

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

**Cover Page**

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<b>Fiscal Year:</b>	FY10
<b>USDA-ARS Agreement ID:</b>	59-0790-7-073
<b>USDA-ARS Agreement Title:</b>	Selection of Defense Peptides to Protect Wheat from Fusarium Head Blight.
<b>FY10 USDA-ARS Award Amount:</b>	\$ 15,074

**USWBSI Individual Project(s)**

<b>USWBSI Research Category*</b>	<b>Project Title</b>	<b>ARS Award Amount</b>
PBG	Effects of Defense Peptides on Fusarium Head Blight.	\$ 15,074
	<b>Total ARS Award Amount</b>	<b>\$ 15,074</b>



Principal Investigator

7/13/2011

Date

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\* 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 continuing to test the concept that antifungal peptides can be used to suppress infection of wheat by sexually produced ascospores of *Gibberella zeae* or macroconidia of the asexual pathogen form, *Fusarium graminearum*. This should ultimately lead to reduced DON accumulation. Two groups of peptides continue to be investigated, the first being native and derivative forms of mating hormones from *F. graminearum* and *Neurospora* discovered by 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. Within the in vitro experiments, *G. zeae* ascospores and *F. graminearum* macroconidia were exposed to individual mating pheromone or combinatorial peptides in 10- $\mu$ l microdroplets mounted on microscope slides. Test concentrations of peptides ranged from 0.2 to 4  $\mu$ M. In planta experiments were conducted with whole wheat heads point-inoculated with *G. zeae* ascospores in the presence of mating pheromone or combinatorial peptides. Wheat heads of similar stages of anthesis were detached from plants and placed in water vials before pathogen inoculation. In each inhibition test, one peptide was reconstituted in dimethylformamide (DMF) prior to mixing with ascospores. A 10  $\mu$ l droplet containing 10  $\mu$ M 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 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):**

**Accomplishment:**

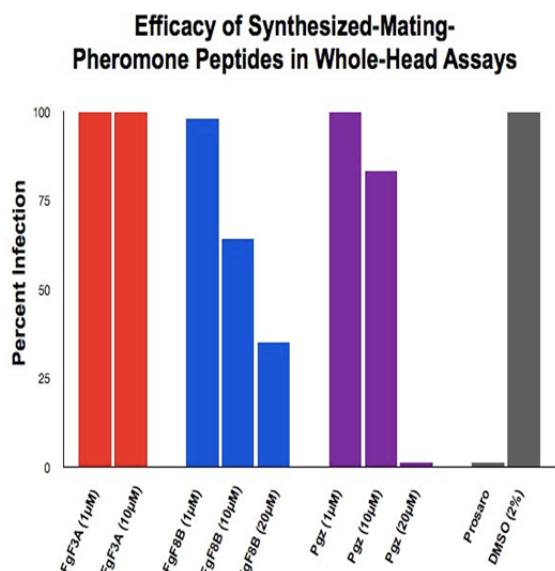
1. At a 20 $\mu$ M concentration, mating pheromone peptide, Pgz, inhibited wheat head infection to levels equivalent to Prosaro fungicide (Fig. 1). Combinatorial peptide FgF8B also significantly reduced infection at this concentration, but peptide FgF3A did not. At a

10 $\mu$ M concentration, mating pheromone peptides derived from *Neurospora crasa* delayed infection of wheat heads, whereas peptides from *F. graminearum* did not (Fig. 2).

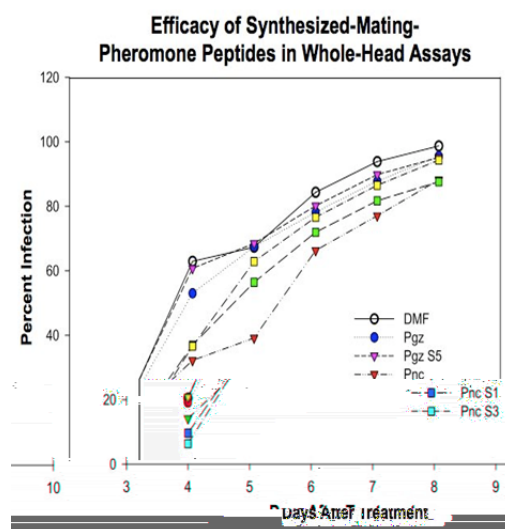
2. Combinatorial peptide, FgF8B, from *F. graminearum* inhibited macroconidium germination equivalently to inhibition of *G. zeae* ascospore germination. Mating pheromone peptides are also being tested for inhibition of macroconidium germination.
3. Design of scaffold-displayed peptides has been confirmed and constructs have been expressed by *P. pastoris* (yeast) fermentation. Characterizations of fermentation products and inhibitory activity were initiated.

### **Impact:**

The results of experiments with wheat head inoculations provided additional evidence that 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.



**Fig. 1.** Protection of wheat heads from infection by *G. zeae* 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  $\mu$ M peptides from *N. crasa* (Pnc, Pnc-S1, Pnc-S3) or *G. zeae* (Pgz, Pgz-S5).

**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.**

**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.