHK_16) +	(Pinnacle, FHK_31) / (Synergy, FHK_31)	(Pinnacle, FHK_42) / (Synergy, FHK_42)	(Pinnacle, FHK_63) / (Synergy, FHK_63)
1			Possible fungal metabolite, (Ex: Leccinine A)	C13 H17 N O3		0.36	235.12093	236.12817	14.260	66769021		3.49e6	2.81e6	4.37e6	4.16e6	6.95e7	6.42e7	2.74e7	3.09e7	-4.37	-4.39	-2.93	-2.90	1.9e-4	5.8e-4	2.3e-2	3.6e-
2			Possible fungal metabolite, furan (Ex: Trienylfuranol A)	C11 H16 O2		0.11	180.11505	181.12233	16.959	997488097		4.51e7	3.81e7	4.69e7	3.68e7	6.50e8	8.27e8	5.08e8	3.37e7	-4.11	-4.51	-3.63	0.24	2.8e-3	2.3e-3	1.9e-3	5.9e-
3			Possible plant alkaloid with multiple rings (Ex: Venezueline B)	C14 H14 N2 O4		0.13	274.09539	275.10267	14.030	40211988		7.18e6	6.63e6	1.11e7	4.41e6	3.51e7	2.69e7	3.20e7	2.64e7	-2.29	-2.02	-1.28	-1.92	7.2e-5	2.5e-2	1.5e-2	7.9e-
4			Probable glycosylated flavonoid (Ex: 6-Methoxyluteolin 7-glucuronide methyl ester)	C23 H22 O13		0.36	506.10622	507.11350	14.738	53590812		7.83e6	1.29e7	1.50e7	1.54e7	2.34e7	4.75e7	4.06e7	4.26e7	-1.62	-1.74	-1.50	-1.21	2.1e-1	3.1e-2	3.0e-2	2.4e-
5			Probable long-chain amine (Ex: N-amyl arachidohoyl amine)	C25 H43 N O		-0.63	373.33423	374.34152	21.903	3157380325		3.62e7	1.12e7	2.54e7	2.75e7	2.83e10	2.54e10	2.91e10	2.79e10	-9.61	-11.18	-10.14	-8.47	4.7e-4	2.0e-3	3.2e-4	2.2e-
6			Probable plant hormone with cyclic structure or long-chain (Ex: Herbarumin III, Traumatin)	C12 H20 O3		0.18	212.14128	213.14855	16.959	816524981		1.83e7	1.97e7	1.90e7	2.00e7	5.00e8	6.32e8	3.92e8	2.44e7	-4.75	-5.23	-4.40	-0.12	1.9e-3	1.2e-3	6.4e-3	7.0e-
7			Unknown	C32 H64 N8 O3		3.71	608.51240	609.51967	22.275	702166885		9.97e6	3.06e6	7.69e6	4.46e6	3.39e8	2.05e8	2.23e8	1.97e8	-6.11	-5.37	-4.91	-5.60	1.3e-2	2.6e-3	3.6e-3	5.9e-
8			Unknown	C35 H61 N5 O2		-2.76	583.48091	584.48820	22.189	473956300		1.54e6	1.87e6	1.32e6	2.96e6	1.22e8	9.41e7	1.58e8	2.93e8	-6.32	-5.73	-6.74	-6.75	8.7e-5	6.6e-3	5.4e-4	5.9e-
9			Unknown	C36 H69 N O6		-0.18	611.51238	612.51966	20.577	338048237		2.20e6	2.23e6	1.85e6	2.31e6	9.37e7	7.58e7	1.22e8	1.60e8	-5.59	-5.09	-5.90	-6.53	7.2e-5	1.5e-3	1.3e-3	5.0e-
10			Unknown	C31 H47 N7 O3		3.49	565.37601	566.38382	20.811	331746756		1.98e6	2.29e6	1.82e6	1.85e6	1.52e8	3.35e8	2.10e8	3.10e8	-6.43	-7.16	-6.85	-7.37	2.2e-3	1.5e-3	2.3e-3	3.5e-
11			Unknown	C37 H65 N5 O		-2.71	595.51730	596.52457	22.417	195169796		1.63e6	1.26e6	2.76e6	7.75e6	5.92e7	5.14e7	9.47e7	9.97e7	-5.35	-5.26	-5.15	-3.62	4.6e-3	2.7e-3	2.5e-3	5.9e-
12			Unknown	C12 H23 N O2 S		0.53	245.14508	246.15236	17.124	115465145		5.36e6	1.62e7	3.52e6	8.40e6	4.90e7	6.00e7	3.02e7	1.46e7	-2.62	-1.89	-2.62	-1.14	2.7e-2	3.7e-2	2.3e-2	9.9e-
