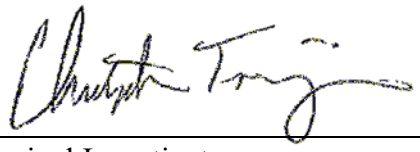
USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY16 Final Performance Report
Due date: July 28, 2017

Cover Page

Principle Investigator (PI):	Christopher Toomajian
Institution:	Kansas State University
E-mail:	toomajia@ksu.edu
Phone:	785-532-0879
Fiscal Year:	2016
USDA-ARS Agreement ID:	59-0206-6-002
USDA-ARS Agreement Title:	Finding *Fusarium graminearum* Genes to Target using Population Genomics.
FY16 USDA-ARS Award Amount:	$ 47,339
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
Project/Grant Reporting Period:	4/21/16 - 4/20/17
Reporting Period End Date:	04/20/17

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Population Genomics to Identify *Fusarium graminearum* Genes to Target.	$ 47,339
	FY16 Total ARS Award Amount	**$ 47,339**



7/26/17

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:

- 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.
- 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.*

A number of the plans for the reporting period had to be adjusted due to unanticipated events, though we were still able to accomplish related activities for the project and expect to complete all major objectives for the project by the end of the 2nd year reporting period. Major greenhouse experiments were scheduled for the winter of 2016-17, but PI Toomajian needed to take paternity leave for much of this season and it was decided it was better to delay these experiments until the fall and winter of 2017-18.

Specific objective 1: This objective included measuring two classes of traits – virulence traits of the *Fg* isolates on wheat as well as saprophytic traits and measures of DON accumulation of isolates in the laboratory.

1) Major activities: A number of the major activities for this objective had to be postponed as explained above. Only preliminary laboratory experiments on isolate growth rates in culture have been performed at this stage of the project.

2) specific objectives: See 'Specific object 1:' above.

3) Significant results: At this stage we have no significant results to report on additional isolate phenotyping.

4) Key outcomes or other achievements: No key outcomes achieved yet for this objective.

Specific objective 2: 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.

1) major activities: As greenhouse trait collection has been postponed, we have no major activities to report for this objective outside of the preliminary GWAS that we had already performed before the current reporting period.

2) specific objectives: See 'Specific object 2:' above.

3) Significant results: At this stage we have no significant results on our planned GWAS.

4) Key outcomes or other achievements: No key outcomes achieved yet for this objective.

Although our research plans had to be adjusted for the current reporting period, we can report accomplishments related to 1) the analysis of patterns of linkage disequilibrium (LD) in our *Fg* isolates, and 2) our reanalysis of phenotypes from the study of a set of 3-ADON and 15-ADON *Fg* isolates collected from New York that had not found any major phenotypic differences between the 2 chemotype groups.

Specific objective 3: To analyze the pattern of decay of LD over physical distance along chromosomes to predict outcomes of our GWAS analysis based on the fraction of the *Fg* genome that may not be covered by our marker set and the average width of association peaks (*i.e.*, how many candidate genes might be included under each peak) that we expected.

1) Major activity: In the course of preparing his dissertation, graduate student Wei reanalyzed patterns of LD decay from subsets of our *Fg* isolates and discovered that PI Toomajian's preliminary results included an error which excluded pairs of SNPs in very low LD. This had caused what appeared to be significant background LD that we initially feared was due to population structure even within smaller subsets of our data.

2) specific objectives: See 'Specific object 3:' above.

3) Significant result: Upon reanalysis, we see that LD decays rapidly within a few kb and that by 100 kb essentially all traces of LD have disappeared.

4) Key outcome: This result indicates that our predicted association peaks should be narrow and include very few genes, allowing rapid progress in moving from association candidates to functionally verified causative genes. Low LD could mean that some causative genes found in regions of the genome with the highest levels of recombination could be missed, but the density of our marker set makes this outcome unlikely.

Specific objective 4: Reanalysis of the results of Spolti *et al*. 2014 in light of GBS genotypes.

1) Major activity: After categorizing NY *Fg* isolates based on the subpopulation to which they belong (using genome-wide SNP data), look for significant trait differences between groups.

