USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY19 Performance Report
Due date: July 24, 2020

Cover Page

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Fiscal Year:	2019
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Novel Microbial Antagonists of *F. graminearum* Rachis Spread and Spore Production
FY19 USDA-ARS Award Amount:	$ 58,419

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Novel Microbial Antagonists of *F. graminearum* Rachis Spread and Spore Production	$ 58,419
	FY19 Total ARS Award Amount	**$ 58,419**

MARTHA VAUGHAN Digitally signed by MARTHA VAUGHAN Date: 2020.07.20 14:55:24 -05'00'

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
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: *Novel Microbial Antagonists of F. graminearum Rachis Spread and Spore Production*

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

Goal 1: Identify beneficial microorganisms that are able to extensively colonize the rachis, constraining *Fusarium* secondary spread.

Goal 2: Identify specialist parasites of *Fusarium* perithecia on crop residue.

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

Goal 1: Identify beneficial microorganisms that are able to extensively colonize the rachis, constraining *Fusarium* secondary spread.

a) What were the major activities?
The *Sarocladium zeae* isolate that was identified and shown to consistently reduce Fusarium head blight disease progression and mycotoxin contamination, was further evaluated to determine the mechanism of control. To determine if the success of *S. zeae* colonization influenced FHB control, samples from the three-disease assay experimental replicates were used to estimate the density of the *S. zeae* within wheat heads. This was done via qPCR and expressed relative to abundance of a wheat gene. Using scanning electron microscopy, we further investigated the extent of endophytic colonization of *S. zeae* isolates. Additionally, the ability of *S. zeae* to influence host plant defenses was assessed. Wheat inflorescence samples were collected 24 hours following treatment with *Sarocladium* isolates and evaluated for the upregulation of defense related phytohormones. Furthermore, the induction of defense related phytohormones was evaluated during the first 48 hours after *F. graminearum* infection to determine if pre-colonization of the *Sarocladium* isolates primed the host plants defenses. Finally, experiments were conducted to determine the potential of introducing endophytic colonization of the biocontrol isolate into two distinct wheat cultivars using a seed treatment method.

b) What were the significant results?
Strains of *S. zeae* differ in their colonization ability, but one *S. zeae* strain was shown to be a systemic endophyte of wheat, successfully colonizing the majority of internal plant organs and surviving within the plant through its life cycle. When allowed to pre-colonize wheat ahead of inoculation with *F. graminearum*, this strain significantly reduced FHB symptoms (57.9% reduction in area under the disease progress curve) and DON content in harvested wheat heads (61.2% reduction). While these protective effects may arise from multiple simultaneously acting mechanisms, we demonstrate that plant hormones related to defense signaling respond to the presence of *S. zeae*, indicating that defense priming may be an important mechanism leading to protection in this system.

c) List key outcomes or other achievements.
We have identified an endophytic biocontrol isolate of *S. zeae* that successfully colonizes wheat tissues and significantly reduced FHB symptoms and DON contamination. Utilizing a long-lasting, systemic endophyte for biological control would provide a number of benefits over conventional biological control at the plant surface. For instance, systemic endophytic biological control agents have the potential to provide protective effects in more than one tissue type, and may be amenable to alternative application methods, such as introduction via coating of seeds prior to planting.

Goal 2: Identify specialist parasites of *Fusarium* perithecia on crop residue.

a) What were the major activities?
Soil samples were collected from local agricultural fields which either utilized cover crops or left fallow during the winter months. Mixtures of bacteria from these different soils were assessed for the ability to reduce *F. graminearum* perithecia formation, and microbiome analyses were conducted and compared between the different soils. The microbiome analyses identified *Stenotrophomonas* as a bacterium that readily colonized *F. graminearum* perithecia, and upon evaluation was shown to delayed perithecia formation. Therefore, we evaluated the ability of several *Stenotrophomonas* isolates to reduce *F. graminearum* perithecia production. Additionally, genome sequencing analysis revealed that a wild-type *F. graminearum* strain, F131, was heavily contaminated with a bacterial symbiont also identified as *Stenotrophomonas*. We compared perithecia formation of F131 to the same strain cured of the bacteria. Furthermore, we evaluated and compared the ability of F131to cause disease on wheat heads.