13			Unknown	C34 H59 N O2		-0.45	513.45435	514.46163	20.844	90288623		1.77e6	2.68e6	1.80e6	4.16e6	3.26e7	5.19e7	5.90e7	6.06e7	-4.25	-4.32	-4.96	-3.78	7.4e-3	1.5e-3	5.3e-3	6.2e-
14			Unknown	C20 H43 N6 O2 P		2.21	430.31946	431.32671	20.491	58461208		2.10e6	2.08e6	1.97e6	2.12e6	4.66e7	1.84e7	2.78e7	1.92e7	-4.57	-3.09	-3.82	-0.29	4.7e-4	3.1e-3	6.8e-3	5.3e-
15			Unknown	C15 H24 O6		0.20	300.15735	301.16462	14.126	57910777		7.65e6	7.48e6	1.15e7	6.34e6	1.61e7	4.45e7	2.10e7	2.50e7	-1.08	-2.57	-1.03	-1.43	1.3e-2	2.8e-2	5.9e-2	3.5e-
16			Unknown	C21 H43 N O		-0.64	325.33426	326.34153	21.178	50898252		2.78e6	1.69e6	3.93e6	3.63e6	3.90e7	2.53e7	3.53e7	2.06e7	-3.49	-3.63	-3.02	-2.08	7.4e-3	1.5e-2	3.3e-3	1.5e-
17			Unknown	C45 H17 N O5 P2		0.66	713.05867	714.06594	15.775	45629076		2.69e6	2.89e6	5.27e6	5.78e6	2.15e7	3.22e7	3.04e7	3.45e7	-3.23	-3.48	-2.43	-2.58	2.2e-2	3.7e-2	1.9e-2	1.5e-
18			Unknown	C18 H22 O8		3.35	366.13269	367.13991	12.552	13120915		1.29e6	3.26e5	2.32e6	8.62e5	1.21e7	2.49e6	7.78e5	1.09e6	-3.19	-2.41	-1.97	-1.38	1.1e-2	7.1e-2	4.5e-2	5.9e-
19			Unknown	C9 H24 Cl N6 P S2		-0.64	346.09278	347.10007	16.970	12013839		7.98e5	5.95e5	1.05e6	7.31e5	9.56e6	1.20e7	7.03e5	5.92e5	-3.77	-4.33	0.11	0.38	1.6e-4	1.2e-3	1.1e-1	7.7e-
20			Unknown, long chain cyclic	C26 H42 N2 O2		-0.35	414.32448	415.33175	20.562	1329520558		4.33e6	1.81e6	1.59e6	1.71e6	1.23e9	2.75e8	4.24e8	2.48e8	-7.89	-7.18	-7.95	-5.39	2.2e-3	2.3e-3	8.2e-4	4.2e-
21			Unknown, long chain cyclic	C26 H43 N O4		-0.52	433.31898	434.32625	18.837	53966233		1.46e6	2.04e6	1.75e6	2.36e6	3.30e7	3.46e7	2.35e7	3.15e7	-4.40	-4.38	-4.52	-3.78	4.0e-3	1.9e-2	2.3e-2	5.9e-
22			Unknown, possible detergent or fatty acid	C24 H41 N O		-0.75	359.31855	360.32582	21.150	1041225260		1.48e7	6.72e6	1.19e7	1.22e7	7.18e8	5.07e8	8.81e8	3.41e8	-5.58	-6.34	-5.83	-3.83	2.2e-3	3.2e-3	6.1e-3	3.5e-
23			Unknown, possible detergent or fatty acid	C24 H41 N O3		-0.40	391.30849	392.31576	18.665	55169064		2.69e6	4.03e6	3.93e6	6.20e6	2.66e7	3.36e7	3.36e7	3.35e7	-3.16	-3.46	-3.39	-2.95	1.9e-1	2.1e-2	5.4e-2	5.9e-
24			Unknown, possible detergent/fatty acid	C25 H43 N O2		-1.26	389.32889	390.33617	19.719	238348288		3.54e6	3.97e6	3.98e6	3.59e6	1.47e8	9.37e7	1.20e8	1.23e8	-5.59	-4.56	-4.79	-3.79	4.3e-3	1.5e-3	5.3e-3	8.1e-
25			Unknown, possible detergent/fatty acid	C25 H43 N O2		-0.67	389.32912	390.33640	20.714	178352376		1.86e6	1.80e6	1.56e6	1.53e6	1.73e8	1.43e8	1.40e8	1.45e8	-6.52	-6.31	-6.44	-6.02	7.2e-5	5.8e-4	2.4e-4	3.5e-

