USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY11 Final Performance Report
July 13, 2012

Cover Page

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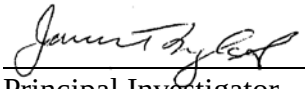
Fiscal Year:	FY11
USDA-ARS Agreement ID:	59-0790-7-073
USDA-ARS Agreement Title:	Selection of Defense Peptides to Protect Wheat from Fusarium Head Blight.
FY11 USDA-ARS Award Amount:	$ 20,172

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Effects of Defense Peptides on Fusarium Head Blight.	$ 20,172
	Total ARS Award Amount	**$ 20,172**



7/13/12

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 be used to suppress infection of wheat by sexually produced ascospores of *Gibberella zeae* or macroconidia of the asexual pathogen form, *Fusarium graminearum*. Previous work in the Leslie laboratory showed that pheromone mating peptides produced by *G. zeae* inhibit infectious ascospores. Initial work in this project confirmed this inhibitory potential and expanded its effect to infectious macroconidia. Subsequent project work showed that mating peptides protected wheat heads in point inoculation experiments conducted under laboratory conditions.

In the past year, we began to evaluate mating peptides for their abilities to protect wheat heads under greenhouse conditions. In this first experiment, wheat heads of similar stages of anthesis were point inoculated at individual florets with a test peptide (in pure synthesized form) and 1,000 macroconidia. Plants were maintained at high humidity for two days and scab development monitored for two weeks.

Also during the project period, we continued to work on a peptide delivery scheme based on the fusion of an inhibitory peptide to a protein scaffold based on cytokinin oxidase/dehydrogenase (CKX). CXK is very stable over a range of environmental conditions and thus, is expected to provide stability to attached peptides. Because we had found our original constructs to be flawed in design, we reconstructed the peptide- scaffolds. In addition to problems with original construct design, problems with expression via fermentation could have been related to the unique biological function of the peptides as pheromone mating factors, an aspect that bears further investigation.

These newly created peptide designs have now been successfully expressed via yeast fermentation. Subsequently, within ongoing in vitro experiments the peptide constructs are added to suspensions of ascospores or macroconidia in microdrops and spore germination monitored over time. The percentage of spore germination and rate of germ tube elongation in the presence of a peptide is being compared to spore germination and growth in the absence of peptide.

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>

- In this first greenhouse experiment, mating peptides (synthesized and not fused to CKX) did not control infection and scab development. The results were not unexpected because the experiment was conducted using a single peptide

concentration that was roughly estimated to be effective based on our previous laboratory experiments. Numerous environmental factors in the greenhouse differ from those of the laboratory that could affect peptide stability and function. Of particular importance in upcoming repetitions of this experiment is the use of higher peptide concentrations and volume of application to provide improved coverage of wheat heads. Increased peptide concentration and application volume will be possible using peptides fused to CKX and produced via fermentation. Because CKX is very stable over a range of environmental conditions, we expect improved peptide performance in subsequent greenhouse experiments.

- Improved peptide-CKX constructs have been created for three peptides proven to be inhibitory (in synthesized format without scaffold) in earlier laboratory experiments. These peptides include Pgz (derived from *G. zeae*), Pnc (derived from *Neurospora crassa*), and Pnc-S1, (derived from *N. crassa* with a single amino acid substitution). Initial testing to confirm the inhibitory the activity of these peptide constructs is in progress. Two additional inhibitory mating peptides, Pgz-S5 and Pnc-S3, are being increased via fermentation for testing when available. The initial testing of these fused peptides will also determine the inhibitory concentrations to test further under greenhouse tests.

Impact:

The results of the first greenhouse experiments are important for optimizing conditions of peptide testing under variable and more realistic environmental conditions. The reconstruction of peptide-scaffold constructs establishes higher quality materials for testing in the laboratory, and more importantly in the greenhouse. Production of peptides via fermentation will provide larger volumes of test materials for use in the experiments that are being addressed in the 2012 phase of the project. 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.

Presentation:
Reduced infection of wheat spikelets inoculated with ascospores of Gibberella 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 2012 annual conference of American Phytopathological Society, Honolulu, HI. Phytopathology 101:S199