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

FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

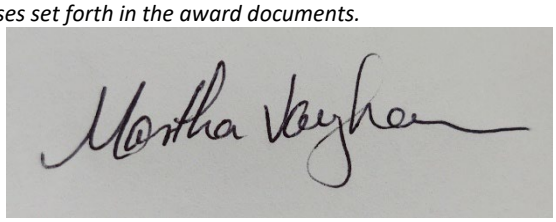
Principle Investigator (PI):	Martha Vaughan
Institution:	USDA-ARS
E-mail:	martha.vaughan@usda.gov
Phone:	309-681-6295
Fiscal Year:	2021
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Silencing Fusarium graminearum Virulence through Bacterial Associations
FY20 USDA-ARS Award Amount:	$51,107
Recipient Organization:	USDA-ARS National Center for Agricultural Utilization Research 1815 N University St., Peoria, IL 61604
DUNS Number:	N/A
EIN:	N/A
Project/Grant Period:	5/1/21 - 4/30/22
Reporting Period End Date:	4/30/2022

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Silencing Fusarium graminearum Virulence through Bacterial Associations	$51,107
	FY21 Total ARS Award Amount	$51,107

I am submitting this report as an: ☒ 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.



Principal Investigator Signature — Date Report Submitted: 7/27/22

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: Silencing Fusarium graminearum Virulence through Bacterial Associations

---

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

   Action Plan Goal: Manipulate bacterial-fungal associations to reduce *Fusarium graminearum* (*Fg*) virulence and/or fitness.

   This goal is being addressed through two objectives:
   Objective 1: Identify bacteria associated with *F. graminearum* (*Fg*) hyphae that can modulate fungal mycelial growth, reproduction, and/or mycotoxin production during plant-fungal interactions.
   Objective 2: Determine the nature of the bacterial- *F. graminearum* associations and identify methods to transfer these associations to other *F. graminearum* isolates.

2. **What was accomplished under these goals or objectives?** *(For each major goal/objective, address these three items below.)*

Objective 1: Identify bacteria associated with *F. graminearum* (*Fg*) hyphae that can modulate fungal mycelial growth, reproduction, and/or mycotoxin production during plant-fungal interactions.

a) What were the major activities?

We identified two *Fg* strains that have associations with bacteria and compared the fitness of the *Fg* with their bacterial associations to respective cured strains.

i) One *Fg* strain, F131+, was found to be associated with a *Stenotrophomonas* sp. This associations significantly reduced the ability of the strain to form perithecia in comparison to the cured strain. We performed three experimental replicates and confirmed the reproducibility of these results (Figure 1). Furthermore, we conducted wheat head inoculation assays and determined that the association does not affect the pathogenicity or aggressiveness of F131+ on wheat.

ii) A second *Fg* strain, Fg 47556+, was identified to have an association with *Paenibacillus illinoisensis.* In contrast to F131+, this association did not affect perithecia production but significantly reduced strain aggressiveness and deoxynivalenol (DON) production on wheat. Three independent experimental replicates revealed that the strain associated with *P. illinoisensis* caused approximately 50% less disease and produced 80% less DON (Figure 2).

Note: The Gz3639 strain was similarly treated with antibiotics following the same procedure used to cure the strains associated with the bacteria and no difference in perithecia or disease progression was observed.

b) What were the significant results?

