USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY14 Final Performance Report
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Cover Page

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Fiscal Year:	FY14
USDA-ARS Agreement ID:	59-0206-1-113
USDA-ARS Agreement Title:	Fungal Genes that Limit or Prevent the Growth of Gibberella zeae.
FY14 USDA-ARS Award Amount:	$ 48,517

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Genotype by Sequencing for Footprints of Selection in Fusarium graminearum.	$ 48,517
	FY14 Total ARS Award Amount	**$ 48,517**

7/14/15

Principal Investigator Date

* MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
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
WES-CP – Western 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 major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

FHB management (and the reduction of the contamination of grains by mycotoxins such as trichothecenes) necessarily involves an integrated approach. For example, though extensive effort has been undertaken in the development of resistant cereal cultivars, genes conferring only partial resistance, such as the QTL *Fhb1*, have been identified. Even with additional sources of partial host resistance, the use of fungicides and biocontrol agents can play an important role in the control of pathogen epidemics. In particular, an integrated approach can target multiple steps in the pathogen disease cycle, including the steps of infection and colonization as well as saprophytic stages important in the development and spread of inoculum. To boost the success of this integrated approach, it is important to understand the biology of the pathogen and identify additional genes that can serve as targets for pathogen control, where the external manipulation of these genes may limit fungal growth, saprophytic survival, virulence, or mycotoxin production.

This project is capitalizing on available *Fusarium graminearum* genome sequences and annotation, as well as the ease of high throughput DNA sequence generation, to identify genes recently affected by natural selection in *F. graminearum* (and thus critical for pathogen fitness) to provide targets for pathogen control. Once we generate high-density genotype data from hundreds of *F. graminearum* isolates from FHB grain, we can perform population genomic analysis of the patterns of genetic variation to infer genes recently affected by natural selection in pathogen populations in an unbiased manner. Additionally, when the genotype data is coupled with phenotype data collected from the same pathogen isolates, we can map the genetic basis of important pathogen traits by statistical association (association mapping), providing other genes that could serve as potential targets for pathogen control.

2. List the most important accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:

Accomplishment: We have adapted our method of preparing genotyping-by-sequencing (GBS) DNA libraries from other *Fusarium* species to *F. graminearum* and have already sequenced GBS libraries representing nearly 400 FHB isolates. Analysis of reads from these libraries mapped back to the annotated reference *F. graminearum* genome provides genotype data from thousands of genetic markers evenly-spaced throughout the genome.

Impact: This method provides a cheaper alternative to whole-genome resequencing that allows the screening of hundreds to thousands of FHB isolates while still capturing the major haplotype blocks and allowing the inference of the majority of common variants found in *F. graminearum* populations. Thus, new allele combinations and allele frequency changes throughout the genome can be monitored.

Accomplishment: We have analyzed the patterns of linkage disequilibrium (LD), or nonrandom association between alleles at nearby loci, in our population samples of *F. graminearum*. We found that the extent of LD varied greatly and matched large-scale heterogeneity in recombination rates across the genome. In high recombination regions, which are enriched in genes related to plant infection, LD decayed within 10 kb, corresponding to excellent expected association mapping resolution.

Impact: Knowing how far apart genetic markers can be on a chromosome and still display a significant amount of LD provides insight into the frequency of outcrossing and the process of genetic recombination. This practical knowledge is also critical to association mapping efforts with unrelated samples, as the extent of LD determines the required marker density as well as the expected mapping resolution.

Accomplishment: Although we are waiting until we have finished collecting and genotyping all of our population samples before performing and interpreting our scans for regions affected by recent natural selection, we have done a few pilot analyses of genetic variation around a handful of genes to see what we can infer about the evolutionary history of variation at these loci. For example, at the major trichothecene gene cluster we can distinguish isolates that are genetically 3-ADON versus 15-ADON. A simple hypothesis for the observed increase in frequency of 3-ADON isolates from the upper Midwest is a selective advantage conferred by this allele, which would produce the characteristic patterns of a selective sweep apparent around the 3-ADON haplotype of the trichothecene cluster. Yet we do not see such a pattern. We can also find genetic variation in our GBS markers linked to the *TRI1* gene that perfectly predicts the presence of the NX-2 haplotype at that gene and confirms its single evolutionary origin, but we need to collect more NX-2 samples before we can determine whether it has increased in frequency recently due to natural selection.

Impact: When our collection of population samples is complete, these types of population genetic analyses can determine not only which genome regions are most variable and whether the genetic differentiation between populations is concentrated on certain chromosomes, but can identify regions of the genome that have experienced recent natural selection in each population. This type of unbiased genomic screen can reveal important genes, pathways, and genetic mechanisms that we otherwise might not have guessed based on our knowledge of the pathogen. These selective sweep signals will spotlight individual candidate genes with alternate alleles that have an important impact on pathogen fitness at some point in the disease cycle, and subsequent functional testing may confirm the differences and reveal key steps and gene targets we can perturb in order to improve its integrated management.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY14 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 FY14 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 FY14 award period?**
   No
   If yes, how many?

3. **Have any post docs who worked for you during the FY14 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   No post docs supported by USWBSI funding.
   If yes, how many?

4. **Have any post docs who worked for you during the FY14 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 post docs supported by USWBSI funding.
   If yes, how many?

Include below a list of all germplasm or cultivars released with full or partial support of the USWBSI during the FY14 award period. List the release notice or publication. Briefly describe the level of FHB resistance. *If not applicable because your grant did NOT include any VDHR-related projects, enter N/A below.*

N/A

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the FY14 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

1. **2015 APS Annual Meeting, Pasadena, CA, August 2015**. **W Yue**, NMI Mohamed Nor, **JF Leslie**, **C Toomajian**. A high-density genetic map in *Fusarium* constructed with Genotyping-by-Sequencing markers. (talk by Yue)
2. **2015 APS Annual Meeting, Pasadena, CA, August 2015**. A Reyes Gaige, **W Yue**, **C Toomajian**, JP Stack. Using Genotyping-by-sequencing (GBS) to study the population genetics of the fungus *Fusarium proliferatum*. (poster presentation)
3. **13th European *Fusarium* Seminar, Martina Franca, Italy, May 2015**. NMI Mohammed Nor, **W Yue**, **C Toomajian** & **JF Leslie**. Pathogenicity of progeny from a cross between *Fusarium fujikuroi* and *Fusarium proliferatum* towards onions. *Proceedings of the 13th European* Fusarium *Seminar*: 60 (talk by Leslie)
4. **28th Fungal Genetics Conference – Satellite Fusarium Workshop, Pacific Grove, CA, March 2015**. **C Toomajian**, **W Yue, JF Leslie**. Population genomics and scans for selection in the plant pathogen *Fusarium graminearum*. (talk by Toomajian)
5. **28th Fungal Genetics Conference, Pacific Grove, CA, March 2015**. **C Toomajian**, **W Yue**, **JF Leslie**. Population genomics and scans for selection in the plant pathogen *Fusarium graminearum*. (poster presentation)
6. **Plant and Animal Genomes Conference XXIII, San Diego, CA, January 2015**. **C Toomajian**, **W Yue**, **JF Leslie**. Using genotyping-by-sequencing to study the population genomics of the plant pathogen *Fusarium graminearum*. (poster presentation)
7. **2014 National Fusarium Head Blight Forum, St. Louis, MO, December 2014**. **C Toomajian, W Yue & JF Leslie.** Genotyping by sequencing for footprints of selection in *Fusarium graminearum*. (talk by Toomajian)