USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY10 Final Performance Report
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Cover Page

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Fiscal Year:	FY10
USDA-ARS Agreement ID:	59-0790-6-072
USDA-ARS Agreement Title:	Enhancing Biological Strategies to Control Fusarium Head Blight and Evaluating Biological Control Agents in Uniform Tests against FHB.
FY10 USDA-ARS Award Amount:	$ 24,113

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
MGMT	Evaluation of Biological Agents for FHB Control.	$ 11,430
MGMT	Effects of Defense Peptides on Fusarium Head Blight.	$ 12,683
	Total ARS Award Amount	**$ 24,113**

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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: *Evaluation of Biological Agents for FHB Control.*

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

   Although more effective fungicides and host resistance in some wheat market classes are now available for scab and DON management, these strategies are not available for all cereal crops nor completely effective. 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 2010 Uniform Biocontrol Trials. The biocontrol agents in this study were Taegro (Novozymes Biologicals, Salem, VA), a commercial product containing *Bacillus amyloliquefaciens* FZB24, and "double yeast" treatment, which consisted of *Cryptococcus flavescens* OH 182.9 (NRRL Y-30216) and *C. aureus* OH 71.4 (NRRL Y-30213) cultured together, supplied by D. Schisler, USDA ARS NCAUR. One application of each agent was made at anthesis and were compared with non-treated controls and the fungicide Prosaro 421 SC alone. Other treatments included Taegro alone at late bloom; a tank mix of Taegro and Prosaro applied at anthesis; Prosaro at anthesis followed by Taegro at late bloom; and Prosaro at anthesis followed by the 'double yeast' at late bloom. A susceptible hard red winter wheat 2137 was used in both locations, as was artificial inoculation with Fusarium-infested grain and mist irrigation. 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:
   Scab-conducive environmental conditions occurring at anthesis resulted in moderate scab development in both locations. Except for Incidence at the Lincoln location, there were significant treatment effects for all field disease parameters, with all treatments involving Prosaro having equal effects in reducing in-field disease measurements compared to the control. The notable exception was the Prosaro at anthesis/ Taegro at late bloom treatment reducing the incidence of scab at Mead below Taegro and Prosaro applied alone (Table 2). Index was reduced by Taegro alone applied at late bloom and double yeast alone applied at anthesis compared to the control at Mead. Combinations of Prosaro with biocontrol agents in the Mead experiment reduced FDK compared to the control, whereas Prosaro and the biologicals alone were ineffective. None of treatments significantly reduced DON levels or elevated yields 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 scab 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 2010 experiments conducted in at two Nebraska locations, Mead (M) and Lincoln (L).

	Incidence (%)		Severity (%)		Index (%)		FDK (%)		DON (ppm)	
Treatment	M	L	M	L	M	L	M	L	M	L
Control	84	94	21	30	19	28	15	61	2.1	1.4
Prosaro at anthesis	70*	80	15*	16*	10*	13*	12	39	1.5	0.6
Taegro at anthesis	80	87	23	26	17	23	20	50	2.5	0.7
Taegro at late bloom	67*	89	22	30	15*	26	13	40	2.7	0.9
Prosaro + Taegro tank mix at anthesis	66*	74	14*	15*	10*	12*	9*	51	2.2	0.8
Prosaro at anthesis/Taegro at late bloom	54*#	80	13*	17*	8*	14*	7*	20	1.3	0.7
Double yeast at anthesis	73	88	21	24	15*	22	15	53	1.6	1.1
Prosaro at anthesis/Double yeast at late bloom	65*	79	16*	19*	11*	15*	8*	33	1.9	0.9
P	0.001	Ns	<.001	0.005	<.001	0.006	0.003	Ns	Ns	Ns
$LSD_{0.05}$	12.9	-	3.8	8.4	3.8	10.2	7.3	-	-	-

* = Value is significantly lower than the control at the 95% confidence level
# = Value is significantly lower than Prosaro at the 95% confidence level
Ns = not significant, i.e., *P*>0.1
- = not applicable

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 continuing to test 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*. This should ultimately lead to reduced DON accumulation. Two groups of peptides continue to be investigated, the first being native and derivative forms of mating hormones from *F. graminearum* and *Neurospora* discovered by the Leslie lab. A second group includes peptides identified in the English lab and derived from combinatorial phage-display peptide libraries. An underlying question of this research is whether all small peptides are equally effective. Previous in vitro experiments in the Leslie lab suggested differences in inhibition potential among peptides.

