

USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 FINAL Performance Report
Due date: August 30, 2019

Cover Page

Principle Investigator (PI):	Christopher Toomajian
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Fiscal Year:	2017 (NCE for FY18)
USDA-ARS Agreement ID:	59-0200-6-002
USDA-ARS Agreement Title:	Finding <i>Fusarium graminearum</i> Genes to Target using Population Genomics.
FY17 USDA-ARS Award Amount:	\$ 47,247
Recipient Organization:	Kansas State University 10 Anderson Hall Manhattan, KS 66506
DUNS Number:	929773554
EIN:	48-0771751
Recipient Identifying Number or Account Number:	AR9855, GAPP603891
Award Reporting Period:	4/21/18 - 8/20/19
Reporting Period End Date:	08/20/19

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Population Genomics to Identify <i>Fusarium graminearum</i> Genes to Target.	\$ 47,247
	FY17 Total ARS Award Amount	\$ 47,247



August 19, 2019

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: *Population Genomics to Identify Fusarium graminearum Genes to Target.*

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

The goal of our project is to identify genes harboring functional variation that contributes to variation in important pathogen traits (pathogenicity, mycotoxin production, measures of fitness) within *F. graminearum* (*Fg*) populations to provide targets for pathogen control.

Project objectives:

- 1) Phenotype approximately 150 *Fg* isolates from the over 500 isolates genotyped in our FY14-15 project, our subset composed of isolates chosen to represent the full genetic diversity of the previously genotyped isolates from the US.
- 2) Perform genome-wide association studies (GWAS) of the above traits, taking into account population structure. Polymorphisms associated with pathogen traits will be compared to the results of the scans for natural selection from our FY14-15 project as well as aggressiveness or mycotoxin production QTL identified by other groups through biparental mapping strategies.

2. What was accomplished under these goals? *Address items 1-4) below for each goal or objective.*

Objective 1

- 1) Specific objectives: This objective included measuring two classes of traits – virulence traits of the *Fg* isolates on wheat as well as saprophytic traits of isolates in the laboratory.
- 2) Major activities: Trainee Dhakal has been testing various procedures and conditions for culturing our *Fg* isolates for measuring their growth rates. Replicated growth measurements have been completed for some isolates and are underway for our remaining isolates. As personnel Dhakal was only brought onboard this project this last spring, we have postponed all virulence trait measurements (of isolates inoculated on wheat) until summer 2019 is over and greenhouse temperatures are suitable for wheat.
- 3) Significant results: By trialing our growth rate experiments, we have an idea of the variation in rates we see across isolates, replicates, and different culture media. We observed some unusual patterns of differences in rates of growth from single spores across replicates from the same isolate, and have worked to minimize this source of variation in our experiments.
- 4) Key outcomes or other achievements: Dhakal's testing has established the optimum conditions for measuring growth rates to allow the detection of minor rate differences between different *Fg* isolates.

Objective 2

- 1) Specific objectives: This objective included performing genome-wide association mapping of traits measured in objective 1 to look for SNPs identified in our previous project associated with these traits.
- 2) Major activities: GWAS-specific activities are postponed until we complete trait measurements from objective 1 above. However, personnel Dhakal has continued previous work re-analyzing population structure in our full set of 570 Fg isolates.
- 3) Significant results: As a result of the re-analysis of population structure in our full Fg sample, which included isolates from three main US regions (NY state, the Upper Midwest, and Louisiana) plus from Uruguay, we identified sets of clonal lineages within our sample. Our sample also includes strains that produce different forms of trichothecenes, from 3-ADON and 15-ADON to NX-2 and NIV. We found that some of our isolates from Louisiana (including most NIV producers and some of both 3-ADON and 15-ADON strains) were genetically very distant from the rest such that they could not be included in a GWAS analysis with the rest of the isolates. We also found important natural recombinant isolates, where genome-wide SNPs group the isolate into a cluster made up largely of isolates of one trichothecene type, while the SNP haplotype of TRI gene clusters identify the isolate as belonging to a different trichothecene type. Interestingly, we find several isolates with the 15-ADON haplotypes on 3-ADON backgrounds, but no isolates with 3-ADON haplotypes on 15-ADON backgrounds.
- 4) Key outcomes or other achievements: A major outcome made possible by the analysis of population structure in our set of Fg isolates has been the final choice of the subset of isolates (between 100 and 150) that will be included in trait measurement experiments and subsequent GWAS.

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

During the current reporting period, the project has provided training for new Ph.D. student Upasana Dhakal, support personnel for this project, collaborator Vero Fumero from Argentina, and collaborator Tamara Krska from Austria. Dhakal received training on modern genotyping technologies, bioinformatics, population genetic analytical techniques, and conditions for culturing *Fusarium graminearum*. Fumero received similar training on genotyping, bioinformatics and population genetics analysis. Krska received training on PCR and DNA sequence analysis. PI Toomajian used data from USWBSI projects in teaching his graduate Population Genetics class to 17 students in spring 2019. PI Toomajian and personnel Dhakal participated in the free, Scab-sponsored and ASA distributed FHB basics and management webinars. Co-PI Leslie provided instruction at the June 2018 International Fusarium Laboratory Workshop at Bari, Italy (including 4 lectures and labs) and the February 2019 Tropical Fusarium Workshop at the University of Brasilia (including 3 lectures with

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labs). He also organized 6 round table discussions on the future of mycotoxin research in China at the April 2019 MyToolBox EU/China meeting in Beijing and helped set-up a new mycotoxin detection lab in Kathmandu, Nepal through the USAID Feed the Future lab. Co-PI Leslie provided professional development opportunities on scientific writing at the USAID Feed the Future Nutrition Conference and Helen Keller International, both in Kathmandu, Nepal and at the University of Brasilia. Finally, both PIs provided instruction and lectures (5 total), and co-PI Leslie coordinated and managed the Fusarium Laboratory Workshop at Kansas State University in June 2019, which featured 11 instructors and 36 participants.

