USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY09 Final Performance Report
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Cover Page

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Fiscal Year:	2009
USDA-ARS Agreement ID:	59-0790-6-064
USDA-ARS Agreement Title:	Genetic Diversity and Genetic Mapping of *Gibberella zeae*.
FY09- USDA-ARS Award Amount:	$ 16,493

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
PBG	Effects of Defense Peptides on Fusarium Head Blight.	$ 16,493
	Total Award Amount	**$ 16,493**

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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 Winter Wheat Region
SWW – Southern Sinter 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 are testing the concept that antifungal peptides can be used to suppress infection of wheat by macroconidia or ascospores of *Fusarium graminearum*. This should ultimately lead to reduced DON accumulation. Two groups of peptides are being investigated, the first being native and derivative forms of mating pheromones from *F. graminearum* and *Neurospora* discovered by the Leslie lab. A second group includes those recently identified in the English lab and derived from combinatorial phage-display peptide libraries.

A first question is whether all small peptides are equally effective. The Leslie lab tested a number of single amino-acid-substituted derivatives of the *Neurospora* and *Fusarium* mating hormone peptides. The peptides were tested for inhibition of macrocondium germination in an *in vitro* assay in which spores were mixed with each peptide (1 to 20 µM) on microscope slides.

Further efforts to test the concept have used the *Pichia pastoris* fermentation system, in use in the English lab, to generate large amounts of mating phermones or combinatorially derived peptides attached to scaffolds that are secreted from the yeast into the culture medium. The scaffold is a secretable plant protein, maize cytokinin oxidase/dehydrogenase (ZmCKX1). Scaffold-peptides purified from the culture extracts are then used for application to wheat in greenhouse experiments.

A third focus has been the evaluation of a selection of chemically synthesized peptides for effects on *F. graminearum*/*G. zeae in vitro* (English lab) and *in planta* (Yuen lab). In the *in vitro* bioassay, ascospores (10^5 per ml) of *G. zeae* were exposed individually to various small peptides (0.2 to 4 µM) in drops mounted on microscope slides.

Two *in planta* bioassays were developed for this project to test small amounts of peptides. One assay evaluated fungal growth on the plant surface; the other evaluated inhibition of scab symptoms. Peptides diluted in 2% DMSO were mixed with ascospores (10^5/ml). In the first assay, 10 µl drops were placed on the surface of detached spikelets on water agar in Petri dishes. Growth of pathogen was examined daily and rated on a 0-4 scale (0=no visible growth; 4=profuse mycelia covering the spikelet). In the second assay, 10 µl volumes of peptide-spore mixtures were inoculated (without damaging tissues) into the middle floret of detached heads maintained in water-filled floral tubes. The inoculated heads were incubated in a moist chamber for 2 weeks, and the percentage of spikelets exhibiting scab symptoms was determined.

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):

Accomplishments:

- Some of the amino-acid-substituted derivatives of the *Neurospora* and *Fusarium* mating phermones were less-efficient at inhibiting growth and macroconidum germination than the native pheromone peptide, and some were more efficient. Increasing the pI of the peptide generally increased inhibition; however, these increases usually were acquired through the addition of arginine residues, which could make the peptide more susceptible to bacterial and other enzymatic degradation. The peptides in hand can inhibit growth and spore germination, but additional peptide sequence variants should be tested to identify even better spore germination and growth inhibitors than the 10 already tested.
- We constructed transformation vectors expressing 4 peptides on the ZmCKX1 scaffold in *Pichia*. Liter amounts of yeast fermentation culture fluid were produced. Logistical

difficulties hindered the final purification of the peptides, but alternative purification equipment has been located, and more purified peptides will be available shortly.

