USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY12 Final Performance Report
July 16, 2013

Cover Page

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Fiscal Year:	FY12
USDA-ARS Agreement ID:	59-0206-1-113
USDA-ARS Agreement Title:	Fungal Genes that Limit or Prevent the Growth of Gibberella zeae.
FY12 USDA-ARS Award Amount:	$ 51,942*

USWBSI Individual Project(s)

USWBSI Research Category**	Project Title	ARS Award Amount
PBG	Vegetative Compatibility Genes for the Control of Fusarium Head Blight.	$ 43,273
PBG	Effects of Defense Peptides on Fusarium Head Blight.	$ 8,669
	Total ARS Award Amount	**$ 51,942**

16 July 2013

Principal Investigator Date

* Partial funding for this research is under ARS agreement # 59-0206-2-087

** MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
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: *Vegetative Compatibility Genes for the Control of Fusarium Head Blight.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Control of Fusarium Head Blight is hampered by a lack of anti-fungal agents and clear targets that can be used in the development of resistance. The goal of this project is to identify some of the genes in the fungus that initiate the apoptotic death process within the fungus. Triggering these genes externally, or mimicking their trigger mechanism could provide another avenue for limiting or eliminating fungal growth.

The genes being targeted are those that control vegetative compatibility. When strains heterozygous at one or more of these loci fuse, the resulting heterokaryotic cell dies and the strains are said to be vegetatively incompatible. The project has two phases: (i) to localize the *vic* genes on an existing genetic map, and (ii) to identify the corresponding genes on the physical map and to test them for activity.

2. List the most important accomplishment and its impact (i.e. how is it being used) to minimize the threat of Fusarium head blight or to reduce mycotoxins. Complete both sections (repeat sections for each major accomplishment):

Accomplishment:

Developing mapping cross and collecting suitable progeny. The mapping cross required a reconstruction of the original cross made by Jurgenson *et al.* with parents that share a common *nit* mutation, but with one of the parents carrying the *MAT* knockout mutation that enables crosses to develop only heterozygous perithecia. From the cross, progeny are selected that can form viable heterokaryons with one of the progeny from the previous cross. These progeny are then used to localize the loci responsible for the vegetative compatibility interactions. We have been unable to recover more than a handful of recombinant progeny of the type needed from the cross. Closer examination of the progeny we had recovered indicated that none were growing normally. The small number of progeny collected has forced us to rethink our approach tom this project.

We generated a 15× Illumina genomic sequence for the MAT knockout strain derived from Z-3639, one of the parents of the cross. We will compare this sequence with that of the partial sequence of Z-3639 currently available on the Broad Institute web site to ensure that there are no major differences between the knockout strain and its wild-type parent. We also will compare these sequences with that of PH-1. Heterozygous *vic* loci will differ in sequence, perhaps in a leader region, between the two strains. To help guide our search we will use the sequences identified recently in *Podospora anserina* (Bidard *et al.*, *G3* 3:1015-1030, 2013) whose expression was increased in non-self interactions.

Impact:

A novel, independent genome sequence for an important strain of *F. graminearum* has been generated. Problems associated with the crosses have forced a re-evaluation of the process for identifying genes involved in the vegetative incompatibility process. The genomic pro-

cess to be followed during the coming year may identify genes that can be targeted for up-regulation as a means of inhibiting or killing fungal cells.

Project 2: *Effects of Defense Peptides on Fusarium Head Blight.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

We are testing the concept that antifungal peptides can suppress infection of wheat by sexually produced ascospores of *Gibberella zeae* or macroconidia of the asexual pathogen form, *Fusarium graminearum*. Previous work showed that pheromone mating peptides of *G. zeae* inhibit infectious ascospores. Initial work in this project confirmed these results and expanded them to infectious macroconidia. Subsequent work showed that mating peptides protected wheat heads in point inoculation experiments conducted under laboratory conditions.

During the past year (May 2012 – May 2013), we evaluated mating peptides attached to a carrier protein, CKX (cytokinin oxidase/dehyrdogenase), for their abilities to protect wheat heads from infection under greenhouse conditions. Several mating peptides, including Pgz, derived from *G. zeae*, and Pnc and Pnc-S1, derived from *Neurospora crassa*, were produced by yeast fermentation. As in the past, peptide production rates were low, but over a period of several months, sufficient quantities of one peptide, Pnc, were produced for testing at a 10 µM concentration. In earlier tests, 10µM synthesized Pnc (not attached to CKX) had noticeably reduced disease development in wheat point inoculation experiments. Unfortunately, in the replicated greenhouse test conducted this year, more than 95% of wheat heads developed disease when sprayed with 10 µM CKX-Pnc peptide alone prior to inoculation with a mixture of pathogen ascospores and macroconidia.

