

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY15 Final Performance Report
Due date: July 15, 2016**

Cover Page

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Fiscal Year:	2015
USDA-ARS Agreement ID:	59-0206-1-113
USDA-ARS Agreement Title:	Fungal Genes that Limit or Prevent the Growth of Gibberella zeae.
FY15 USDA-ARS Award Amount:	\$ 48,469
Recipient Organization:	Kansas State University 10 Anderson Hall Manhattan, KS 66506
DUNS Number:	929773554
EIN:	48-0771751
Recipient Identifying Number or Account Number:	AR9933 / GAPP602116
Project/Grant Reporting Period:	04/21/15-04/20/16
Reporting Period End Date:	04/20/16

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Genotype by Sequencing for Footprints of Selection in Fusarium graminearum.	\$ 48,469
	FY15 Total ARS Award Amount	\$ 48,469

Principal Investigator

7/8/16

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: Genotype by Sequencing for Footprints of Selection in *Fusarium graminearum*.

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

The overall goal of our project is to identify genes recently affected by natural selection in *F. graminearum* (*Fg*) to provide additional targets for pathogen control. In response to speculation that natural selection directly acts on trichothecene chemotypes to cause recent *Fg* population shifts, we posit that natural selection may be favoring a variant at some other gene, and one trichothecene gene cluster genotype ('chemotype') has managed to hitchhike along to higher frequency. Genome scans for the molecular traces of selective sweeps in population-level variation data should identify, in an unbiased manner, biologically and ecologically relevant loci that affect fungal fitness which would be difficult to identify based on predicted function.

Project objectives:

1. Genotype a large sample of *Fg* from the US using a newly developed genotype by sequencing (GBS) protocol that will result in an unprecedented density of markers.
2. Analyze population structure using the GBS markers to infer clusters, compare cluster membership with collection location and trichothecene genotype, and see how differentiation varies by genomic location.
3. Scan the genome for footprints of recent selection in our population to identify the regions responsible for population shifts, and from these regions identify candidate genes that can be targeted for fungal control.
4. Measure linkage disequilibrium (LD) between GBS markers and determine how rapidly LD decreases with physical distance between markers to lay the groundwork for genome-wide association studies in *Fg*.

2. What was accomplished under these goals?

1) **major activities:** Our major activity has been the acquisition of hundreds of *Fg* isolates from different US regions collected within the last 20 years (with generous help from colleagues who have shared samples with us) for the purpose of performing high-density genotyping and population genetic analyses of population structure, linkage disequilibrium, and scans for natural selection in addition to inferring other aspects of the evolutionary history of US *Fg* populations. We have also used trait data gathered from a subset of these isolates to perform preliminary GWAS (genome-wide association study) in search of genetic markers associated with certain traits and thus potentially linked to causative alleles.

2) **specific objectives:** We sequenced GBS libraries from 570 *Fg* isolates, generating sequence data from > 30,000 loci and uncovering genetic variation at ~ 20,000 of these loci.

We have performed population structure analyses using both Principal Components Analysis and the model-based software STRUCTURE in order to explore the relationship between the clusters and the trichothecene gene cluster genotypes and sampling location of isolates.

We have analyzed patterns of LD between over 6000 high quality single nucleotide polymorphisms (SNPs) using hundreds of our isolates in order to see how patterns of variation tend to be autocorrelated along chromosomes.

We have also performed a preliminary GWAS scan using our SNP data and a subset of our isolates in order to explore the feasibility of this approach for finding the genetic basis of trait variability in *Fusarium graminearum*.