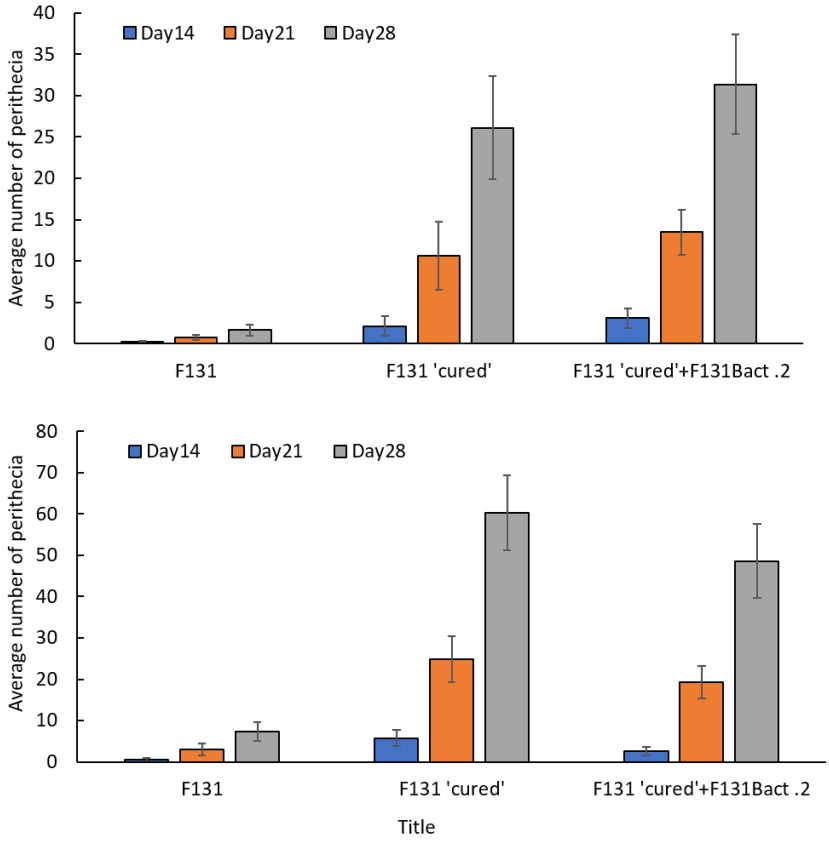
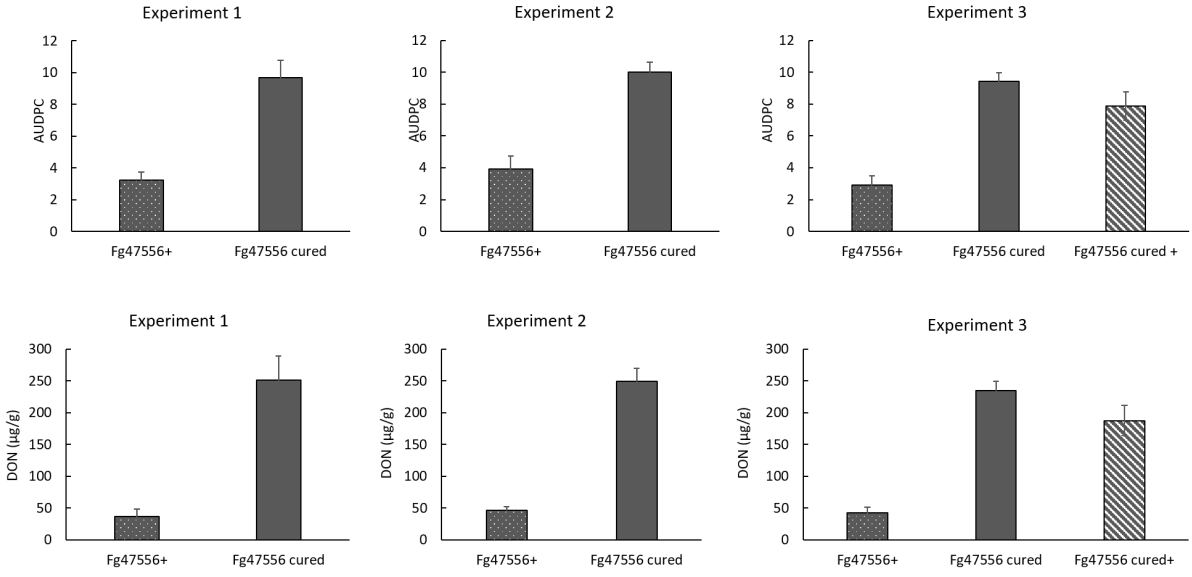


Figure 1. *Fg* strain, F131 which has endosymbiotic association with *Stenotrophomonas*, produces significantly less perithecia than the corresponding cured strain. Additionally, simply mixing the cured strain with the bacterial symbiont did not reconstitute the observed phenotype.



(Form – PPR21)

Figure 2. *Fg* strain, Fg47446+ which has ectosymbiotic association with *P. illinoisensis*, is significantly compromised in pathogenicity and mycotoxin production on wheat. Additionally, simply mixing the cured strain with the bacterial symbiont did not reconstitute the observed phenotype of the association.

c) List key outcomes or other achievements.

