USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY20 Annual Performance Progress Report
Due date: July 29, 2021

Cover Page

Principle Investigator (PI):	Martha Vaughan
Institution:	USDA-ARS
E-mail:	martha.vaughan@usda.gov
Phone:	309-681-6295
Fiscal Year:	2020
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Silencing *Fusarium graminearum* Virulence through Bacterial Associations
FY20 USDA-ARS Award Amount:	$ 51,107
Project/Grant Reporting Period:	5/1/20 - 4/30/21
Reporting Period End Date:	4/30/2021

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Silencing *Fusarium graminearum* Virulence through Bacterial Associations	$ 51,107
	FY20 Total ARS Award Amount	**$ 51,107**

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 *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.

MARTHA VAUGHAN Digitally signed by MARTHA VAUGHAN
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Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R- Research
S – Service (DON Testing Labs)
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
EC-HQ – Executive Committee-Headquarters
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Soft Winter 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 *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- *Fg* associations and identify methods to transfer these associations to other *Fg* 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?**

   Objective 1: We identified two *Fg* strains associated with bacteria: a) F131+, was found to be associated with a *Stenotrophomonas* species and b) 47556+, was identified to have an association with *Paenibacillus illinoisensis.* The *Fg* strains were cured from their bacterial associations and the cured and uncured strains fitness were compared by evaluating the ability of the strains to produce deoxynivalenol (DON) in liquid culture, produce perithecia on wheat straw in microcosms, and cause disease on wheat.
   The *Fg* strains were cured of bacterial associations using a mixture of antibiotics. PCR was used to validate that the *Fg* strains were cured from the bacteria. The bacteria were isolated from the fungal strains and sequenced for identification. As a control, Gz3639 was similarly treated with antibiotics following the same procedures.
   To compare fitness of the strains we evaluated differences in toxin production, perithecia production and disease formation on wheat. The cured and uncured strains were grown in mycotoxin inducing agmatine media and the amount of DON produced was estimated from extracted cultures using GCMS. The strains were used to inoculate sterile wheat straw in microcosms and the number of perithecia produced were counted on day 14, 21 and 28. Apogee wheat was inoculated with the cured and uncured strain and the number of FHB symptomatic florets were scored on day 7, 10, 14, 17, and 21. After scoring on day 21, the inoculated heads were collected, lyophilized, pulverized and separated for biochemical and molecular analyzes. The amount of DON was estimated via GCMS analysis and the amount of *Fg* and associated bacteria were determined using quantitative PCR. Three experimental replicates were performed to assess reproducibility of fitness traits using Gz3639 as a

control to ensure that the antibiotic treatment did not result in any unintended fitness costs.

Objective 2: We utilized microscopy and molecular biology techniques to elucidate the nature of the bacterial associations and attempted to reconstitute the natural association. Under the microscope, bacteria could be observed on the hyphae of 47556+ but not F131+. However, if the hyphae of F131+ were crushed the bacteria was visible. DNA extraction and PCR amplification with species-specific primers validated the endosymbiotic association in the absents of external visualization. To determine if 47556+ also had an endosymbiotic association in addition to its visible ectosymbiotic association with *Paenibacillus,* the fungus was repeatedly washed with 0.02% tween water over a filter that was large enough for the bacteria to pass through but not the fungal hyphae (40 micron). The washed hyphae were grown on V8 media and visualized under the microscope, but the bacterial cells were no longer visible. Additionally, the hyphae were collected, DNA was extracted, and quantitative PCR was used to verify that no bacteria remained.

Once we had validated that *Stenotrophomonas* had an endosymbiotic association with *Fg* strain F131 and *P. illinoisensis* had an ectosymbiotic association with 47556. We tested if the bacterial associations could be vertically transferred with the spores. Microcosms containing wheat straw were inoculated with the different strains, after perithecia were formed, fired spores where collected from the lids of the dishes, and regrown on media. The *Fg* hyphae were then assessed for bacterial associations as above.

Attempts to reconstitute the bacterial associations with fungal hyphae by mixing the bacteria back with the cured *Fg* strain were unsuccessful and did not restore the phenotype of reduced *Fg* fitness. Further experiments using more complex reassociation methods were postponed due to ongoing laboratory occupancy limitations due to the pandemic.

b) What were the significant results?

We discovered that *Stenotrophomonas* and *Paenibacillus* bacteria can form associations with *Fg* strains that reduce the fungal pathogens fitness.