Table 4. The second part of top 50 metabolomic compound list (26-50)

	Compound of interest	Synergy Up*	Pinnacle Up	Name	Formula	Predicted Compositions*	Annot.	Calc. MW	m/z	RT [min]	Area (Max	MS2	Group Areas								Log2 Fold Change				Adj. P-value			
													Pinnacle_FHK_16*	Pinnacle_FHK_31	Pinnacle_FHK_42	Pinnacle_FHK_63	Synergy_FHK_16	Synergy_FHK_31	Synergy_FHK_42	Synergy_FHK_63	(Pinnacle_FHK_16) / (Synergy_FHK_16)*	(Pinnacle_FHK_31) / (Synergy_FHK_31)	(Pinnacle_FHK_42) / (Synergy_FHK_42)	(Pinnacle_FHK_63) / (Synergy_FHK_63)	(Pinnacle_FHK_16) / (Synergy_FHK_16)*	(Pinnacle_FHK_31) / (Synergy_FHK_31)	(Pinnacle_FHK_42) / (Synergy_FHK_42)	(Pinnacle_FHK_63) / (Synergy_FHK_63)
26				Unknown, possible detergent/fatty acid	C24 H41 N O		-0.79	359.31853	360.32581	21.454	125648512		1.78e6	2.14e6	1.82e6	2.41e6	8.74e7	6.22e7	7.92e7	5.38e7	-5.68	-5.00	-5.25	-4.26	8.4e-4	1.5e-3	3.2e-4	3.9e-
27				Unknown, possible detergent/fatty acid	C24 H42 O2		-0.86	362.31817	363.32544	22.980	28858338		1.85e6	1.99e6	2.20e6	1.88e6	2.64e7	2.53e7	1.80e7	1.06e7	-3.73	-3.06	-2.85	-2.45	2.2e-3	9.1e-3	2.3e-2	3.6e-
28				Unknown, possible sterol	C26 H45 N O		-0.70	387.34984	388.35712	22.404	673070624		1.24e6	1.17e6	1.13e6	1.52e6	5.47e8	5.07e8	6.20e8	4.57e8	-8.79	-8.66	-9.06	-7.56	1.6e-4	5.7e-5	5.6e-6	3.4e-
29				Possible cyclic fungal metabolite (Ex: Cyathiformine B)	C12 H16 O8		0.12	288.08455	289.09181	13.921	44841948		2.37e7	2.27e7	2.28e7	1.28e7	4.26e5	8.05e5	6.20e5	1.47e6	5.32	4.82	4.86	2.97	4.1e-4	1.1e-2	7.4e-3	8.6e-
30				Possible fungal metablomite, ester (Ex: Yanuthone E)	C29 H42 O7		0.02	502.29307	503.30038	18.010	512581487		4.77e8	3.55e7	8.30e7	7.68e7	1.35e8	2.16e7	1.59e7	1.09e8	1.48	0.84	2.43	0.65	7.9e-2	2.4e-1	1.9e-2	7.5e-
31				Possible fungal metabolite, cyclic (Ex: Malassezione)	C19 H16 N2 O		0.42	288.12638	289.13366	16.305	278028197		2.23e8	1.13e8	2.83e8	1.02e8	1.23e8	6.46e7	8.12e7	2.70e7	0.75	1.25	1.34	2.55	7.6e-1	4.5e-1	1.3e-1	5.9e-
32				Possible fungal metabolite, cyclic (Ex: Penicibenzoxepinol)	C10 H10 O2		0.30	162.06813	163.07540	17.739	41467383		3.42e7	3.68e7	3.48e7	3.89e7	5.30e6	6.09e6	1.64e7	3.07e7	2.69	2.60	1.08	0.53	4.0e-3	9.1e-3	1.6e-1	8.6e-
33				Possible fungal metabolite, cyclic (Ex: Quinolactacin B)	C15 H16 N2 O2		-0.32	256.12109	257.12837	15.687	26202970		1.70e7	1.53e7	2.57e7	1.78e7	4.67e6	3.29e6	5.39e6	6.59e6	1.78	2.34	2.04	1.82	1.6e-4	3.5e-3	1.9e-2	3.9e-