b) What were the significant results?
Bacteria from soil samples collected from fields that utilized cover crops reduced *F. graminearum* perithecia formation to a greater extent than soil bacteria from fields left fallow. *Stenotrophomonas* isolates readily colonized *F. graminearum* perithecia and reduce perithecia formation to approximately half when evaluated on day 14. The bacterial association between F131 and *Stenotrophomonas* significantly inhibited perithecia formation. In comparison to F131 that had been cured of the bacterium via antibiotics, the F131 that was associated with *Stenotrophomonas* produced 4-fold fewer perithecia. However, this association did not inhibit F131 from causing FHB on wheat.

c) List key outcomes or other achievements.
The use of cover crops may facilitate the establishment of beneficial bacterial microbiomes that reduce *F. graminearum* inoculum in the field. *Stenotrophomonas* isolates can delay or reduce perithecia formation. We have identified a strain of *Stenotrophomonas* that forms a close association with *F. graminearum* and can reduce perithecia formation by 73%.

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

On 3/25/2020 the IL governor issues a shelter at home order and the USDA initiated a maximum telework mandate. Although the shelter at home order has been lifted the maximum telework manded is still in place because the number and trend of COVID-19 cases in our area do not meet the designated standards. Therefore, we have been unable to complete final experiment needed to prepare the manuscript regarding the identification of bacteria that colonize *F. graminearum* perithecia. Specifically, we must complete final experimental replicates to ensure that the *Stenotrophomonas* isolates consistently reduce or delay perithecia formation.

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

This project provided an Oak Ridge Institute for Science and Education (ORISE) trainee, Nathan Kemp, with an exceptional experience in a research setting. Nathan received hands-on experience in a wide range of microbiology and molecular biology techniques. His training as an ORISE participant helped shape and enhance his talents as a researcher and scientist, inspiring him to seek full-time employment as a research technician within the National Center for Agricultural Utilization Research (NCAUR) in Peoria, IL. ORISE has featured Nathan's success story for the program on their website: https://orise.orau.gov/usda-ars/profiles/kemp.html.

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

Results from the first goal have been published in the Biological Control journal. The manuscript is entitled, "*Sarocladium zeae* is a systemic endophyte of wheat and an effective biocontrol agent against Fusarium head blight." The results from the second goal have been presented at the USWBSI 2019 National FHB Forum. Furthermore, these results are scheduled to be presented at the Plant Biology 2020 Worldwide Summit on July 27-31.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (N/A). 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 FY19 award period?**
   Not applicable.
   If yes, how many?

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY19 award period?**
   Not applicable.
   If yes, how many?

3. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   Not applicable.
   If yes, how many?

4. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   Not applicable.
   If yes, how many?

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 FY19 award period. 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** (S, MS, MR, R, where R represents your most resistant check)	**FHB Rating** (0-9)	**Year Released**

Add rows if needed.

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

Abbreviations for Grain Classes

Barley - BAR
Durum - DUR
Hard Red Winter - HRW
Hard White Winter - HWW
Hard Red Spring - HRS
Soft Red Winter - SRW
Soft White Winter - SWW

Publications, Conference Papers, and Presentations

Instructions: Refer to the FY19-FPR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY19 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (N/A**) 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:

De Wolf, E., D. Shah, P. Paul, L. Madden, S. Crawford, D. Hane, S. Canty, R. Dill-Macky, D. Van Sanford, K. Imhoff and D. Miller. 2019. "Impact of Prediction Tools for Fusarium Head Blight in the US, 2009-2019." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum*, Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY. p. 12.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

Kemp, N.D., Vaughan, M.M., McCormick, S.P., Brown, J.A., and Bakker, M.G. 2020. "*Sarocladium zeae* is a systemic endophyte of wheat and an effective biocontrol agent against Fusarium head blight. Biological Control 149: 104329.
Status: Published https://doi.org/10.1016/j.biocontrol.2020.104329
Acknowledgement of Federal Support: YES

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

Nothing to Report

Other publications, conference papers and presentations.

Kemp, N.D., Bakker, M.G., McCormick, S.P., and Vaughan, M.M. 2019. "*Stenotrophomonas* Bacteria Readily Colonizes *Fusarium graminearum* Perithecia and Reduced Perithecia Formation." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum*, Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY. p. 73.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)