USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY07 Final Performance Report (approx. May 07 – April 08)
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Cover Page

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Fiscal Year:	2007
USDA-ARS Agreement ID:	59-0790-7-073
USDA-ARS Agreement Title:	Selection of Defense Peptides to Protect Wheat from Fusarium Head Blight.
FY07 ARS Award Amount:	$ 39,024

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
PGG	Selection of Defense Peptides to Protect Wheat from Fusarium Head Blight.	$39,024
	Total Award Amount	**$ 39,024**

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Principal Investigator Date

* CBCC – Chemical, Biological & Cultural Control
EEDF – Etiology, Epidemiology & Disease Forecasting
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GET – Genetic Engineering & Transformation
HGR – Host Genetics Resources
HGG – Host Genetics & Genomics
IIR – Integrated/Interdisciplinary Research
PGG – Pathogen Genetics & Genomics
VDUN – Variety Development & Uniform Nurseries

Project 1: *Selection of Defense Peptides to Protect Wheat from Fusarium Head Blight.*

1. What major problem or issue is being resolved and how are you resolving it?

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.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishment:

The first step in developing defense peptides for scab protection is selection of candidate peptides that bind to *Fusarium* germlings and disrupt growth and development. We have made initial selections by screening a library of random phage-display peptides for affinity binding with germlings derived from macroconidia. The original library included about one billion peptide variants. Each peptide variant is 8 amino acids in length. Numerous phage-peptide clones were recovered after three rounds of screening and amplification. Evaluation of recovery rates indicated selectivity of phage-peptide clone recovery. A collection of 35 randomly selected clones were sequenced initially. Of these clones, about 25% displayed identical peptide inserts. Additional motifs with sequence redundancy were also identified along with several unique sequences. This pattern of recovery is indicative of appropriate sampling of the initial peptide pool for affinity binding.

We have performed initial evaluations of the inhibitory potential of affinity-selected, phage-display peptides. In these evaluations, we have found phage-peptide clones that significantly slow germling development in relation to water and non-selected phage library controls. We are also testing selected peptides for inhibitory effects against germlings derived from ascospores.

We have begun to incorporate candidate defense peptides into plant-derived protein carrier molecules. These fused peptide-protein scaffolds will be tested for germling inhibition by both in vitro and in planta assays.

In addition to developing and testing scaffold-displayed peptides, we have made additional selections of germling-binding peptides from a different library that contains peptides 12-amino acids in length. Recovered peptides are also being assessed for germling inhibition. We are a

Impact:

We have developed the first set of peptides that have the potential to directly disrupt germling penetration of wheat flowers and kernals, 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.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:

Genomic and proteomic studies are elucidating what proteins and other factors produced by *Fusarium* are critical to scab development. These molecules are targets for disruption by application of defense peptides. The platform technologies developed in this project will be made available to the scab research community and enable researchers to develop defense peptides against targets of interest.

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

Inhibition of *Fusarium graminearum* germling development caused by combinatorially selected defense peptides. N. W. GROSS (1), Z. D. Fang (1), C. J. Murphy (1), B. Cooper (2), J. T. English (1) (1) Division of Plant Sciences, University of Missouri, Columbia, MO, USA; (2) Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD 20705. American Phytopathological Society Annual Meeting. San Diego, CA, July 2007.

Inhibition of *Fusarium graminearum* germling development caused by combinatorially selected defense peptides. N. W. Gross[1], Z. D. Fang[1], B. Cooper[2], and J. T. English[1.] [1]Division of Plant Sciences, University of Missouri, Columbia, MO 65211 and [2]Soybean genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD 20705. National Fusarium Head Blight Forum. Kansas City, MO, December 2007.