In the past year, we continued to evaluate and compare chemically synthesized peptides for inhibition of *F. graminearum*/*G. zeae* in vitro and in planta. Within the in vitro experiments, *G. zeae* ascospores and F. graminearum macroconidia were exposed to individual mating pheromone or combinatorial peptides in 10-µl microdrops mounted on microscope slides. Test concentrations of peptides ranged from 0.2 to 4 µM. In planta experiments were conducted with whole wheat heads point-inoculated with *G. zeae* ascospores in the presence of mating pheromone or combinatorial peptides. Wheat heads of similar stages of anthesis were detached from plants and placed in water vials before pathogen inoculation. In each inhibition test, one peptide was reconstituted in dimethylformamide (DMF) prior to mixing with ascospores. A 10 µl droplet containing 10 µM peptide and 1,000 ascospores was applied to a single spikelet from the center of the rachis of each of 6 replicate wheat heads. Inoculated wheat heads were incubated in moist chambers for 2 days to optimize infection conditions. Percentage infection of wheat heads, based on scab symptom development, was assessed for 12 or more days. Pathogen development on inoculated wheat heads was also assessed and rated on a scale of 0-4 (0= no visible mycelia; 4= tufts of pigmented, sporulating mycelia on tissue).

We have continued to refine constructs of peptides attached to a protein delivery scaffold based on maize cytokinin oxidase/dehydrogenase (ZmCKX1) for scale-up production via our *Pichia pastoris* fermentation system. Scaffold-peptides purified from the culture extracts are to be used for application to wheat in greenhouse experiments.

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>

1. At a 20µM concentration, mating pheromone peptide, Pgz, inhibited wheat head infection to levels equivalent to Prosaro fungicide (Fig. 1). Combinatorial peptide FgF8B also

significantly reduced infection at this concentration, but peptide FgF3A did not. At a 10µM concentration, mating pheromone peptides derived from *Neurospora crasa* delayed infection of wheat heads, whereas peptides from *F. graminearum* did not (Fig. 2).

2. Combinatorial peptide, FgF8B, from *F. graminearum* inhibited macroconidium germination equivalently to inhibition of *G. zeae* ascospore germination. Mating pheromone peptides are also being tested for inhibition of macroconidium germination.

3. Design of scaffold-displayed peptides has been confirmed and constructs have been expressed by *P. pastoris* (yeast) fermentation. Characterizations of fermentation products and inhibitory activity were initiated.

Impact: The results of experiments with wheat head inoculations provided additional evidence that mating pheromone and combinatorial peptides can significantly protect wheat from infection by both infectious spore types (ascospores and macroconidia) produced by the head blight pathogen. 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.

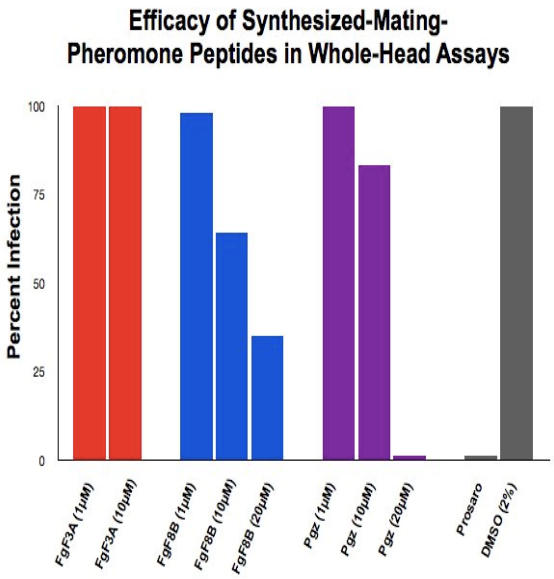

Fig. 1. Protection of wheat heads from infection by *G. zeae* ascospores in the presence of peptides Pgz and FgF8B. Peptide FgF3A did not provide effective protection from infection.