Stenotrophomonas formed an endosymbiotic association with the *Fg* strain, F13. This association was not vertically transferred with the fungal spores, but significantly compromised the strains reproductive fitness by reducing perithecia production by approximately 83% in comparison to the cured strain (Figure 1). Furthermore, the association did not affect the strains ability to produce DON or its ability to cause disease on wheat.

Paenibacillus formed an ectosymbiotic association with *Fg* strain, 47556. The association was vertically transferred with spores but did not affect perithecia production. However, the association significantly reduced the strains ability to produce DON and cause disease in wheat. Three independent experimental replicates revealed that the strain associated with *P. illinoisensis* caused approximately 50% less visual disease, 70% less pathogen biomass, and produced 80% less DON (Figure 2).

Mixing the cured *Fg* strains with the bacteria was not sufficient to reconstitute the association and resulting phenotype of less disease. In planta the relative amount of *Paenibacillus* (Pb DNA) to wheat DNA (Ta DNA) or Fusarium (Fg DNA) was not correlated with DON concentration but there was a visible difference between the naturally associated strain Fg47556+ and the Fg47556 cured with the *Paenibacillus* added back (Figure 3).

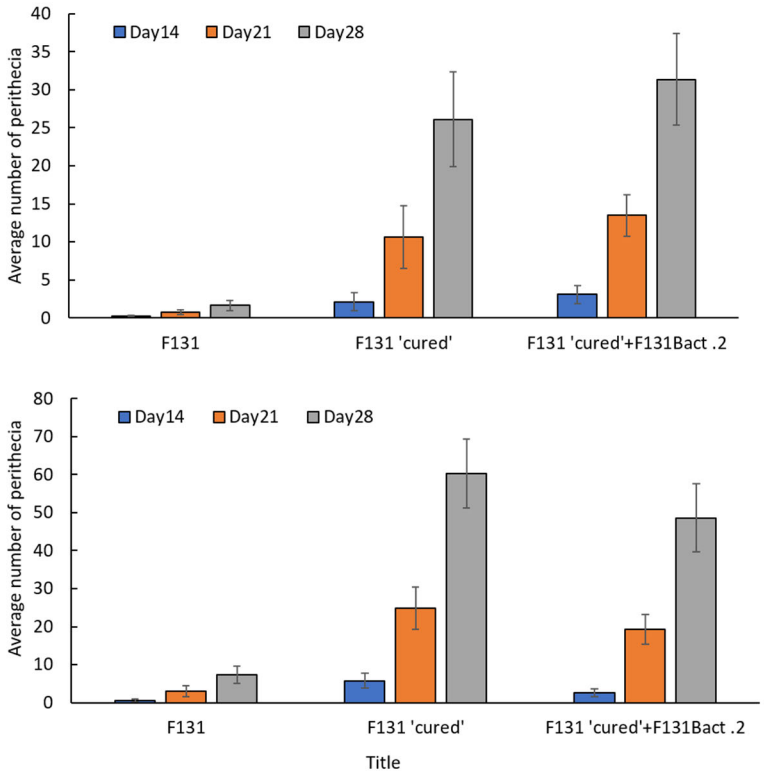

Figure 1. *Fg* strain, F131 which has endosymbiotic association with *Stenotrophomonas*, produces significantly less perithecia than the corresponding cured strain. Additionally, simply mixing the cured strain with the bacterial symbiont did not reconstitute the observed phenotype.

