USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY10 Final Performance Report
July 15, 2011

Cover Page

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Fiscal Year:	FY10
USDA-ARS Agreement ID:	59-0790-6-064
USDA-ARS Agreement Title:	Genetic Diversity and Genetic Mapping if *Gibberella zeae*.
FY10 USDA-ARS Award Amount:	$ 14,682

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Effects of Defense Peptides on Fusarium Head Blight.	$ 14,682
	Total ARS Award Amount	**$ 14,682**

15 July 2011

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?

In this project, we are testing the concept that antifungal peptides can suppress infection of wheat by reducing or preventing the germination of ascospores and/or macroconidia of *Fusarium graminearum*. Reduced infection also should reduce DON accumulation. Two groups of peptides are being evaluated: the first group consists of native and derivative forms of mating phermones from *F. graminearum* and *Neurospora* discovered in the Leslie lab. A second group includes peptides identified in the English lab and derived from combinatorial phage-display peptide libraries. An underlying question of this research is whether all small peptides are equally effective. Previous *in vitro* experiments in the Leslie lab suggested differences in inhibition potential among peptides.

In the past year, we continued to evaluate and compare chemically synthesized peptides for inhibition of *F. graminearum*/*G. zeae in vitro* and *in planta*. In the *in vitro* experiments, both ascospores and macroconidia were exposed to individual peptides in 10-µl microdrops on microscope slides. Test concentrations of the peptides ranged from 0.2 to 4 µM. *In planta* experiments were conducted with whole wheat heads point-inoculated with ascospores in the presence of a peptide. Wheat heads at similar stages of anthesis were detached from plants and placed in water vials before pathogen inoculation. In each inhibition test, a peptide was reconstituted in dimethylformamide (DMF) prior to mixing with ascospores. A 10-µl droplet containing 10 µM peptide and 1,000 ascospores was applied to a single spikelet from the center of the rachis of each of 6 replicate wheat heads. Inoculated wheat heads were incubated in moist chambers for 2 days to optimize infection conditions. Percentage infection of wheat heads, based on scab symptom development, was assessed for 12 or more days. Pathogen development on inoculated wheat heads was also assessed and rated on a scale of 0-4 (0 = no visible mycelia; 4 = tufts of pigmented, sporulating mycelia on the tissue).

We have continued to refine constructs of peptides attached to a protein delivery scaffold based on maize cytokinin oxidase/dehydrogenase (ZmCKX1) for scale-up production via our *Pichia pastoris* fermentation system. Scaffold-peptides purified from the culture extracts are to be used for application to wheat in greenhouse experiments.

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):

<u>Accomplishments:</u>

- At 20µM, mating pheromone peptide, Pgz, inhibited wheat head infection to levels equivalent to Prosaro fungicide (Fig. 1). Combinatorial peptide FgF8B also significantly reduced infection at this concentration, but combinatorial peptide FgF3A did not. At

10µM, mating pheromone peptides derived from *Neurospora crassa* delayed infection of wheat heads, whereas mating peptides from *F. graminearum* did not (Fig. 2).

- Combinatorial peptide, FgF8B inhibited germination of macroconidia and ascospores equivalently. Mating pheromone peptides also are being tested for the inhibition of macroconidial germination.

- Design of scaffold-displayed peptides has been confirmed and constructs have been expressed by *P. pastoris* (yeast) fermentation. Characterization of the fermentation products and inhibitory activity have begun.

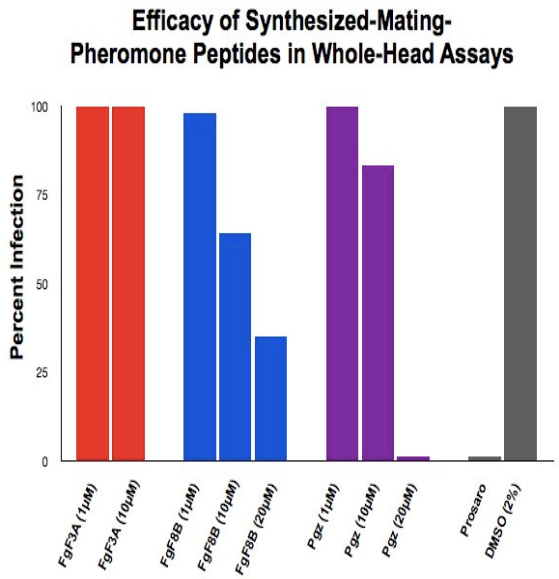

Fig. 1. Protection of wheat heads from infection by ascospores in the presence of peptides Pgz and FgF8B. Peptide FgF3A did not provide effective protection from infection.

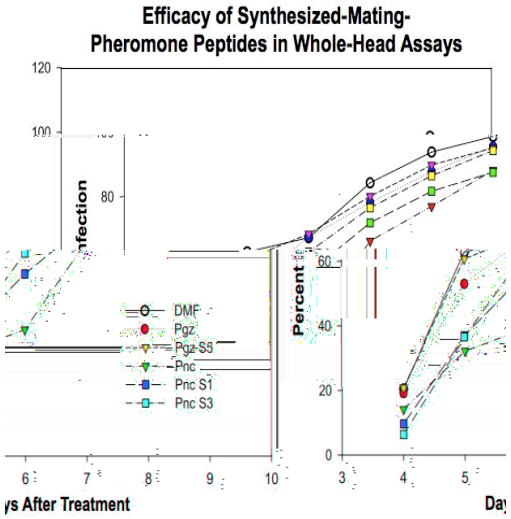

Fig. 2. Delays in wheat head infection after inoculation with ascospores in the presence of 10 µM peptides from *N. crassa* (Pnc, Pnc-S1, Pnc-S3) or *G. zeae* (Pgz, Pgz-S5).

Impact:

The results of experiments with wheat head inoculations provide additional evidence that particular mating pheromone and combinatorial peptides can significantly protect wheat from infection by both infectious spore types (ascospores and macroconidia) produced by the head blight pathogen. Completion of experiments assessing the protective potential of scaffold-displayed peptides will enable development of disease management strategies based on protective spray applications or deployment of inhibitory peptides in enhanced wheat germplasm.

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 (2010):

Leslie, J. F. & J.-R. Xu. 2010. *Fusarium* genetics and pathogenicity. In: *Cellular and Molecular Biology of Filamentous Fungi* (K. A. Borkovich & D. Ebbole, eds.), pp. 607-621. ASM Press, Washington, DC.

Summerell, B. A., M. Laurence, E. C. Y. Liew & **J. F. Leslie**. 2010. Biogeography and phylogeography of *Fusarium*: A review. *Fungal Diversity* **44:** 3-13.

Voss, H.-H., R. L. Bowden, **J. F. Leslie** & T. Miedaner. 2010. Variation and transgression of aggressiveness among two *Gibberella zeae* crosses developed from highly aggressive parental isolates. *Phytopathology* **100:** 904-912.

Presentations:

Reduced infection of wheat spikelets inoculated with ascospores of *Giberella zeae* in the presence of fungal mating pheromone peptides. G.Y. Yuen, C.C. Jochum, N.W. Gross, J.T. English & J.F. Leslie. Poster presented at annual conference of American Phytopathological Society, Charlotte, NC; August, 2010.

Evaluation of mating pheromone peptides for inhibition of wheat spikelet infection by *Fusarium graminearum*. G.Y. Yuen, C.C. Jochum, N.W. Gross, J.T. English, & J.F. Leslie. Poster presented at 2010 National Fusarium Head Blight Forum, Milwaukee, WI; December 2010.