USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY13 Final Performance Report
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Cover Page

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Fiscal Year:	FY13
USDA-ARS Agreement ID:	59-0206-1-122
USDA-ARS Agreement Title:	New Management Tools for Fusarium Head Blight.
FY13 USDA-ARS Award Amount:	$ 26,120

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
MGMT	Evaluation of Biological Agents for FHB and DON Control.	$ 11,539
MGMT	Effects of Defense Peptides on Fusarium Head Blight.	$ 14,581
	FY13 Total ARS Award Amount	**$ 26,120**

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 and DON Control.*

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

While more effective fungicides and host resistance to scab in some wheat market classes have been developed, these strategies are not completely effective in DON management or available for all cereal crops. Biological control measures that can be effective in diverse environments are needed to augment current chemical and resistance strategies. Results from previous years' Uniform Biocontrol Trials indicated that while biologicals applied alone were not effective or consistent alternatives to chemical fungicides, the combination of chemical fungicides followed by a late application of a biological could provide better control of DON than a chemical alone. The Yuen laboratory conducted a field experiment at the University of Nebraska Agricultural and Research Development Center near Mead, NE as part of the 2013 Uniform Biocontrol Trials. The primary objective of the trial was investigate the benefits of applying a commercial biological control product Taegro™ (Novozymes Biologicals, Salem, VA, containing *Bacillus amyloliquefaciens* FZB24) as a late application after treatment with of one of the chemical fungicides tebuconazole or Prosaro®. Each of the fungicides was sprayed at Feekes 10.51, while Taegro with or without canola oil and nitrogen amendment (to enhance bacterial colonization and antibiotic production) were applied 5 days later. Comparison treatments included a nontreated control, each of the chemical fungicides applied alone, Taegro alone (late application), Taegro with canola oil and nitrogen amendment (late application), and canola oil-nitrogen alone. A susceptible hard red winter wheat was used in the experiment, as was artificial inoculation with Fusarium-infested grain, spray inoculation with Fusarium conidia, and mist irrigation, to stimulate scab development. 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 accomplishments and their impact (i.e. how are they 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 resulted in moderate scab incidence and low head severity in the Nebraska trial. As expected, Prosaro alone provided superior control of scab and DON over tebuconzole alone, while Taegro alone was not effective in reducing disease or DON levels in comparison to the non-treated control (Table 1). There were no significant differences, however, in the results between the fungicides alone and the fungicides with late applications of Taegro. Amendment of Taegro with canola oil and nitrogen did not improve the performance of the biological control product.

Impact:

The results from the experiment conducted in Nebraska did not support previous work showing that combinations of biocontrol agents with fungicides have the potential to provide better control of DON than fungicide alone. In context of the 2013 Uniform Biocontrol Trials, the Nebraska results are in line with those from other states in which the combining of Prosaro or tebuconazole with Taegro had no appreciable effects on the fungicide's ability to suppress scab and DON.

Table 1. Results from 2013 Uniform Biocontrol Trial – ARDC, Nebraska

Treatment	Head Severity	% Incidence	Index	Yield (BU/A)	% FDK	DON (ppm)
Prosaro	3.7 c	7 d	0.3 b	12.1 a	11 b	0.1 d
Prosaro @ 10.51 and Taegro late	6.4 bc	9 d	0.7 b	10.5 abc	14 b	0.5 d
Prosaro @ 10.51 and Taegro + 1% Canola Oil + nitrogen late	4.1 c	4 d	0.2 b	11.7 ab	14 b	0.1 d
Tebuconazole	10.3 bc	54 bc	5.5 b	9.2 bcd	24 a	3.2 bc
Tebuconazole @ 10.51 and Taegro late	12.3 b	44 c	5.6 b	10.5 abc	23 a	2.0 c
Tebuconazole @ 10.51 and Taegro + 1% Canola Oil + nitrogen late	12.4 b	48 c	6.2 b	8.3 cd	23 a	3.0 c
Taegro late	24.6 a	79.5 a	19.4 a	7.4 d	29 a	4.4 ab
Taegro + 1% Canola Oil + nitrogen late	21.7 a	65 ab	14.0 a	7.1 d	31 a	5.0 a
1% Canola oil + nitrogen late	28.5 a	68 ab	19.7 a	7.5 d	28 a	5.7 a
Non-treated	23.2 a	76 a	18.4 a	8.0 cd	25 a	5.7 a

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 conducted under laboratory conditions showed that mating peptides protected wheat heads in point inoculation experiments, i.e. pathogen inoculum and mating peptides placed together in the floral tube (stigmatic channel).

