USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY19 Final Performance Progress Report
Due date: August 31, 2021

Cover Page

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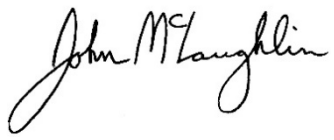
Phone:	848-932-6359
Fiscal Year:	2019
USDA-ARS Agreement ID:	59-0206-8-213
USDA-ARS Agreement Title:	Suppression of FHB by Green Leaf Volatiles (GLVs)
FY19 USDA-ARS Award Amount:	$ 38,760
Recipient Organization:	Rutgers, The State University of New Jersey Division of Grant and Contract Accounting ASB 111, 3 Rutgers Plaza New Brunswick, NJ 08901·8559
DUNS Number:	00-191-2864
EIN:	22-6001086
Recipient Identifying Number or Account Number:	824625 (Oracle # 124243)
Project/Grant Reporting Period:	7/1/19 - 6/30/21
Reporting Period End Date:	6/30/2021

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Suppression of FHB by Green Leaf Volatiles (GLVs)	$ 38,760
	FY19 Total ARS Award Amount	**$ 38,760**



8/30/21

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R – Research
S – Service (DON Testing Lab)
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: *Suppression of FHB by Green Leaf Volatiles (GLVs)*

1. **What are the major goals and objectives of the research project?**
   1) Determine the effect of volatile treatment on susceptibility of wheat to F. graminearum.
   2) Determine if the volatile treatment induces expression of the defense genes in wheat.
   3) Determine if FHB resistance can be improved by increasing the production of volatiles in wheat.

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?

   Previous research in the lab found that green leaf volatiles (GLV) are potent inhibitors of *Fusarium graminearm* growth and that certain concentrations are lethal to the fungus. We set out to investigate how GLV impact fungal grown on wheat. Using point inoculation assays of wheat seedlings, we identified concentrations of GLV that were able to significantly reduce fungal growth on the plant. At higher concentrations, to our surprise, GLV exposure encourages fungal growth relative to the mock (ethanol) treatment. Using both stress and defense-related genes to monitor green leaf volatile (GLV) exposure response in wheat, we were able to identify that at high concentrations of the volatile, wheat stress genes like heat shock protein 90 (HSP90) are greatly induced. We identified concentrations which greatly reduced stress-associated genes and increased defense-associated genes. We found that reducing the concentration of the GLV from 1 ppm to 0.01 ppm and pre-treating the plants significantly increased resistance of wheat to the fungus.

   b) What were the significant results?

   We found that the GLV (E)-2-hexenal can act to either suppress or promote fungal growth on wheat, depending on the concentration of the GLV. We found that the plant responds quickly to the GLV even at low concentrations (0.001 ppm) and within a short time period (1 hour). Expression analysis shows that GLV upregulates wheat defense-related genes, such as TaPR1 (Plant Resistance1) at low concentrations (0.01 ppm and below). At higher concentrations (0.05 ppm and above) we see significant induction of stress related genes like TaHSP90.1 (Heat Shock Protein 90.1). TaHSP90.1 was especially responsive to the GLV with a 1 ppm exposure leading to a >400 fold increase relative to mock treatment. Pre-treatment of wheat seedlings with GLV can significantly impact resistance to *F. graminearum*. At lower concentrations (0.01 ppm) we see a significant increase in resistance to the fungus as measured 5 days post inoculation, while at higher concentrations, closer to the EC50 for the fungus alone (calculated to be 0.6 ppm), we see a significant decrease in resistance at 5 days post inoculation. This indicates that the GLV can modulate plant defense genes but that concentrations in the 0.05 ppm and above range are detrimental to the plant in terms of resistance to *F. graminearum*. The pre-treatment of wheat plants with the GLV

lead to the induction of plant defense genes and likely contributes to enhanced resistance to the fungus.

c) List key outcomes or other achievements.

Our earlier work which found that (E)-2-hexenal is a potent inhibitor of *F. graminearum* (an EC_{50} of 0.06 ppm for the GLV vs fungal plug was estimated). Together with the finding that wheat responds to the GLV in a dose-dependent manner (0 to 1 ppm) in terms of the induction of TaHSP90.1, suggests that measurement of FgHSP genes may offer a way to identify toxic concentrations of the GLV before visual impact on fungal growth is evident. After identifying the concentrations and time course of GLV exposure, we can move forward to performing RNAseq with this material to identify the spectrum of genes which are regulated in response to the volatile. We are preparing a paper on these findings.

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.

Early on, this research was impacted by the pandemic by limiting research hours in the laboratory.

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

The project has provided for the training for four undergraduate students (Maha Kahn, Noura AlDarwish (RISE Scholar), Waner Zheng (Douglas Scholar), and Jeffrey Garcia-Sanchez (McNair Scholar and Honors Thesis project) and one PhD student (Khadija Abdulhafid). The students have learned how to grow *Fusarium graminearum*, grow wheat/barley in the greenhouse, isolate Fusarium conidia and count the spores with a hemocytometer and flow cytometry, how to inoculate plants, how to perform protein isolations and analysis using SDS-PAGE/Western, how to isolate high quality plant DNA, and how to perform qPCR. The students gave presentations during lab meetings and learned how to prepare and present both posters and presentations on their results. Jeffrey Garcia-Sanchez successful defended his Honors Thesis project working with me on this project. The title of his undergraduate thesis was "Combating *Fusarium graminearum* in wheat: Testing the overexpression of nsLTPs and measuring the impact of exposure to green leaf volatiles". Jeffrey will attend the Biological Sciences Molecular, Cellular, & Developmental Biology program at the University of South Carolina this fall. https://lsamp-nb.rutgers.edu/lsamp-class-2020-cohort

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

Through poster presentations and abstracts in conference proceedings.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the **FY19 award period (7/1/19 - 6/30/21)**. 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 FY19 award period?**
   ☐Yes ☐No ☒ Not Applicable
   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 FY19 award period?**
   ☐Yes ☐No ☒ Not Applicable
   If yes, how many? Click to enter number here.

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?**
   ☐Yes ☐No ☒ Not Applicable
   If yes, how many? Click to enter number here.

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?**
   ☐Yes ☐No ☒ Not Applicable
   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 **FY19 award period (7/1/19 - 6/30/21)**. 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
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
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

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 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 (7/1/19 - 6/30/21**) 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)

Nothing to Report

Journal publications.

None.

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

None.

Other publications, conference papers and presentations.

John E. McLaughlin, Khadija Abdulhafid and Nilgun E. Tumer. Green Leaf Volatiles (GLVs) Effectively Inhibit *Fusarium graminearium* but the Impact on Infection in Wheat Exposed to Exogenous Supplied GLVs is Complicated. 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. 53.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)