2) specific objectives: See 'Specific object 3:' above.

3) Significant result: Isolates grouped by subpopulation showed significant differences at many more traits than the 1 trait difference related to type of DON produced when they were grouped based on TRI genotypes. Though hinted at in our FY16 proposal, the implications of this result have become clear after spending more time with our population structure data.

4) Key outcome: Finding that just by recategorizing a few isolates ("recombinant" in the sense that they carry the TRI genotype associated with the 3-ADON chemotype but belong to the subpopulation predominated by isolates with the 15-ADON chemotype, or vice versa) the difference in the average of several traits could become significant suggests a resolution to the contradictory results of trait differences between these two chemotypes. Many tests between chemotype groups could show significant differences if they don't include any "recombinant" isolates. Yet without "recombinant" isolates, any number of loci besides the TRI cluster that systematically differ between subpopulations can explain the trait differences.

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

During the current reporting period, the project has provided training for 3 Ph.D. students, including Wei Yue, support personnel for this project, and visiting Ph.D. student Vero Fumero. Both received training on modern genotyping technologies relevant for Fusarium as well as bioinformatics and population genetic analytical techniques. Additionally, co-PI Leslie provided professional development opportunities by his participation in the 2016 Fusarium Laboratory Workshop in Pretoria, South Africa, and both PIs provided instruction and lectures in the 2017 Fusarium Laboratory Workshop in Manhattan, KS.

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 following conferences: attendance at the 2016 Scab Forum by co-PI Leslie, attendance with poster presentation at the PAG XXV conference by graduate student Wei, attendance with poster presentation at the 29th GSA Fungal Genetics Conference and attendance with poster presentation at the 2016 APS annual conference by collaborator Reyes-Gaige.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY16 award 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?**
   Yes

   If yes, how many? 2

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?

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 FY16 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *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 FY16-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY16 grant. Only include citations for publications submitted or presentations given during your award period (4/21/16 - 4/20/17). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.

Liuzzi, V.C., V. Mirabelli, M.T. Cimmarusti, M. Haidukowski, J.F. Leslie, A.F. Logrieco, R. Caliandro, F. Fanelli and G. Mule. Enniatin and beauvericin biosynthesis in *Fusarium* species: Production profiles and structural determinant prediction. Toxins 9(2):2017. 45.
Status: Published
Acknowledgement of Federal Support: No, publication resulted from other funding source.

Minnaar-Ontong, A., L. Herselman, W.-M. Kriel and J.F. Leslie. Morphological characterization and trichothecene genotype analysis of a Fusarium Head Blight population in South Africa. *Eur J Plant Pathol* 148:2017. 216-269.
Status: Published
Acknowledgement of Federal Support: No, publication resulted from other funding source.

Reyes Gaige, A., W. Yue, C. Toomajian and J. Stack. Introduction and seed transmission of *Fusarium proliferatum* in the field. (Abstr.) *Phytopathology* 106:2016. S4.29.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: No, publication resulted from other funding source.

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

Other publications, conference papers and presentations.

Conference presentations:

29th Fungal Genetics Conference, Pacific Grove, CA, March 2017. Yue, W., C. Toomajian, N.M.I. Mohamed Nor and J.F. Leslie. Quantitative mapping of pathogenicity factors in the *Fusarium fujikuroi* complex with an interspecific genetic cross. (conference poster/abstract)
Status: Abstract printed in abstract book and poster presented
Acknowledgement of Federal Support: No

Plant and Animal Genomes Conference XXV, San Diego, CA, January 2017. Yue, W., J.F. Leslie, C. Toomajian. Investigating species differences in fungal plant pathogens with a high-density inter-species genetic map and a draft genome. (conference poster/abstract)
Status: Abstract printed and poster presented
Acknowledgement of Federal Support: No (abstract), yes (poster)

Invited presentations (Leslie):

FABI, University of Pretoria, Pretoria, South Africa, May 2016. Faculty of Agriculture, University of Venda, Thohoyandou, South Africa, June 2016

USAID Mission, Kabul, Afghanistan, July 2016. Ministry of Agriculture, Dar es Salaam, Tanzania, October 2016.