USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY09 Final Performance Report
July 15, 2010

Cover Page

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Fiscal Year:	2009
USDA-ARS Agreement ID:	59-0790-7-073
USDA-ARS Agreement Title:	Selection of Defense Peptides to Protect Wheat from Fusarium Head Blight.
FY09- USDA-ARS Award Amount:	$ 15,750

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
PBG	Effects of Defense Peptides on Fusarium Head Blight.	$ 15,750
	Total Award Amount	**$ 15,750**

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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?

In this project, we are testing the concept that antifungal peptides can be used to suppress infection of wheat by macroconidia of *Fusarium graminearum* or ascospores of the sexual pathogen form, *Gibberella zeae*. 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 by an in vitro assay in which spores were mixed with each peptide (1 to 20 µM) on microscope slides.

Further efforts in testing the concepts have focused on using the *Pichia pastoris* fermentation system, in use in the English lab, to generate large amounts of mating hormone 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 will then be 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). Within the in vitro bioassay, ascospores (10^5 per ml) of *G. zeae* were exposed to individual mating hormone or combinatorially selected peptides (0.2 to 4 µM) in drops mounted on microscope slides.

Two in planta bioassays were developed for this project specifically to test small amounts of peptides. One assay examined effects of peptides on growth of the fungus on the plant surface; the other evaluated peptides for inhibition of scab symptoms. Peptides diluted in 2% DMSO were mixed with to ascospores (10^5 per ml). In the first assay, 10µl drops were deposited on the surface of detached spikelets supported 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 in planta assay, 10µl volumes of peptide-spore mixtures were inoculated (without damaging tissues) into the middle floret of detached heads maintained in water-filled floral tubes. After the inoculated heads were incubated in a moisture chamber for 2 weeks, 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):

<u>Accomplishment:</u>

1. In testing amino-acid-substituted derivatives of the *Neurospora* and *Fusarium* mating hormone peptides, some substitutions were less-efficient at inhibiting macroconidum germination and further growth than the native pheromone peptide, and in some cases more efficient. Patterns included that increasing the pI of the peptide generally increased inhibition. However, these increases usually were acquired through the addition of additional arginine residues, which could make the peptide more susceptible to bacterial and other enzymatic degradation. Although the peptides in hand certainly can inhibit growth and spore

germination, additional changes in peptide sequence need to be tested to identify those that are even better inhibitors of spore germination and fungal growth than the 10 already tested.

2. We have we have constructed transformation vectors for expression of 4 peptides on the ZmCKX1 scaffold in *Pichia*. Liter amounts of yeast fermentation culture fluid have been produced. Logistical difficulties have hindered the final purification of peptides from the culture fluids, but alternative purification equipment recently has been found, so purified peptides will be available shortly for greenhouse experiments.
3. Chemically synthesized mating pheromone peptide, Pgz, and two combinatorially derived peptides, F3A and F8B, were found to inhibit the germination of ascospores in vitro.
   Pzg and F8B inhibited growth of *F. graminearum* when mixtures of peptides and ascospores were spotted onto detached on spikelets (Fig. 1 left). This effect was concentration-dependent; Pgz at 20µM completely suppressed mycelial growth while lower concentrations acted in slowing fungal growth (Fig. 1 right). The same peptides inhibited scab development, also in a concentration-dependent manner, when peptide-ascospore mixtures were inoculated into florets on intact heads (Table 1). The effect of Pzg at 20µM on scab severity was the same as that of Prosaro fungicide. Another combinatorial peptide F3A had no effect on growth of the pathogen or scab severity.

Impact:

The results provide evidence towards proving the concept that application of specific peptides to wheat heads can affect infection by *F. graminearum*. The spikelet and whole head bioassays developed in this study will be used to screen a number of candidate peptides and to optimize application parameters prior to testing of peptides produced by fermentation for scab and DON control in large scale greenhouse experiments.

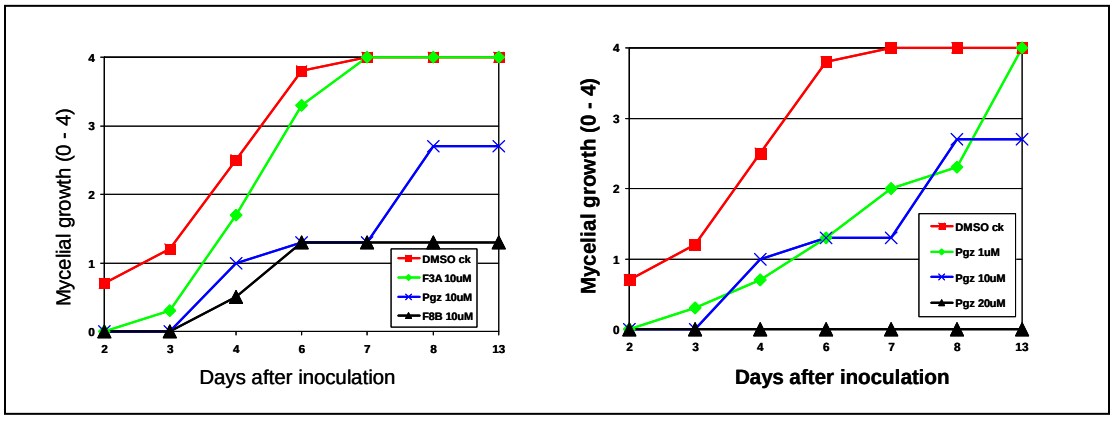

Figure 1. Effects of peptides on growth of *Fusarium graminearum* on detached spikelets: left) comparison of mating hormone peptide Pzg and combinatorial peptides F3A and F8B, all at 10µM; right) comparison of different concentrations of hormonal peptide Pzg.

Table 1. 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#
F3A 10µM	100 A
Combinatorial peptide F8B 1µM	98 A
F8B 10µM	64 B
F8B 20µM	35 B
Hormonal peptide Pgz 1µM	100 A
Pgz 10µM	83 AB
Pgz 20µM	1 C
Prosaro fungicide	1 C
DMSO 2% (diluent)	100 A

Letters denote significant differences at P=0.05

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.

Peptide Technologies for Management of Fusarium Head Blight. J. T. English. Invited presentation made at the 2009 National Fusarium Head Blight Forum, Dec. 7-9, Orlando, FL.