- Chemically synthesized mating pheromone peptide, Pgz, and two combinatorially derived peptides, F3A and F8B, inhibit the germination of ascospores *in vitro*.
- Pzg and F8B inhibit growth of *F. graminearum* when peptide/ascospore mixtures are spotted on detached spikelets (Fig. 2 left). This effect is concentration-dependent; Pgz at 20 µM completely suppresses mycelial growth with lower concentrations slowing fungal growth (Fig. 2 right). These peptides also inhibit scab development in a concentration-dependent manner when peptide-ascospore mixtures are inoculated into florets on intact heads (Table 3). The effect of Pzg at 20 µM on scab severity is the same as that of Prosaro fungicide. Combinatorial peptide F3A alters neither pathogen growth nor scab severity.

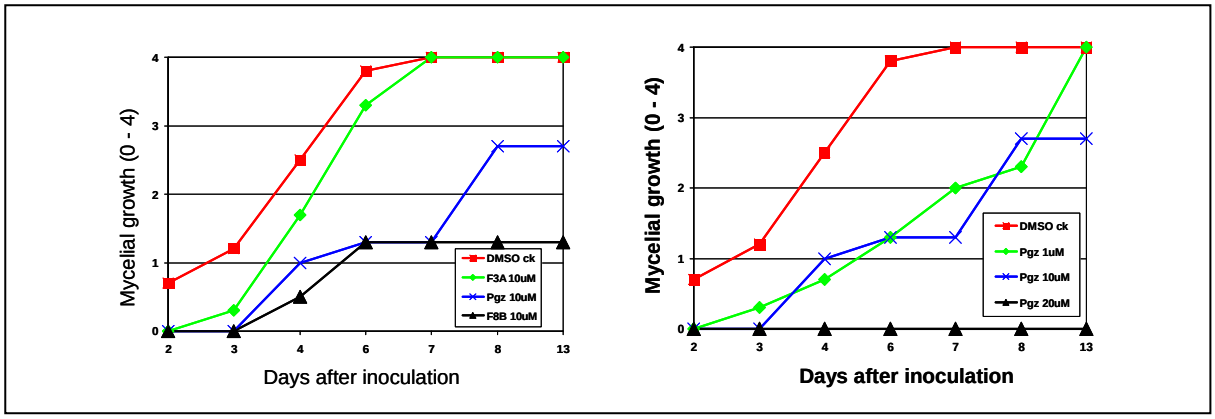

Figure 2. Effects of peptides on growth of *Fusarium graminearum* on detached spikelets: (left) comparison of mating hormone peptide Pzg and combinatorial peptides F3A and F8B, all at 10µM; (right) comparison of different concentrations of phermone peptide Pzg.

Table 3. Effects of mating hormone Pzg and combinatorial peptides F3A and F8B at different concentrations on severity of scab caused by *Fusarium graminearum*.

Treatment & concentration	Percent infected spikelets
Combinatorial peptide F3A 1µM	100 A[1]
F3A 10µM	100 A
Combinatorial peptide F8B 1µM	98 A
F8B 10µM	64 B
F8B 20µM	35 B
Phermone peptide Pgz 1µM	100 A
Pgz 10µM	83 AB
Pgz 20µM	1 C
Prosaro fungicide	1 C
DMSO 2% (diluent)	100 A

[1]Values followed by the same letters are not significantly different at $P = 0.05$

Impact:

The application of some small peptides to wheat heads can alter infection by *F. graminearum*. The spikelet and whole head bioassays we developed will be used to screen additional peptides and to optimize application parameters prior to testing peptides produced by fermentation for scab and DON control in larger scale greenhouse experiments.

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:

1. Bentley, A. R., M. G. Milgroom, J. F. Leslie, B. A. Summerell & L. W. Burgess. 2009. Spatial aggregation in *Fusarium pseudograminearum* populations from the Australian grain belt. *Plant Pathology* **58:** 23-32.
2. Lee, J., I.-Y. Chang, H. Kim, S.-H. Yun, J. F. Leslie & Y.-W. Lee. 2009. Lineage composition and toxin production of *Fusarium graminearum* populations from rice in Korea. *Applied and Environmental Microbiology* **75:** 3289-3295.