34				Possible fungal metabolite, cyclic (Ex: Quinolactacin E)	C16 H18 N2 O2		0.30	270.13691	271.14419	17.305	119329535		9.50e7	9.96e7	1.35e8	9.76e7	1.56e7	1.93e7	3.73e7	5.05e7	2.04	2.32	1.83	0.71	2.2e-2	5.8e-4	7.3e-3	7.9e-
35				Possible plant steroid (Ex: Carthamosterone)	C29 H42 O8		0.17	518.28806	519.29536	17.388	181635988		1.50e8	1.34e7	1.15e7	2.38e7	1.77e7	2.55e6	4.60e6	3.99e7	2.80	2.00	1.66	-0.49	2.0e-2	2.3e-2	8.9e-2	5.5e-
36				Unknown	C14 H21 N O7		0.01	315.13181	316.13908	14.835	1887536910		9.62e8	9.97e8	1.09e9	9.35e8	1.08e8	1.21e8	8.05e7	1.22e8	3.16	3.47	3.37	2.56	8.7e-3	1.2e-2	1.1e-2	5.2e-
37				Unknown	C14 H21 N O7		-0.06	315.13178	316.13906	14.615	1753742269		1.17e9	8.10e8	1.36e9	1.23e9	3.04e7	6.55e7	4.94e7	1.44e8	5.27	3.59	4.62	3.09	2.2e-3	9.1e-3	3.5e-2	4.1e-
38				Unknown	C30 H52 O6		0.10	508.37644	509.38373	15.722	330518435		1.43e8	2.16e8	1.21e8	4.81e6	4.96e7	4.88e7	1.35e7	3.79e6	1.31	2.64	3.16	0.77	7.0e-1	3.9e-1	4.6e-2	9.4e-
39				Unknown	C23 H37 N5 O4		0.62	447.28483	448.29210	16.548	324275584		2.43e7	6.41e7	5.78e7	1.65e8	8.90e6	3.02e7	2.32e7	2.33e7	2.12	1.08	1.32	1.47	3.1e-2	7.7e-1	1.9e-1	7.0e-
40				Unknown	C16 H22 N6 O		1.02	314.18583	315.19310	18.476	183279602		1.41e8	1.62e8	1.74e8	2.70e8	5.45e7	7.62e7	9.55e7	1.74e8	1.40	1.46	0.83	0.58	4.4e-1	1.3e-1	2.2e-1	3.0e-
41				Unknown	C34 H34 O16		0.13	698.18478	699.19205	15.133	67895483		1.74e7	2.09e7	2.56e7	4.35e7	4.64e6	7.52e6	5.11e6	9.83e6	1.82	1.47	1.98	1.62	2.2e-2	2.0e-2	2.6e-2	5.2e-
42				Unknown	C9 H4 Cl2 N2 O16 F		-0.49	527.84105	528.84833	1.560	48000637		4.72e7	4.13e7	4.57e7	4.88e7	2.36e7	1.48e7	1.55e7	1.15e7	1.09	1.60	1.47	1.45	9.0e-3	9.1e-3	8.5e-3	8.1e-
43				Unknown	C32 H43 N O3		0.19	489.32439	490.33166	17.760	43627096		3.35e7	3.22e7	4.16e7	1.66e7	9.96e5	6.66e6	1.94e7	1.76e7	4.52	1.95	1.12	-0.53	4.8e-3	2.4e-1	5.3e-2	5.9e-
44				Unknown	C17 H11 Cl N2 O15		0.37	707.82086	708.82814	1.559	15326093		1.56e7	1.36e7	1.46e7	1.60e7	7.67e6	4.29e6	4.51e6	3.88e6	1.36	1.66	1.75	2.15	3.0e-1	9.7e-3	1.0e-2	5.1e-
45				Unknown	C17 H28 N2 O5 P2		-0.76	434.11908	435.12636	11.450	13916717		1.45e6	4.14e6	2.79e6	1.64e7	2.06e5	5.58e5	2.88e5	9.45e5	2.11	2.45	3.28	4.08	1.1e-1	1.2e-2	4.4e-2	1.6e-
46				Unknown	C30 H13 N4 O18 P3		0.00	969.80412	970.81140	1.553	6385875		6.68e6	5.83e6	7.07e6	4.86e6	3.58e6	1.92e6	2.48e6	1.57e6	0.90	1.35	1.31	1.49	1.3e-1	7.0e-3	2.0e-2	3.0e-