3) **significant results:** In our population structure analyses, we have found support for two main genetic clusters that strongly but imperfectly correlate with trichothecene gene cluster genotype. A more detailed investigation indicates that South American isolates can be distinguished from those of the US, and reveals an unexpected pattern of grouping for the recently identified NX-2 trichothecene gene cluster genotype. It is also clear that most 15-ADON and 3-ADON isolates differ from each other at many genetic loci throughout the genome rather than just at the trichothecene biosynthetic gene clusters. We found that LD extends the shortest distance in the regions of the genome previously noted as having both high recombination rates (as would be expected) and high genetic variation, while LD is much more extensive in the rest of the genome. This finding has important implications for understanding the species' evolutionary potential, as natural selection is expected to be most efficient in the regions where LD is least extensive. As some of the isolates shared with us had been measured for several pathogenic and saprophytic fitness traits, we realized we could run a GWAS to look for the genetic basis of these traits, but our sample size (n=38) was too small to be very conclusive. However, our analysis of the average of fitness traits for the isolates grouped by genotype indicated that after grouping based in STRUCTURE results, 6 traits were significantly different, while only 1 trait different when isolates were grouped based on trichothecene gene cluster genotype. While a full scan for footprints of recent selection (objective 3) has been delayed (full sample genotyping just finished last month), our preliminary analysis indicates that our data are not consistent with our null hypothesis that natural selection has acted on the 3-ADON haplotype to produce a selective sweep.

4) **key outcomes or other achievements:** Though we have demonstrated the efficiency of our population genomics approach using GBS markers, the data we collect can be used to address numerous questions that we have not had time for in the 2 years of this project. Some delays in gathering samples and data mean the full analysis, including predictions of loci under selection, will take a few more months, but our preliminary data has already launched our next funded Scab project on GWAS that will continue to use the data collected here.

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

The project has provided training for 5 Ph.D. students and 3 undergraduates. Ph.D. student Wei Yue has been trained on culturing of *Fusarium* species, the preparation of GBS libraries, and the analysis of high-throughput next-generation sequencing (NGS) data to infer population genetics parameters and the evolutionary history of *Fusarium* populations. Wei had the opportunity to present research on a related project at the 2015 APS meeting. An

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additional two Ph.D. students have been trained in the production of Fusarium GBS libraries, and an additional three Ph.D. students have been trained in population genetics analysis of Fusarium. The three undergrads received training on the molecular genetic analysis of Fusarium isolates and the analysis of NGS data. Finally, the project has helped to provide training to over 50 participants in Fusarium laboratory workshops.

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

Results of this project have been disseminated through attendance and presentations at the 2014 and 2015 Scab Forum (see below) as well as presentations at the 2015 Plant and Animal Genome Conference and the 28th GSA Fungal Genetics Conference.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY15 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 FY15 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 FY15 award period?**

Yes

If yes, how many?

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- 3. Have any post docs who worked for you during the FY15 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 FY15 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 FY15 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

Refer to the FY15-FPR_Instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY15 grant. If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.

Toomajian, C., Yue, W., and Leslie, J.F. (2015). A genome-wide view of population structure and genetic differentiation of *Fusarium graminearum* populations from the Americas. In: S. Canty, Clark, S. Vukasovich and D. Van Sanford (Eds.), *Proceedings of the 2015 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. p. 59.

Status: Abstract published and poster presented

Acknowledgement of Federal Support: YES

Toomajian, C., Yue, W., Spolti, P., Bergstrom, G.C., and Leslie, J.F. (2015). A small pilot GWAS for the genetic basis of pathogenic and saprophytic fitness in a sample of New York *Fusarium graminearum* isolates from wheat. In: S. Canty, Clark, S. Vukasovich and D. Van Sanford (Eds.), *Proceedings of the 2015 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. p. 60.

Status: Abstract published and poster presented

Acknowledgement of Federal Support: YES

Chiara, M., Fanelli, F., Mulè, G., Logrieco, A.F., Pesole, G., Leslie, J.F., Horner, D.S., and Toomajian, C. (2015). Genome sequencing of multiple isolates highlights subtelomeric genomic diversity within *Fusarium fujikuroi*. *Genome Biol. Evol.* 7(11):3062-3069.