It is not clear why the mating peptide attached to CKX did not protect wheat heads from pathogen infection. It is possible that CKX interferes with mating peptide binding to or uptake by fungal hyphae. Further studies would be required to understand deleterious effects of CKX as a carrier protein.

Based on these greenhouse results, we focused on synthesized peptides (without attached CKX) in follow-up field experiments. For this purpose, we had two mating peptides, Pgz and Pnc commercially synthesized. In on-going field trials beyond this project period, each peptide will be applied to flowering winter wheat in replicate treatment plots at two locations in Nebraska. After spray application, plants will be misted for 5 days to provide environmental conditions conducive to pathogen infection. Scab ratings will be made two weeks after this infection period.

2. List the most important accomplishment and its impact (i.e. how is it 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 overcame technical problems of yeast fermentation and produced sufficient quantities of a candidate inhibitory mating peptide for greenhouse spray tests. The lack of protection provided by mating peptide attached to the CKX carrier protein focused our efforts on synthesized peptides for further testing under field conditions.

Impact:

The field trials currently underway will determine whether mating peptides can be effectively applied as a spray to protect wheat from scab. If spray applications are ineffective, there will be a need to develop transgenic wheat for production and delivery of inhibitory mating peptide within susceptible plant tissues.

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 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Peer-reviewed articles and book chapters (2012):

- Lee, J., H. Kim, J.-J. Jeon, H.-S. Kim, K. A. Zeller, L. L. A. Carter, **J. F. Leslie** & Y.-W. Lee. 2012. Population structure and mycotoxin production of *Fusarium graminearum* from maize in Korea. *Applied and Environmental Microbiology* **78:** 2161-2167.
- **Leslie, J. F.** 2012. Genetics and *Fusarium oxysporum*. In: *Fusarium Wilts of Greenhouse Vegetables and Ornamental Crops* (A. Garibaldi, J. Katan & M. L. Gullino, eds.), 39-46. APS Press, St. Paul, Minnesota.
- Lima, C., L. Pfenning, S. Costa, L. Abreu & **J. F. Leslie**. 2012. *Fusarium tupiense* sp. nov., a new species in the *Gibberella fujikuroi* complex causing mango malformation in Brazil. *Mycologia* **104:** 1408-1419.
- Postic, J., Cosic, D. Jurkovic, K. Vrandecic, A. A. Saleh & **J. F. Leslie**. 2012. Diversity of *Fusarium* species isolated from weeds and plant debris in Croatia. *Journal of Phytopathology* **160:** 76-81.
- Saleh, A. A., J. P. Esele, A. Logrieco, A. Ritieni & **J. F. Leslie**. 2012. *Fusarium verticillioides* from finger millet. *Food Additives and Contaminants: Part A* **29:** 1762-1769.
- Shiraishi, A., **J. F. Leslie**, S. Zhong & J. Y. Uchida. 2012. Amplified fragment length polymorphisms, pathogenicity and vegetative compatibility group analyses of *Fusarium oxysporum* and *Fusarium pseudocircinatum* from *Acacia koa*. *Plant Disease* **96:** 1111-1117.
- Studt, L., C. Troncoso, F. Gong, P. Hedden, C. Toomajian, **J. F. Leslie**, H.-U. Humpf, M. C. Rojas & B. Tudzynski. 2012. Segregation of gibberellin and mycotoxin biosynthesis in hybrids of *Fusarium fujikuroi* and *Fusarium proliferatum*. *Fungal Genetics and Biology* **49:** 567-577.

- Summerell, B. A. & **J. F. Leslie**. 2012. Introducing the Genus *Fusarium*. In: *Control of* Fusarium *Diseases* (F. M. Alves-Santos & J. J. Diez-Casero, eds.), pp. 1-17. Research Sign-posts, Trivandrum, Keral, India.

Presentations (2012):

- College of Tropical Agriculture & Human Resources, University of Hawaii, Honolulu, Hawaii, January 2012.
- Tropical *Fusarium* Workshop, National University of Lavras, Lavras, Brazil, February 2012.
- Department of Plant Protection, King Saud University, Riyadh, Saudi Arabia, April, 2012.
- CRC for Plant Biosecurity Science Exchange, Perth, Australia, May, 2012.
- FABI, University of Pretoria, Pretoria, South Africa, September, 2012.
- University of the Free State, Bloemfontein, South Africa, October, 2012.
- Korean Society for Plant Pathology, 50th Anniversary symposium, Seoul, Korea, October 2012.