47				Unknown	C10 H14 Cl N8 O17		-0.36	805.79789	806.80517	1.560	5852886		5.80e6	4.92e6	5.47e6	6.02e6	2.69e6	1.14e6	1.43e6	1.20e6	1.62	2.10	1.92	1.32	9.8e-2	2.7e-3	2.3e-3	9.9e-
48				Unknown, cyclic	C26 H27 N3 O2		-0.23	413.21023	414.21751	18.136	101393504		6.98e7	1.04e7	5.02e7	1.26e7	1.72e7	6.56e6	2.11e7	6.48e6	2.15	1.72	1.36	2.11	5.5e-2	1.9e-1	8.7e-2	8.1e-
49				Unknown, long chain cyclic	C42 H64 N10 O3		0.79	756.51688	757.52416	19.116	34441862		1.55e7	3.26e7	1.55e7	1.08e7	1.12e7	7.07e6	5.40e6	5.04e6	0.62	2.15	1.23	1.17	2.2e-1	8.0e-2	6.3e-2	5.0e-
50				Unknown, possible long chain ether	C12 H26 O3		0.03	218.18820	219.19553	17.413	29765712		2.62e7	2.11e7	3.08e7	1.80e7	6.90e5	8.74e5	1.74e7	2.96e7	5.10	4.41	0.85	-0.77	4.1e-4	8.9e-2	7.1e-1	4.7e-

c) List key outcomes or other achievements.

In December 2022, we hired an Oak Ridge Institute for Science and Education (ORISE) fellow who helps us conduct all the labor-intensive works including DNA extraction, genome assembly and infection assay. Less than year now from December, we have been successfully generated all the results by team's hard work (Please see the above significant result section). This result brought us a biggest accomplishments and achievements to meet all the milestones this year for each objective. We continue to finish up rest of the project to meet multiple objectives and milestones for following year.

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

I have trained the ORISE fellow on CLC Genomics Workbench for genome assembly, submit batch job scrips to SCINet (high performance cluster, Ceres) to annotate genomes by using AUGUSTUS and run python script for adding locus tag feature into genome annotation GenBank file. The ORISE fellow also learned how to generate phylogenetic tree for evolutionary aspect, conduct infection assay, and analyze the fungal biomass and metabolomic data.

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

Not yet but we have a lot of result to present this year so I am attending 2023 National FHB Forum that will be held in Cincinnati, Ohio (December 3-5, 2023) and will present this work in the scab meeting.

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?

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