USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY12 Final Performance Report
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Cover Page

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Fiscal Year:	FY12
USDA-ARS Agreement ID:	59-0206-1-122
USDA-ARS Agreement Title:	New Management Tools for Fusarium Head Blight.
FY12 USDA-ARS Award Amount:	$ 26,657*

USWBSI Individual Project(s)

USWBSI Research Category**	Project Title	ARS Award Amount
MGMT	Evaluation of Biological Agents for FHB and DON Control.	$ 11,550
MGMT	Effects of Defense Peptides on Fusarium Head Blight.	$ 15,107
	Total ARS Award Amount	**$ 26,657**

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Principal Investigator Date

* Partial funding for this research is under ARS agreement # 59-0206-9-055

** 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: *Evaluation of Biological Agents for FHB and DON Control.*

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

   More effective fungicides and host resistance in some wheat market classes are now available for scab and DON management, but these strategies are not completely effective or available for all cereal crops. Biological control measures that can be effective in diverse environments are needed to augment current strategies. The Yuen laboratory conducted field experiments in two Nebraska locations (Lincoln and Mead) as part of the 2012 Uniform Biocontrol Trials. A susceptible hard red winter wheat was used in both locations, as was artificial inoculation with Fusarium-infested grain and mist irrigation. The trials examined efficacy of Taegro™ (Novozymes Biologicals, Salem, VA), a commercial product containing *Bacillus amyloliquefaciens* FZB24, applied either alone at five to seven days after Feekes growth stage 10.5.1, or following application of a triazole fungicide at Feekes growth stage 10.5.1, and with or without canola oil as an adjuvant. A nontreated control was included. Scab severity, incidence, and index were determined in the field. Percent Fusarium diseased kernels (FDK), DON levels, and seed yield were measured after harvest.

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:

   Because of the occurrence of high temperatures and drought, very low levels of scab incidence <5%) and severity (<20%) were recorded and DON was at undetectable levels (<0.05 ppm) in all plots. There were no significant treatment effects for any disease or yield parameter at either location.

   Impact:

   Because adverse weather conditions prevented disease development, the results from the experiments conducted in Nebraska in the 2012 growing season provided no useful information on the any of the treatments.

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*. 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.

   During the past year (May 2012 – May 2013), we continued to evaluate mating peptides attached to a carrier protein, CKX (cytokinin oxidase/dehyrdogenase), for their abilities to protect wheat heads from infection under greenhouse conditions. Several mating peptides for testing, including Pgz, derived from *G. zeae*, and Pnc and Pnc-S1, derived from *Neurospora crassa*, were produced by yeast fermentation. As in the past, rates of peptide production were low, but over a period of several months, sufficient quantities of one peptide, Pnc, were produced for testing at a 10µM concentration. In earlier tests, 10µM synthesized Pnc (not attached to CKX) had noticeably reduced disease development in wheat point inoculation experiments. Unfortunately, in the replicated greenhouse test conducted this year, more than 95% of wheat heads developed disease when sprayed with 10 µM CKX-Pnc peptide alone prior to inoculation with a mixture of pathogen ascospores and macroconidia.

   It is not clear why the mating peptide attached to CKX did not protect wheat heads from pathogen infection. It is possible that CKX interferes with mating peptide binding to or uptake by fungal hyphae. Further studies would be required to understand deleterious effects of CKX as a carrier protein.

   Based on these greenhouse results, we decided to focus on synthesized peptides (without attached CKX) in follow-up field experiments. For this purpose, we had two mating peptides, Pgz and Pnc commercially synthesized. In on-going field trials beyond this project period, each peptide will be applied to flowering winter wheat in replicate treatment plots at two locations in Nebraska. After spray application, plants will be misted for 5 days to provide environmental conditions conducive to pathogen infection. Scab ratings will be made two weeks after this infection period.

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>

   We were able to overcome technical problems of yeast fermentation and produce sufficient quantities of a candidate inhibitory mating peptide for greenhouse spray tests.

The lack of protection provided by mating peptide attached to the CKX carrier protein directed our focus to synthesized peptide for further testing under field conditions.

Impact:

The field trials currently underway will determine whether mating peptides can be effectively applied as a spray to protect wheat from scab. If spray applications are ineffective, there will be a need to develop transgenic wheat for production and delivery of inhibitory mating peptide within susceptible plant tissues.

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.

M. McMullen, G. Bergstrom, J. Cummings, C. Jochum, G. Yuen, B.H. Bleakley, N.K.S. Murthy, and K. Ruden, 2012. Uniform Tests of Biological Control Agents for Management of FHB and DON, 2012. In: S. Canty, A. Clark, A. Anderson-Scully and D. Van Sanford (Eds.), Proceedings of the 2012 National Fusarium Head Blight Forum (p. 21). East Lansing, MI/Lexington, KY: U.S. What & Barley Scab Initiative.