- We have identified a strain of *Stenotrophomonas* that can form an association with *Fg* and compromise its reproductive fitness by reducing perithecia production by approximately 83%.
- We have identified a strain of *Paenibacillus illinoisensis* that can form a vertically transferrable ectosymbiotic association with *Fg* and significantly reduce its pathogenic fitness resulting in 50% less disease and 80% less DON of wheat.
- These strains represent novel biocontrol agents that could be used to reduce a) pathogen inoculum formation and b) crop losses to disease and mycotoxin contamination.

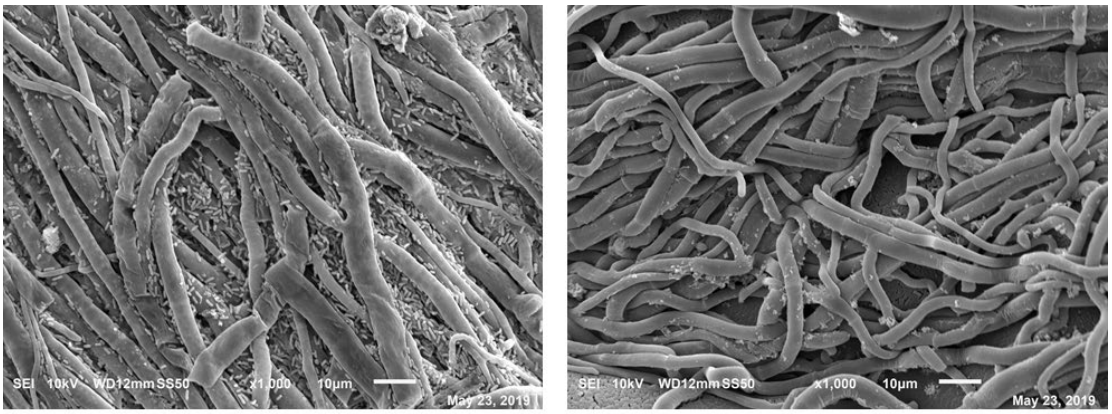
Objective 2: Determine the nature of the bacterial- *F. graminearum* associations and identify methods to transfer these associations to other *F. graminearum* isolates.

a) What were the major activities?

We conducted various experiments to determine the nature of the bacterial- *F. graminearum* associations.

i) The *Stenotrophomonas* likely has endosymbiotic association with *Fg* strain F131. It was not visible on hyphae observed with microscopic imaging but was detectible via quantitative PCR. To determine if the association vertically transferred with the spores, we captured the spores fired from the perithecia on the lid of the microcosms and used PCR to test for the presence of *Stenotrophomonas*. However, the bacterial association was not transferrable. Furthermore, we evaluated if the association between the bacteria and cured *Fg* isolate could be reformed by mixing the two species. But the phenotype of compromised perithecia production could not be reconstituted by simply mixing the bacteria back with the cured *Fg* strain (Figure 1). Thus, more complex reassociation methods are likely needed. Since this association did not affect disease and mycotoxin contamination, we focused primarily on attempting to reconstitute the association with *P. illinoisensis.*

ii) The *P. illinoisensis* appeared to have an ectosymbiotic association with *Fg*47556. The rod-shaped bacterial cells of *P. illinoisensis* were visible on the *Fg*47556+ hyphae Figure 3. Furthermore, the *P. illinoisensis* was vertically transferred with the spores. However, the association and phenotype of reduced pathogenicity and DON production could not be reconstituted by simply mixing the bacteria back with the cured *Fg* strain (Figure 2).



a) 47556+ b) 47556 cured

Figure 3. Scanning electron micrographs showing (a) bacterial cells on the surface of the hyphae of *Fg* strain 47556+, and (b) the absence of bacterial cells on 47556 cured by antibiotic treatment.

