USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY13 Final Performance Report
July 15, 2014

Cover Page

PI:	John Leslie
Institution:	Kansas State University
Address:	Department of Plant Pathology 4024 Throckmorton Hall Manhattan, KS 66506-5502
E-mail:	jfl@ksu.edu
Phone:	785-532-1363
Fax:	785-532-2414
Fiscal Year:	FY13
USDA-ARS Agreement ID:	59-0206-1-113
USDA-ARS Agreement Title:	Fungal Genes that Limit or Prevent the Growth of Gibberella zeae.
FY13 USDA-ARS Award Amount:	$ 58,182

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Effects of Defense Peptides on Fusarium Head Blight.	$ 14,951
PBG	Vegetative Compatibility Genes for the Control of Fusarium Head Blight.	$ 43,231
	FY13 Total ARS Award Amount	**$ 58,182**

Principal Investigator Date

* 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: *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 tested the concept that antifungal peptides can be used to suppress infection of wheat by sexually- produced ascospores or asexually produced macroconidia of *Fusarium graminearum*. Previous work in the Leslie laboratory showed that pheromone mating peptides produced by *F. graminearum* and some of their analogs inhibit ascospores germination and growth. Initial work in this project confirmed this inhibitory potential and expanded its effect to infectious macroconidia. Subsequent project work conducted under laboratory conditions showed that mating peptides protected wheat heads in point inoculation experiments, *i.e.*, pathogen inoculum and mating peptides placed together in the floral tube (stigmatic channel).

During FY 12 we found that pheromone mating peptides attached to a protein carrier, CKX (cytokinin oxidase/dehydrogenase) and produced via fermentation in a yeast did not protect wheat as we had expected, based on our experience with other plant diseases. Consequently, we decided to focus on synthesized free peptides (without attached CKX) in follow-up experiments during FY 13.

During the past year (May 2013 – May 2014), we conducted two greenhouse trials to evaluate the ability of mating peptides in the free form (not attached to a protein carrier) to protect wheat from infection and scab development. Mating peptides Pnc and Pgz were synthesized commercially. Each of these peptides inhibits germination and development of ascospores in *in vitro* experiments. In the greenhouse experiments, the peptides were tested separately at a 20µM concentration, which was inhibitory to the pathogen in previous studies.

In each trial, peptides Pnc and Pgz were sprayed onto florets until run-off. Immediately after application, florets were spray-inoculated with ascospores (10,000 spores/ml). A control treatment was included in which plants were sprayed to run-off with deionized water prior to ascospore inoculation. Inoculated plants of all treatments were placed immediately into a humidity chamber, and were examined 2 weeks later for scab symptoms. In trials 1 and 2, scab incidence in the control treatment was 100 and 84%, respectively. In each trial, the scab incidence for florets treated with Pnc or Pgz did not differ significantly from the respective control treatment.

The reason for a lack of significant scab control by mating peptides sprayed onto florets is not clear. It is possible that peptide concentrations following application were depleted by run-off or degradation. Thus, the lack of scab control from sprayed peptides could be due to insufficient contact of the peptides with the pathogen on the surface of the florets. Higher peptide concentrations in the spray or longer periods of contact between peptides and pathogen may be needed to limit infection, tissue colonization and disease. Such longer-term exposure and inhibition could be better achieved in plants that have been transformed to produce a consistent supply of peptides, such as Pnc or Pgz, over time.

To develop mating peptides as a tool for scab control, the stability of the peptides when applied to plant surface must be evaluated and ways to improve peptide dispersal and stability may need to be identified. It also would be beneficial to determine the range of fungi whose germination and growth is limited by these mating peptides is limited to various *Fusarium* spp., or whether the peptides have broader inhibitory potential against other asco-

mycetous pathogens of importance on wheat, *e.g.*, *Pyrenophora*. The inhibitory range of these peptides is important in assessing priorities for developing transgenic wheat disease resistance.

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 determined that inhibitory mating peptides, when applied to the surface of wheat florets, do not effectively limit infection by *F. graminearum* and subsequent scab development.

Impact:

Results of these experiments suggest a need to develop transgenic wheat for effective production and delivery of inhibitory mating peptides within susceptible plant tissues.

Project 2: *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 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:

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 recovered indicated that none were growing normally. The small number of progeny collected has forced us to rethink our approach to 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 and compared it to that of the partial sequence of Z-3639 currently available on the Broad Institute web site. There are a number of differences between these sequences. These differences suggest that there are significant differences between the original parent and the transformed strain that functions only as a female parent. It is likely that one or more *vic* genes were altered as well. Thus, the original premise upon which the experiment was based is flawed.

Impact:

The differences in strain sequence suggest that the current approach to identifying these genes needs to be rethought and an alternative strategy used in its place.

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 FY13 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Geiser, D. M., T. Aoki, C. W. Bacon, S. Baker, M.K. Bhattacharyya, M. E. Brandt, D. W. Brown, L. W. Burgess, S. N. Chulze, J. J. Coleman, J. C. Correll, S. F. Covert, P. W. Crous, C. A. Cuomo, G. S. de Hoog, A. di Pietro, W. H. Elmer, L. Epstein, R. J. N. Frandsen, S. Freeman, A. E. Glenn, T. R. Gordon, T. R., K. E. Hammond-Kosack, L. E. Hanson, M. del Mar Jímenez-Gasco, S. Kang, H. C. Kistler, G. A. Kuldau, J. F. Leslie, A. Logrieco, G. Lu, E. Lysøe, L.-J. Ma, S. P. McCormick, Q. Migheli, A. Moretti, F. Munaut, K. O'Donnell, L. Pfenning, R. C. Ploetz, R. H. Proctor, S. A. Rehner, V. A. R. G. Robert, A. P. Rooney, B. bin Salleh, M. M. Scandiani, J. Scauflaire, E. Steenkamp, H. Suga, B. A. Summerell, D. A. Sutton, U. Thrane, F. Trail, A. van Diepeningen, H. D. VanEtten, A. Viljoen, C. Waalwijk, T. J. Ward, M. J. Wingfield, J.-R. Xu, X.-B. Yang, T. Yli-Mattila & N. Zhang. 2013. One fungus, one name: Defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. *Phytopathology* **103:** 400-408.

Leslie, J. F. 2013. Saying good-bye to *Gibberella zeae*. USWBSI Annual Meeting (Milwaukee, Wisconsin). Abstract and invited oral presentation.

Leslie, J. F. 2014. Innovative technologies to control mycotoxin contamination. American Association for the Advancement of Science Annual Meeting (Chicago, Illinois). Abstract and invited oral presentation.

Leslie, J. F. & R. L. Bowden. 2013. *Fusarium graminearum*: Species or clade? European *Fusarium* seminar (Bordeaux, France). Abstract and invited oral presentation.

Leslie, J. F. & A. Logrieco. 2014. *Mycotoxin Reduction in Grain Chains: A Practical Guide*. John Wiley & Sons, Ames, Iowa. (352 pp.).

Nik M. I. Mohamed Nor, N. M. I., B. Salleh, C. Toomajian, J. P. Stack & J. F. Leslie. 2013. Interspecific hybrids between *Fusarium fujikuroi* and *Fusarium proliferatum*. MycoRed Closing seminar (Martina Franca, Italy). Abstract and invited oral presentation.