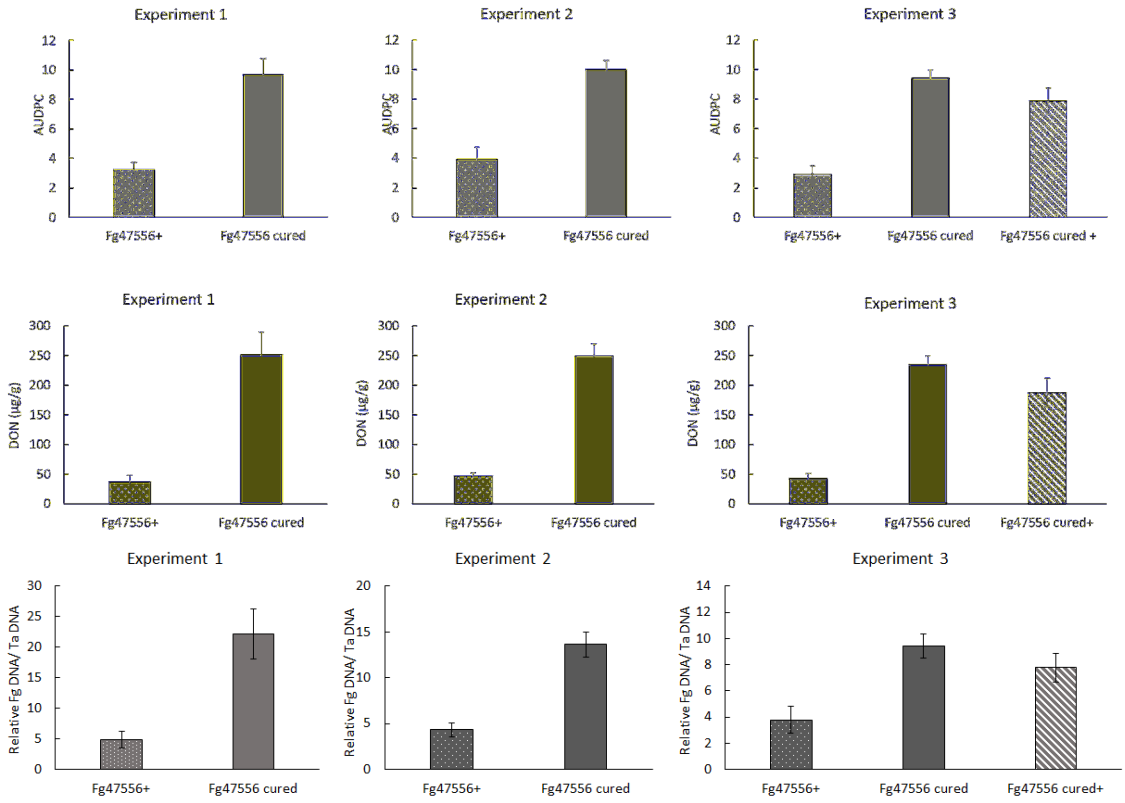

Figure 2. *Fg* strain, Fg47446+ which has ectosymbiotic association with *P. illinoisensis*, is significantly compromised in pathogenicity and mycotoxin production on wheat. Additionally, simply mixing the cured strain with the bacterial symbiont did not reconstitute the observed phenotype of the association.