During FY 12 we found that the pheromone mating peptides attached to a protein carrier, CKX (cytokinin oxidase/dehydrogenase) and produced via fermentation in a yeast did not protect wheat as we had expected, based on our experience with other plant diseases. Consequently, we decided to focus on synthesized free peptides (without attached CKX) in follow-up field experiments during FY 13.

During the past year (May 2013 – May 2014), we conducted two greenhouse trials to evaluate the ability of mating peptides in the free form (not attached to a protein carrier) to protect wheat from infection and scab development. Two mating peptides, Pnc and Pgz, were synthesized commercially. Each of these had been shown in previous in vitro experiments to inhibit germination and development of infective G. zeae ascospores. In the greenhouse experiments, each of these peptides was tested individually at a 20µM concentration, which was shown to be inhibitory to the pathogen in previous studies.

In each trial, peptides Pnc and Pgz were sprayed onto to florets until run-off. Immediately after application, florets were spray-inoculated with ascospores (10,000 spores/ml) of G. zeae. A control treatment was included in which plants were sprayed to run-off with deionized water prior to ascospore inoculation. Inoculated plants of all treatments were placed immediately into a humidity chamber, and were examined 2 weeks later for scab symptoms. In trials 1 and 2, scab incidence in the control treatment was 100 and 84%, respectively. In each trial, the scab incidence for florets treated with Pnc or Pgz did not differ significantly from the respective control treatment.

The reason for a lack of significant scab control by mating peptides sprayed onto florets is not clear. It is possible that peptide concentrations following application were depleted by run-off or degradation. Thus, the lack of scab control from sprayed peptides might be related to an insufficient contact of the peptides with the pathogen on the surface of the florets. It is likely that a higher peptide concentration in the spray or a longer period of contact between peptides and pathogen is necessary to limit infection, tissue colonization and disease. Such longer-term inhibition could be better achieved in plants that have been transformed to produce a consistent supply of peptides, such as Pnc or Pgz, over time.

In order to develop mating peptides in a tool for scab control, it will be important to evaluate the stability of the peptides when applied to plant surface. Then it may be necessary to identify measures to improve peptide dispersal and stability. It would also be beneficial to determine whether the mating peptides that inhibit *G. zeae* are limited in their activity to this pathogen, or whether they have broader inhibitory potential against other ascomycetous pathogens of importance on wheat, such as *Pyrenophora*. Determination of the inhibitory range of mating peptides inhibition will contribute to assessment of priorities in developing transgenic wheat for disease resistance.

2. List the most important accomplishments and their impact (i.e. how are they 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 determined that inhibitory mating peptides, when applied in their unmodified forms to the surface of wheat florets, do not effectively limit infection by *G. zeae* and subsequent scab development.

Impact:

Results of these experiments suggest a need to develop transgenic wheat for effective production and delivery of inhibitory mating peptides within susceptible plant tissues. Alternatively, further investigation into the stability of the peptides and better understanding of their mode of action on *G. zeae* is needed before they can be developed into a new fungicidal tool.

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 FY13 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Publication:

B.H. Bleakley, N.K.S. Murthy, E. Byamukama, G. Redenius, K. Ruden, G. C. Bergstrom, J. Cummings, G.Y. Yuen and C. Jochum. 2013. "Uniform tests of biological control agents for management of FHB and DON, 2013." In: S. Canty, A. Clark, Y. Salat, and D. Van Sanford (Eds.), *Proceedings of the 2013 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. p. 97.

Presentation:

Uniform Tests of Biological Control Agents for Management of FHB and DON, 2013. B.H. Bleakley, N.K.S. Murthy, E. Byamukama, G. Redenius, K. Ruden, G. C. Bergstrom, J. Cummings, G.Y. Yuen and C. Jochum. Poster presented at the 2013 National Fusarium Head Blight Forum, Dec. 3-5, Milwaukee, WI.