USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY11 Final Performance Report
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Cover Page

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Fiscal Year:	FY11
USDA-ARS Agreement ID:	59-0206-1-122
USDA-ARS Agreement Title:	New Management Tools for Fusarium Head Blight.
FY11 USDA-ARS Award Amount:	$ 24,244

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
MGMT	Effects of Defense Peptides on Fusarium Head Blight.	$ 12,683
MGMT	Evaluation of Biological Controls in Nebraska.	$ 11,561
	Total ARS Award Amount	**$ 24,244**

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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
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.

Project 2: *Evaluation of Biological Controls in Nebraska.*

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 2011 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, used alone or in various tank mix or sequential combinations with Prosaro (Bayer CropScience), a 50:50 blend of tebuconazole and prothioconazole, and chelated manganese (Mn) which was found previously to enhance

antibiotic production by *Bacillus* spp. Treatments applied as a single application at Feeke's growth stage 10.51 included i) Taegro alone, ii) Taegro + Prosaro, iii) Taegro + Prosaro + chelated manganese (Mn), and iv) Prosaro alone. Treatments applied sequentially at Feeke's 10.51 and 5 to 7 days later included v) Taegro and Taegro, vi) Taegro + Prosaro and Taegro, and vii) Taegro + Prosaro + Mn and Taegro. 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:

Environmental conditions at and following anthesis were not conducive to scab and resulted in moderate scab incidence and low severity in both locations. There were no significant treatment effects for any disease parameter at the Lincoln location. Some combination with all treatments involving Prosaro having equal effects in reducing in-field disease measurements compared to the control (Table 1). Some of the Taegro+Prosaro tank mix combinations and Prosaro alone reduced scab index compared to the control in the Mead experiment (Table 2). The most notable result in that experiment was the reduction in DON by Taegro+Prosaro tank mix combinations, whereas Prosaro applied alone did not reduce DON compared to the control.

Impact:

The results from the experiments conducted in Nebraska provide further evidence that combinations of biocontrol agents with fungicides have the potential to provide better control of DON than fungicide alone. In context of the Uniform Biocontrol Trials, the Nebraska results are in line with those from other states in which the Taegro-Prosaro combinations were effective in controlling scab in the field and reducing DON levels.

Table 1. Results from 2011 Uniform Biocontrol Trial – Mead, NE

Treatment	% Head Severity	% Incidence	Index	Est. Yield (BU/A)	% FDK	DON (ppm)
Untreated check	13	72	9	13.1	3	0.2
Taegro @ 10.51	13	77	10	12.3	2	0.3
Taegro+Prosaro @ 10.51	10	72	7	13.0	2	0.1
Taegro+Prosaro+ chelated manganese @ 10.51	17	71	12	14.8	3	<0.05
Taegro @ 10.51 + late Taegro	12	73	9	15.7	2	0.2
Taegro+Prosaro @ 10.51 + late Taegro	14	77	11	16.9	3	0.4
Taegro+Prosaro+chelated manganese @ 10.51 late Taegro	11	64	6	13.3	3	0.3
Prosaro @ 10.51	12	56	7	12.3	2	0.4
p	NS	NS	NS	NS	NS	NS

Table 2. Results from 2011 Uniform Biocontrol Trial – Lincoln, NE

Treatment	% Severity	% Incidence	Index	Est. Yield (BU/A)	% FDK	DON (ppm)
Untreated check	10	50	5.5 ab	5.18	9.3 a	0.63 bc
Taegro @ 10.51	9	54	5.3 ab	5.16	9.6 a	1.17 a
Taegro+Prosaro @ 10.51	10	50	4.1 bc	6.25	7.2 ab	0.18 d
Taegro+Prosaro+ chelated manganese @ 10.51	7	39	2.7 c	6.03	6.3 b	0.18 d
Taegro @ 10.51 + late Taegro	11	61	6.6 a	5.05	9.8 a	0.87 ab
Taegro+Prosaro @ 10.51 + late Taegro	7	45	3.1 c	5.67	7.2 ab	0.18 d
Taegro+Prosaro+chelated manganese @ 10.51 + late Taegro	8	47	3.7 bc	5.85	7.8 a	0.37 cd
Prosaro @ 10.51	7	41	2.9 c	6.52	5.8 b	0.30 cd
p	NS	NS	0.0043	NS	0.0496	<.0001
$LSD_{0.05}$			2.1		3	0.41

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.

Presentations:

G. Y. Yuen, C. C. Jochum, N. W. Gross, J. T. English, J. F. Leslie. Reduced infection of wheat spikelets inoculated with ascospores of *Gibberella zeae* in the presence of fungal mating pheromone peptides. Poster presented at 2012 Annual Meeting of the American Phytopathological Society, Honolulu, HI. Phytopathology 101:S199

S. Halley, G. Yuen, C. Jochum, B.H. Bleakley, K.R. Ruden, K. Waxman, G. Bergstrom and L.E. Sweets. Uniform Biological Fungicide Evaluations for Control of Fusarium Head Blight and Deoxynivalenol in Wheat. Poster presented at 2011 Fusarium Head Blight Forum, St. Louis, MO. Abstract published in Proceedings of 2011 Fusarium Head Blight Forum, St. Louis, MO, pages 140-141.