USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY08 Final Performance Report (approx. May 08 – April 09)
July 15, 2009

Cover Page

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Fiscal Year:	2008
USDA-ARS Agreement ID:	59-0790-7-073
USDA-ARS Agreement Title:	Selection of Defense Peptides to Protect Wheat from Fusarium Head Blight.
FY08 USDA-ARS Award Amount:	$ 45,533

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
PBG	Evaluation of Defense Peptides to Protect Wheat from Fusarium Head Blight.	$45,533
	Total Award Amount	**$ 45,533**

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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
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: *Evaluation of Defense Peptides to Protect Wheat from Fusarium Head Blight.*

1. **What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?**
2.

There is a continuing need for enhanced wheat resistance to scab. In this regard, recently developed biotechnology tools have promise for designing unique new forms of resistance that can complement current wheat breeding efforts.

Scab is caused by several *Fusarium* species, including in particular, *F. graminearum*. Wheat is susceptible to infection by *Fusarium* from the time of initial flowering through the soft dough stage of kernel development. Numerous studies have shown that initial infection is caused by germinating ascospores and macroconidia (germlings) of the pathogen. Beyond initial infection, pathogen hyphal growth occurs beneath the cuticle layer of floral parts, and eventually hyphal penetration of cell walls of parenchyma cells leads to cell degradation and development of scab symptoms.

This project addresses the need to specifically protect flowers and kernels from *Fusarium* infection and colonization. We are developing defense peptides that can be deployed in wheat plants for protection of flowers during these early stages of pathogenesis. Peptides are selected from combinatorial phage-display peptide libraries that allow the identification of peptides that bind to multiple cell-surface molecules of the pathogen. Based on experience with several other plant pathogens, some of these binding peptides will disrupt pathogen development and limit disease. This platform peptide technology will complement ongoing resistance breeding that is directed against scab.

3. **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:

We have continued the evaluation of peptides that might be used to defend wheat from Fusarium head blight. We have accomplished two important tasks during this year. The first of these was the development of techniques to produce ascospores of the pathogen. As background, our initial work in screening peptides in the previous year was based on evaluations of inhibition of macroconidium growth and development. It was later determined that we need to focus more specifically on ascospores rather than macroconidia, because these spores serve more commonly as the inoculum that initiates disease.

In developing our ability to produce ascospores, we initially worked with isolates of the pathogen available in collections from Missouri made in years past. We were unable, initially, to produce ascospores using methods described in the scientific literature.

From Frances Trail (MSU), we obtained additional advice and a pathogen isolate known to produce spores efficiently. With this assistance, we subsequently were able to produce ascospores and manipulate them effectively for incorporation into our peptide screening assays.

We have also recently made new collections of the pathogen from infected wheat in Missouri. We are now characterizing these isolates for ascospore production and for inclusion in our peptide-screening experiments.

The second important accomplishment was the completion of screening of a new peptide library against germinating ascospores (germlings). This library consists of more than one billion peptide variants. Each peptide variant is 12 amino acids in length. Based on this diversity, we expect to find peptides that bind to and disrupt cell-surface components of the pathogen that are critical to its development and ability to cause disease. We tested several strategies for peptide screening to find the most efficient means of recovering peptides that bind strongly to germlings. With an effective strategy finally in hand, we were able to collect a pool of candidate peptides for inhibition testing.

We are now evaluating members of the candidate peptide pool for sequence diversity. We are also beginning to test representative members of the peptide pool for their ability to halt or slow the growth of germlings derived from ascospores. In addition, peptides that were selected the previous year from screenings against macroconidia are being tested for their inhibitory potential against ascospores.

With our focus, this year, on technical aspects of ascospore production and new library screens, we delayed experiments initiated in the previous year to incorporate peptides derived from macroconidium screens into a protein delivery scaffold for further germling inhibition testing both in vitro and in planta. We are now restarting those experiments by incorporating candidate ascospore-inhibitory peptides into scaffolds.

Impact:

We have developed two pools of peptide candidates. We expect that members of these pools have the potential to directly disrupt germling (whether derived from ascospores or macroconidia) development and penetration of wheat flowers and kernels, and thus slow or inhibit scab development. Completion of proof of concept for the use of defense peptides will open the door for development of unique wheat germplasm with defensive traits that can enhance existing resistance established by traditional breeding methods.

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.

Poster Presentations:

N. W. Gross, Z. D. Fang, F. J. Schmidt, and J. T. English. Defense Peptides Derived from Combinatorial Libraries as a Novel Means of Protection against *Fusarium* Head Blight. University of Missouri Life Sciences Week. Columbia, MO. April 2008.

N. W. Gross, Z. D. Fang, F. J. Schmidt, and J. T. English. Defense Peptides Derived from Combinatorial Libraries as a Novel Means of Protection against *Fusarium* Head Blight. Annual conference of the American Phytopathological Society, Minneapolis, MN. August 2008.

N. W. Gross, F. J. Schmidt, Z. D. Fang, and J. T. English. Combinatorially-Selected Antimicrobial Peptides Provide Novel Means of Resistance to *Fusarium* Head Blight of Wheat. National FHB Forum, Indianapolis, IN. December, 2008.

If your FY08 USDA-ARS Grant contained a VDHR-related project, include below a list all germplasm or cultivars released with full or partial support of the USWBSI. List the release notice or publication. Briefly describe the level of FHB resistance. If this is not applicable (i.e. no VDHR-related project) to your FY08 grant, please insert 'Not Applicable' below.