This past year (FY21) we focused on further evaluating the *P. illinoisensis* association with Fg47556. We designed an experiment to test whether the ectohyphal *Paenibacillus* could be manually removed by successive rinses. Replicate cultures of Fg47556+ were grown up overnight in either Tryptone Glucose Yeast Extract TGY) Broth or Potato Dextrose (PD) Broth. The cultures were then poured through a 40 µM filter basket to remove the media and washed once with sterile water. The fungal hyphae were then either removed and stored in a 1.5 mL microcentrifuge tube (which served as the 0x wash control treatment) or moved to a new filter basket and successively rinsed with sterile 0.04% Tween20 (a mild surfactant) 5x times, after which no bacteria were visible under a phase contrast microscope. Approximately half of the hyphae (0x and 5x washed sets) were transferred into a 15 mL conical tube, where they were manually crushed using sterile glass beads on a vortex machine. DNA was extracted from all treatments (2 medias x 2 wash levels x 2 yes/no crush treatments = 8 treatments). To quantitatively determine the nature of this relationship, we optimized primer sets design to amplify *Paenibacillus* using qPCR (primers MR-18/MR-19; Ranieri et al 2012 doi:10.1128/AEM.01361-12). These primer sets were used to analyze extracted DNA.

Additionally, we conducted experiments to re-establish the *P. illinoisensis* association with 47556. We designed an experiment that incorporated multiple treatments to test 1) whether mild forms of nutrient stress applied to Fg47556cured and *Paenibacillus* cultures prior to mixing would allow for the reassociation and 2) whether mixing the Fg47556cured and *Paenibacillus* at different concentrations would affect the success of reestablishment. Phenotypic differences were observed between the Fg47556+ and Fg47556cured strains grown on PDA. The cured strain had a distinct red color while the Fg47556+ strain with the *Paenibacillus* association was orange Figure 4.

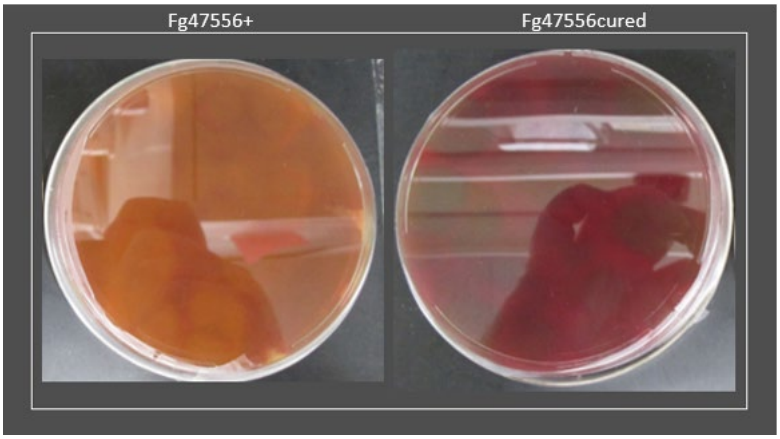


Figure 4. *Difference in color between* Fg47556+ and Fg47556cured strains grown on PDA.

Paenibacillus and Fg47556cured were grown in medias of reduced concentration (0.1x and 0.01x PD-Broth or 0.1 and 0.01x TGY-Broth) to test whether the reduced concentration would induce stress and increase likelihood of successful re-establishment. Initial trials showed that the *Paenibacillus* did not grow well in PDB, thus the 0.1x TGYB was selected for additional tests. Next, we used absorbance readings from a spectrophotometer to create mixing ratios of the 47556cured and *Paenibacillus* at 3:1, 5:1, 7:1, and 10:1 ratios. After 1 week, the different ratios of Fungus:Bacteria were plated onto three replicate plates per each of four different media types, 2% Water Agar (WA), PD-Agar (PDA), 0.1x TGY-Agar (0.1 TGYA), or V8-Agar (V8). After an additional 10 days, plates were visually inspected and compared to 47556+ and 47556cured cultures that had been put through the same mixing process.

Furthermore, to determine if mixing the bacteria back with the cured *Fg* strain resulted in similar ratios of the two species in the association, we designed species specific primers and evaluated the relative amount of *Paenibacillus* and *Fg* recovered from wheat heads using qPCR. Our results showed that the *Fg*47556+ had significantly more *Paenibacillus* than the 1:1 mix of bacterial cells and fungal spores (Figure 5). Thus, further experiments with greater amounts of *Paenibacillus* to *Fg* are currently underway.

b) What were the significant results?

