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:	59-0206-1-113
USDA-ARS Agreement Title:	Fungal Genes that Limit or Prevent the Growth of Gibberella zeae.
FY11 USDA-ARS Award Amount:	$ 58,146

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Vegetative Compatibility Genes for Control of Fusarium Head Blight.	$ 36,097
PBG	Effects of Defense Peptides on Fusarium Head Blight.	$ 22,049
	Total ARS Award Amount	**$ 58,146**

13 July 2012

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: *Vegetative Compatibility Genes for Control of Fusarium Head Blight.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Control of Fusarium Head Blight is hampered by a lack of anti-fungal agents and clear targets that can be used in the development of resistance. The goal of this project is to identify some of the genes in the fungus that initiate the apoptotic death process within the fungus. Triggering these genes externally, or mimicking their trigger mechanism could provide another avenue for limiting or eliminating fungal growth.

The genes being targeted are those that control vegetative compatibility. When strains heterozygous at one or more of these loci fuse, the resulting heterokaryotic cell dies and the strains are said to be vegetatively incompatible. The project has two phases: (i) to localize the *vic* genes on an existing genetic map, and (ii) to identify the corresponding genes on the physical map and to test them for activity. At this time the gene mapping activities are the current focus.

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:
Developing mapping cross and collecting suitable progeny. The mapping cross requires a reconstruction of the original cross made by Jurgenson *et al.* with parents that share a common *nit* mutation. The cross also needs to be made with one of the parents carrying the *MAT* knockout mutation that enables crosses to develop only heterozygous perithecia. From the cross, progeny are selected that can form viable heterokaryons with one of the progeny from the previous cross. These progeny are then used to localize the loci responsible for the vegetative compatibility interactions.

Impact:
Suitable *nit* mutants have been made in both the parents of the projected cross. These strains could cross and produce fertile ascospores. The strain carrying the *MAT* knockout did note readily yield *nit* mutants. To get a strain with the *nit* mutation and the *MAT* knockout, an isogenic cross between the *MAT* knockout and a *nit* mutant derived in the same strain without the *MAT* knockout was made. The *nit* mutant and the *MAT* locus were on the same chromosome, so selecting a recombinant with both traits was time-consuming as all progeny had to be screened microscopically. We have adapted the technology for collecting the near-isogenic progeny from *F. verticillioides*. At this time we have several progeny for use in the mapping process, but they are much rarer than expected. We will not characterize these progeny further until we have collected a large enough number to be used for the complete analysis. At this time the project is progressing somewhat slower than expected due to the relatively small number of progeny collected to date.

Project 2: *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*. There is a need for additional anti-fungal agents that can stop or slow the germination of spores or the resulting hyphal colonies. These peptides are of interest because it will be difficult for the fungus to develop resistance to them without losing its ability to produce the sexual stage, an essential part of the life cycle for the wheat disease cycle.

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 evaluating 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.

During the current 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 oxdase/ 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 be related to the unique biological function of the peptides as pheromone mating factors. This problem could indicate a potential fermentation scale-up product and perhaps the need to express these peptides in an inactive form that is processed to an active form outside the cell. Similar problems could arise if the peptides are constitutively expressed in a transgenic wheat plant.

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):

Accomplishment:

- In the 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 with only 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 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 for 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.

Peer-reviewed articles and book chapters (2011):

- Leslie, J. F. & B. A. Summerell. 2011. In search of new *Fusarium* species. *Plant Breeding and Seed Science* **63:** 93-102.
- Summerell, B. A. & J. F. Leslie. 2011. Fifty years of *Fusarium*: How could nine species ever have been enough? *Fungal Diversity* **50:** 135-144.
- Summerell, B. A., J. F. Leslie, E. C. Y. Liew, M. H. Laurence, S. Bullock, T. Petrovic, A. R. Bentley, C. G. Howard, S. A. Peterson, J. L. Walsh & L. W. Burgess. 2011. *Fusarium* species associated with plants in Australia. *Fungal Diversity* **46:** 1-27.

Presentations:

- Leslie, J. F. (2011) Vegetative compatibility - A native fungal mechanism for inducing death in *G. zeae*. Oral presentation at the 2011 USWBSI National Fusarium Head Blight Forum, St. Louis, Missouri.
- Yuen, G. Y., C. C. Jochum, N. W. Gross, J. T. English & J. F. Leslie. (2012) Reduced infection of wheat spikelets inoculated with ascospores of *Gibberella zeae* in the presence of fungal mating pheromone peptides. Poster presented at 2012 annual conference of American Phytopathological Society, Honolulu, HI. *Phytopathology* 101:S199.

Other presentations made that contained some material from these and previous USBSI supported projects:

- International Society for Mycotoxicology (African meeting), Capetown, South Africa – April, 2011.
- Hungarian Academy of Sciences (Inaugural Lecture), Budapest, Hungary – May, 2011.
- IFA, University of Life Sciences and Natural Resources (BOKU) – Tulln, Austria – May, 2011.
- Dong-A University, Busan, Korea – September, 2011.
- International Society for Mycotoxicology (South American meeting), Mendoza, Argentina – November, 2011.