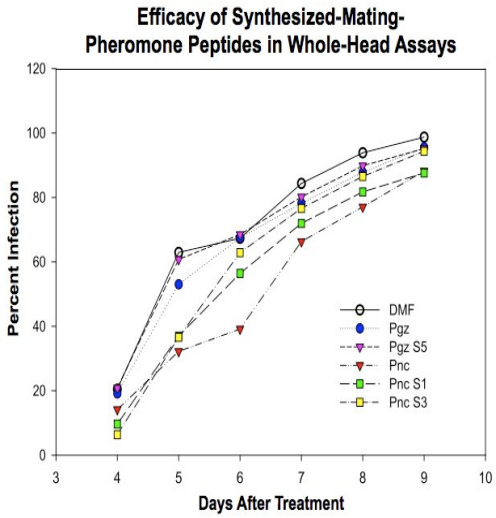

Fig. 2. Delays in wheat head infection after inoculation with ascospores in the presence of 10 µM peptides from *N. crassa* (Pnc, Pnc-S1, Pnc-S3) or *G. zeae* (Pgz, Pgz-S5).

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.

Publications

Non-peer reviewed articles:

Yuen, G.Y., Jochum C.C., Du, L., Arreguin, I., and Gale, L.R. 2010. Preinoculation of wheat heads with a non-toxigenic *Fusarium*isolate inhibits deoxynivalenol production by a toxigenic pathogen. In: S. Canty, A. Clark, A. Anderson-Scully, D. Ellis, and D.A. Van Sanford (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2010 Dec 7-9; Milwaukee, WI. Lexington, KY: University of Kentucky. p. 57.

Yuen, G.Y., Jochum, C.C., Halley, S.A., Sweets, L.E., Kirk, W., Schisler, D.A., 2010 Uniform biological control trials – preliminary results. 2010. In: S. Canty, A. Clark, A. Anderson-Scully, D. Ellis, and D.A. Van Sanford (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2010 Dec 7-9; Milwaukee, WI. Lexington, KY: University of Kentucky. p.112.

Abstract:

Yuen, G.Y., Jochum, C. C., Gross, N. W., English, J. T., Leslie, J.F. 2010. Evaluation of mating pheromone peptides for inhibition of wheat spikelet infection by *Fusarium graminearum*. In: S. Canty, A. Clark, A. Anderson-Scully, D. Ellis, and D.A. Van Sanford (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2010 Dec 7-9; Milwaukee, WI. Lexington, KY: University of Kentucky. p. 116

Presentations

Yuen, G.Y., Jochum C.C., Du, L., Arreguin, I., and Gale, L.R. Preinoculation of wheat heads with a non-toxigenic *Fusarium* isolate inhibits deoxynivalenol production by a toxigenic pathogen. Poster presented at National Fusarium Head Blight Forum; 2010 Dec 7-9; Milwaukee, WI.

Yuen, G.Y., Jochum, C.C., Halley, S.A., Sweets, L.E., Kirk, W., Schisler, D.A. Uniform biological control trials – results 2010. Poster presented at National Fusarium Head Blight Forum; 2010 Dec 7-9; Milwaukee, WI.

Yuen, G.Y., Jochum, C. C., Gross, N. W., English, J. T., Leslie, J.F. Evaluation of mating pheromone peptides for inhibition of wheat spikelet infection by *Fusarium graminearum*. Poster presented at National Fusarium Head Blight Forum; 2010 Dec 7-9; Milwaukee, WI.