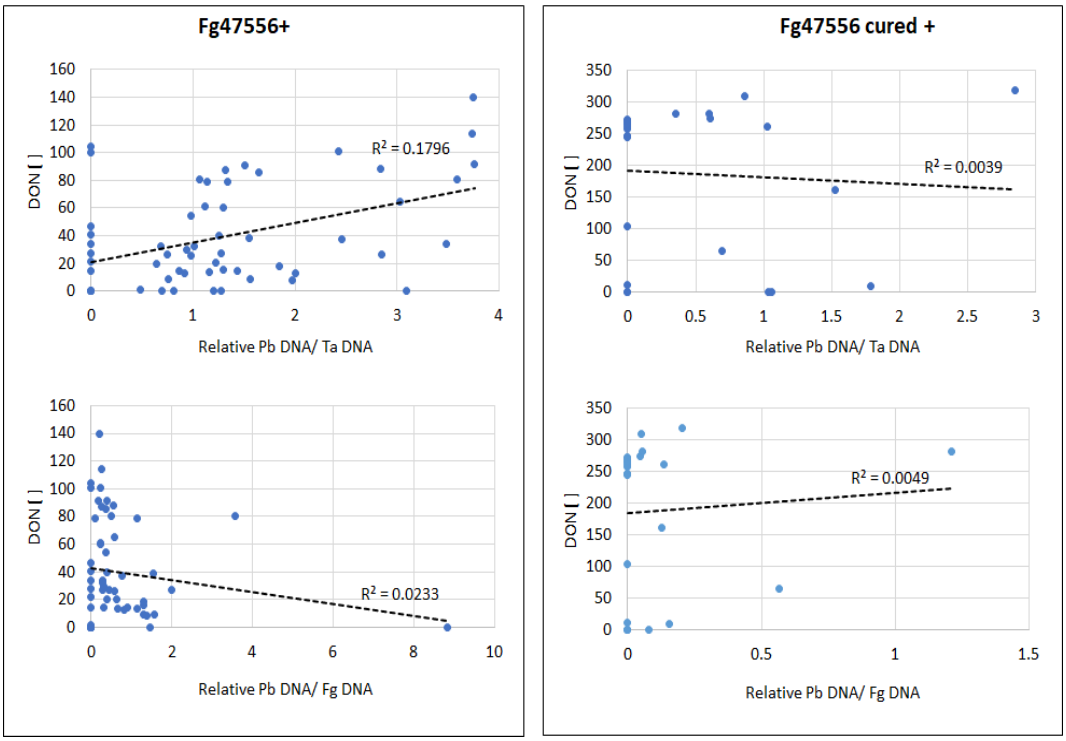

Figure 3. In planta, the relative amount of *Paenibacillus* (Pb DNA) to wheat DNA (Ta DNA) or Fusarium (Fg DNA) was not correlated with DON concentration ([ ]). Mixing the cured strain with the bacterial symbiont did not reconstitute the observed phenotype of less DON and the relative amounts of Pb DNA/ Fg DNA are much lower in the mix.

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.

Key outcome: Demonstrated that bacteria associations with *Fusarium graminearum* have the potential to be used as novel biocontrol agents to reduce a) pathogen inoculum formation and b) crop losses to disease and mycotoxin contamination.

3. **Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.**

   Since March of 2020 the USDA ARS had been operating under a mandatory maximum telework posture due to the pandemic. Onsite work is restricted to 25% normal capacity. While under these restrictions we have diligently worked to meet milestones, but regardless some of the proposed disease assays which required consistent everyday plant care were not possible to complete. Particularly, we were unable to complete the characterization of *Fusarium graminearum* disease phenotypes after attempting the bacterial re-associate. Furthermore, we still have several re-association methods, which we have not attempted. The USDA leadership has indicated that they do not anticipate us being back to full staff normal operation till Jan 2021.

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

   During the first year of this project, a trainee, Nathan Kemp, was employed via the USDA Agricultural Research Service (ARS) Research Participation Program, which is administered through the US Department of Energy's Oak Ridge Institute for Science and Education (ORISE). This Research Participation Program provides opportunities for students, postgraduates, established scientists and faculty to participate in programs, projects and activities at ARS-designated facilities to help ARS solve agricultural problems of high national priority. The program pairs young individuals who are focused on science, technology, engineering and math (STEM) with ARS research scientists and allows these individuals to gain experience in all aspects of the scientific research process in one of the ARS laboratory settings.

   Nathan's research activities as an intern included data collection and analysis. He contributed to experimental design and expanded his knowledge on a variety of new culturing methods, research assays and use of specialized equipment. Nathan also helped prepare a poster which was presented at last year's USWBSI. I am very proud to report that Nathan recently obtained a permanent technical support research position with another scientist at the USDA.

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

   These results have been presented at the USWBSI forum and a manuscript is currently in preparation.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (5/1/20 - 4/30/21). The term "support" below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student's stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY20 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY20 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

3. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

4. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY20 award period (5/1/20 - 4/30/21). All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

NOTE: Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.

Name of Germplasm/Cultivar	Grain Class	FHB Resistance	FHB Rating (0-9)	Year Released
Not applicable to this project.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
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Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Publications, Conference Papers, and Presentations

Instructions: Refer to the PR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY20 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (5/1/20 - 4/30/21**) should be included. If you did not publish/submit or present anything, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each citation, you **must** indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in the publication/ presentation. See example below for a poster presentation with an abstract:

Z.J. Winn, R. Acharya, J. Lyerly, G. Brown-Guedira, C. Cowger, C. Griffey, J. Fitzgerald, R.E. Mason and J.P. Murphy. 2020. "Mapping of Fusarium Head Blight Resistance in NC13-20076 Soft Red Winter Wheat." In: S. Canty, A. Hoffstetter, and R. Dill-Macky (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum (*p. 12.), Virtual; December 7-11. Online: https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

None.

Books or other non-periodical, one-time publications.

None.

Other publications, conference papers and presentations.

M.M. Vaughan, N. Kemp, M. G. Bakker and S. P. McCormick. 2020. "Identification of a Bacterial-Fungal Association that Silences *Fusarium graminearum* Virulence." In: S. Canty, A. Hoffstetter, and R. Dill-Macky (Eds.), *Proceeding of the 2020 National Fusarium Head Blight Forum* (p 94), Virtual; December 7-11. Online: https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Published
Acknowledgement of Federal Support: Yes