Results from the qPCR test of the ectohyphal relationship showed that broth type was the greatest determinant of the bacteria presence. The *Paenibacillus* was essentially unable to grow in PD-Broth, relative to TGY-Broth, regardless of any efforts to manually remove the ectopic bacteria from the surface of the hyphae. On the other hand, the results from the efforts to manually remove *Paenibacillus* in TGYB were mixed. Successive washing 5x with the mild surfactant did reduce bacterial concentrations relative to the 0x wash control. However, manually crushing the hyphae also resulted in reduced *Paenibacillus* DNA, which was unexpected. Given the media specific outcome of this experiment, we plan to characterize the 47556-WT association in the future using fluorescent microscopy. The Live-Dead bacterial stain kit has been used in other systems to characterize endohyphal/ectohyphal bacteria in fungi (Herdari et al 2021; doi:10.1007/s00203-020-02122-4).

All attempts at reintroduction failed to re-establish the *Fg*47556+ phenotype. All plates had the same phenotype as the Fg47556cured plates, regardless of media type or fungal:bacterial ratio at mixing. *Fg*47556+ and Fg47556cured had the greatest phenotypic differences on the PDA and V8A media plates. Specifically, the Fg47556cured had a greater number of white hyphal spots on the PDA and less haphazard pink hyphal spots on the V8A. Minor differences were also noted on the 0.1x TYGA plates, with slightly more hyphal growth for the Fg47556cured strains relative to the *Fg*47556+.

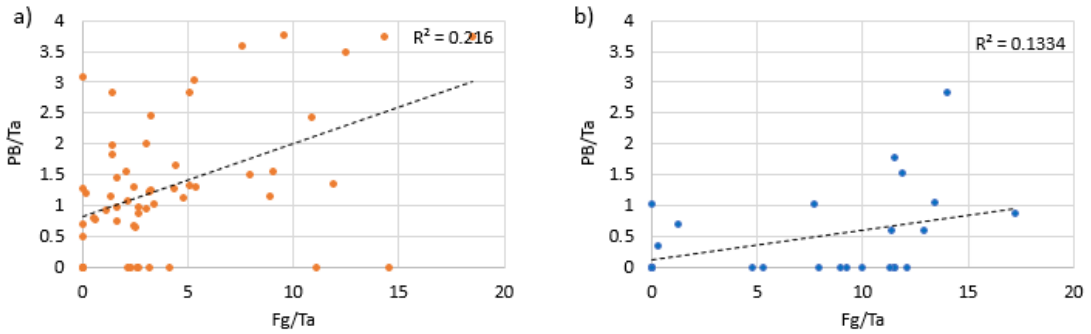


Figure 5. Relative amounts of *Paenibacillus illinoisensis* and *Fusarium graminearum* in *Fg*47556+ (a) and *Fg*47556cured+ inoculated Apogee wheat plants. *P. illinoisensis, F. graminearum and Triticum aestivum* specific primers were used to estimate the relative biomass of *P. illinoisensis* and *Fg* in the inoculated wheat heads.

c) List key outcomes or other achievements.

Determined *Paenibacillus illinoisensis* can form an ectohyphal association with *Fusarium graminearum* that results in reduced *F. graminearum* aggressiveness and DON contamination. The ratio of *Paenibacillus illinoisensis to Fusarium graminearum* appears to be important in developing the association between the species and likely the efficacy of being able to use this association as a means of disease and mycotoxin control.

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

This project provided the opportunity for training and professional development for an Oak Ridge Institute for Science and Education (ORISE) fellow (Nathan Kemp) and an early career scientist (Dr. Briana Whitaker).

Having completed his ORISE fellowship, which was funded by this USWBSI grant, Nathan Kemp gain the necessary experience to be competitive for permanent employment within the USDA. He is now a full-time federal employee at the same institution working with Dr. William Hay. His success story was featured: USDA ARS Research Participant Shapes a Career in Biological Science | Agricultural Research Service (ARS) Research Participation Program (orau.gov).

Dr. Briana Whitaker joined the team two years ago and was given the opportunity to work on this project as well. Her contributions earned her an invitation to present this research at 2021 USWBSI forum. She has not received USWBSI funding of her own as both a PI and co-PI.

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

These results were presented at the USWBSI forum as a poster in 2020 by Martha Vaughan, and the results were presented at an invited talk in 2021 by Briana Whitaker. A manuscript is currently in preparation.

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 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.