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

During the current period, results from this project have been disseminated through participation at the 30th Fungal Genetics Conference (Pacific Grove, CA, March 2019, with poster presentation) and its satellite Fusarium Workshop, the NC1183 Annual Meeting at Rutgers University, and the 2nd MycoKey Conference in Wuhan, China. Both PIs participated in Kansas State University's annual open house for the purpose of outreach to enhance public understanding of plant pathology and increase interest in careers in this field.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY16-NCE (FY18) period. 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 FY16 award period?**

No

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 FY16 award period?**

No

If yes, how many?

3. **Have any post docs who worked for you during the FY16 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**

No

If yes, how many?

4. **Have any post docs who worked for you during the FY16 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**

No

If yes, how many?

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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 FY17-NCE (FY18) 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

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Publications, Conference Papers, and Presentations

Instructions: Refer to the FY16_NCE-FY18_FPR-Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY16 award. Only include citations for publications submitted or presentations given during your NCE period (4/21/18 - 4/20/19). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each reference/citation, you must indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in publication/presentation.

Journal publications.

Bushula, V.S., J. Jebril, **J.F. Leslie**, T. Tesso, C.R. Little. 2018. Stalk rot of sweet sorghum caused by genetically diverse *Fusarium thapsinum* strains (Abstr.) *Phytopathology* 108(10):S.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: No, publication resulted from other funding source.

Vismer, H.F., G.S. Shephard, L. van der Westhuizen, P. Mngqawa, V. Bushula-Njah, **J.F. Leslie**. 2019. Mycotoxins produced by *Fusarium proliferatum* and *F. pseudonygamai* on maize, sorghum and pearl millet grains *in vitro*. *Int. J. Food Microbiology* 296: 31-36. DOI: org/10.1016/j.ijfoodmicro.2019.02.016.

Status: Published

Acknowledgement of Federal Support: Indirectly, through the inclusion of Kansas Agricultural Experiment Station manuscript number

Mohamed Nor, N.M.I., B. Salleh and **J.F. Leslie**. 2019. *Fusarium* species from sorghum in Thailand. *The Plant Pathology Journal*.

Status: In press

Acknowledgement of Federal Support: Indirectly, through the inclusion of Kansas Agricultural Experiment Station manuscript number

Torres, A.M., S.A. Palacios, N. Yerkovich, J.M. Palazzini, P. Battilani, **J.F. Leslie**, A.F. Logrieco and S.N. Chulze. 2019. Fusarium head blight and mycotoxins in wheat: Prevention and control strategies across the food chain. *World Mycotoxin Journal*.

Status: In press

Acknowledgement of Federal Support: Indirectly, through the inclusion of Kansas Agricultural Experiment Station manuscript number

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Ferrara, M., M. Haidukowski, A.F. Logrieco, **J.F. Leslie**, and G. Mulè. 20xx. A CRISPR-Cas9 system for genome editing of *Fusarium proliferatum*.

Status: Submitted

Acknowledgement of Federal Support: Indirectly, through the inclusion of Kansas Agricultural Experiment Station manuscript number

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

Other publications, conference papers and presentations.

Conference presentations

NC1183 Annual Meeting, Rutgers University, May 18, 2018, **Leslie, J.F.**, “2017 Mycotoxins in Kansas”

Status: Oral presentation given

Acknowledgement of Federal Support: No, presentation resulted from other funding source.

US Culture Collection Network Annual Meeting, August 22, 2018, **Leslie, J.F.**, “Role of the Fungal Genetics Stock Center in the Community of Living Culture Collections”

Status: Oral presentation given

Acknowledgement of Federal Support: No, presentation resulted from other funding source

Neurospora Information Conference, Pacific Grove, CA, October 18-21, 2018. **Leslie, J.F.**, “Status of the Fungal Genetics Stock Center”

Status: Oral presentation given

Acknowledgement of Federal Support: No, presentation resulted from other funding source

30th Fungal Genetics Conference, Pacific Grove, CA, March 2019. Fumero, M., W. Yue, M. Chiotta, S. Chulze, **J. Leslie**, **C. Toomajian**. Population genomics reveals patterns of divergence and gene flow between populations of *Fusarium subglutinans* and *Fusarium temperatum* in Argentina. (conference poster/abstract)

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (poster), NO (abstract)

Invited presentations

USAID Feed the Future Post-Harvest Loss Innovation Lab, May 1, 2018, **Leslie, J.F.**, “Mycotoxins in wheat in Afghanistan”

Status: Oral presentation given

Acknowledgement of Federal Support: No, presentation resulted from other funding source

Plant Biosecurity CRC Closeout Science Exchange, May 30, 2018, **Leslie, J.F.**, “K-State’s Role in International Biosecurity”

Status: Oral presentation given

Acknowledgement of Federal Support: No, presentation resulted from other funding source

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Ecological Genomics Research Forum, Kansas State University, Manhattan KS, July 2018.

Toomajian, C., Population genomics of fungal maize pathogens from Argentina.

Status: Oral presentation given

Acknowledgement of Federal Support: No, work resulted from other funding source.

2nd MycoKey Conference, Wuhan, China, September 14-20, 2018, **Leslie, J.F.**, “Populations of Mycotoxigenic Fungi” as well as organized Round Table discussion on future research on mycotoxins in wheat.

Status: Oral presentation given

Acknowledgement of Federal Support: No, presentation resulted from other funding source