Status: Published

Acknowledgement of Federal Support: Publication resulted from other funding source

Hernandez Nopsa, J.F., Daghish, G.J., Hagstrum, D.W., Leslie, J.F., Phillips, T.W., Scoglio, C., Thomas-Sharma, S., Walter, G.H., and Garrett, K.A. (2015). Ecological networks in stored grain: key postharvest nodes for emerging pests, pathogens, and mycotoxins. *BioScience* 65:985-1002.

Status: Published

Acknowledgement of Federal Support: Publication resulted from other funding source

Yue, W., Mohamed Nor, N.M.I., Leslie, J.F., and Toomajian, C. (2015) A high-density genetic map in *Fusarium* constructed with Genotyping-by-Sequencing markers. (Abstr.) *Phytopathology* 105(Suppl. 4):S4.155

Status: Abstract published and oral presentation given (by PhD candidate Wei Yue).

Acknowledgement of Federal Support: Publication resulted from other funding source

Reyes Gaige, A., Yue, W., Toomajian, C., and Stack, J.P. (2015) Using Genotyping-by-sequencing (GBS) to study the population genetics of the fungus *Fusarium proliferatum*. (Abstr.) *Phytopathology* 105(Suppl. 4):S4.118

Status: Abstract published and poster presented

(Form – FPR15)

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Acknowledgement of Federal Support: Publication resulted from other funding source

Mohammed Nor, N.M.I., Yue, W., Toomajian, C., and Leslie, J.F. (2015) Pathogenicity of progeny from a cross between *Fusarium fujikuroi* and *Fusarium proliferatum* towards onions. *Proceedings of the 13th European Fusarium Seminar*: 60

Status: Abstract published and oral presentation given by J.F. Leslie

Acknowledgement of Federal Support: Publication resulted from other funding source

Fanelli, F., Chiara, M., Toomajian, C., Pesole, G., Logrieco, A.F., Leslie, J.F., Mulè, G., and Horner, D.S. (2015) Genome sequencing of multiple isolates highlights sub-telomeric genomic diversity within *Fusarium fujikuroi*. *Proceedings of the 13th European Fusarium Seminar*: 119

Status: Abstract published and poster presented

Acknowledgement of Federal Support: Publication resulted from other funding source

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

None

Other publications, conference papers and presentations.

Conference presentations:

13th Annual Ecological Genomics Symposium, November 2015, Manhattan KS. C.

Toomajian. Population differentiation in New York populations of the plant pathogen *Fusarium graminearum*.

Status: Abstract printed in symposium program and poster presented

Acknowledgement of Federal Support: YES

US Culture Collection Network 2015 National Center for Genetic Resources Preservation Meeting, October 2015, Fort Collins, CO

Status: John F. Leslie presented

Acknowledgement of Federal Support: Resulted from other funding source

Other presentations:

Workshops:

***Fusarium* Laboratory Workshop, June 2015, Kansas State University, Manhattan KS**

10 instructors and 27 participants

John F. Leslie: Workshop organizer and instructor - presented 3 lectures

Christopher Toomajian: Instructor - presented 2 lectures

Status: Lectures presented and slides distributed to workshop participants

Acknowledgement of Federal Support: Resulted from other funding source

Tropical Fusarium Workshop, February 2016, Maringá Brazil

7 instructors and 25 participants

Status: John F. Leslie, Instructor, presented 2 lectures

Acknowledgement of Federal Support: Resulted from other funding source

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Other:

For all presentations below:

Status: oral presentation given by John F. Leslie

Acknowledgement of Federal Support: Resulted from other funding source

May 2015, BOKU (Universität für Bodenkultur Wien), Tulln, Austria

May 2015, China Agricultural University, Beijing, China

May 2015, Northwest Agriculture and Forestry University, Yangling, China

July 2015, USAID Bureau of Food Security, Washington DC

October 2015, NCR-1183 Annual Meeting, University of Kentucky

October 2015, Feed the Future Director's Meeting, Washington DC

March 2016, Afghanistan Food Safety and Security, New Delhi, India