USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY09 Preliminary Final Performance Report
No Cost Extension for FY10
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Cover Page

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USDA-ARS Agreement Title:	Control of DON Production in Grain with Non-Toxigenic Strains of Fusarium.
FY09- USDA-ARS Award Amount:	$ 9,916

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
PBG	Control of DON Production in Grain with Non-Toxigenic Strains of Fusarium.	$ 9,916
	Total Award Amount	**$ 9,916**

Liangcheng Du June 30, 2010
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 Winter Wheat Region
SWW – Southern Sinter Wheat Region

Project 1: *Control of DON Production in Grain with Non-Toxigenic Strains of Fusarium.*

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

While host genetic resistance and fungicides have proven to be useful in managing Fusarium head blight (FHB) and deoxynivalenol (DON) accumulation, new methods are needed to augment these strategies, particularly in controlling DON. We tested the concept that pre-application of a non-toxigenic (Tox-) hypovirulent strain of *Fusarium graminearum* to wheat heads can inhibit floret infection by toxigenic (Tox+) virulent pathogen strains and, ultimately, reduce DON accumulation in the grain. This concept is based on the theory that the Tox- strain can compete with the Tox+ pathogen for niches and substrates on florets and potentially can induce host resistance mechanisms. A wild, Tox- isolate (WG-9) provided by Gale was tested against the standard Tox+ strain PH-1. Seven greenhouse experiments are completed by Yuen in which WG-9 and PH-1 were co-inoculated onto flowering heads of scab susceptible spring wheats 'Bobwhite' and 'Wheaton'. Inoculation was performed in one set of experiments by spray-inoculating WG-9 first and PH-1 one day later, both at either 10^5 or 10^4 spores/ml. In another set of experiments, the two strains were simultaneously point inoculated in the center spikelet of individual heads. In both sets of experiments, disease severity was determined one week after inoculation. Seed were harvested at maturity for determination of Fusarium diseased kernels (FDK). Diseased and asymptomatic seed were assayed for DON by University of Minnesota Diagnostic Lab. In addition, the identity of the *Fusarium* strain infesting individual seed were determined in first set of experiments by Du (more than 210 samples). A multiplex PCR system with primers based on *TRI3* and *TRI12* gene sequences was used for the identification procedure (Figure 1).

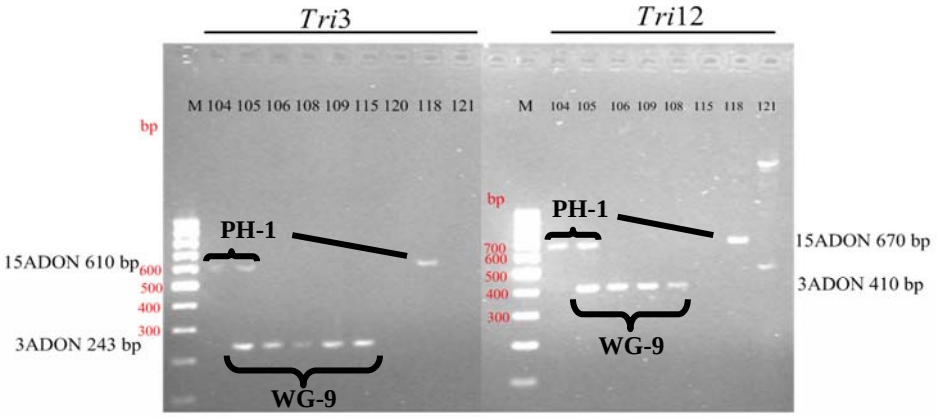

Figure 1. Representative electrophoresis results from *TRI3* (left) and *TRI12* (right) multiplex PCR of DNA extracted from plated seed and mycelia. *Fg* PH-1 (TOX+) and *Fg* WG-9 (TOX-) are indicated by amplicons of different size in each multiplex**.**

The primer mixture for *TRI3*:

3CON	5'-TGGCAAAGACTGGTTCAC-3'	(common to all chemotypes)
3D15A	5'-ACTGACCCAAGCTGCCATC-3'	(for PH-1, giving 610 bp)
3D3A	5'-CGCATTGGCTAACACATG-3'	(for WG-9, giving 243 bp)

The primer mixture for *TRI12*:

12CON	5'-CATGAGCATGGTGATGTC-3'	(common to all chemotypes)
12-15F	5'-TACAGCGGTCGCAACTTC-3'	(for PH-1, giving 670 bp)
12-3F	5'-CTTTGGCAAGCCCGTGCA-3'	(for WG-9, giving 410 bp)

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.

Accomplishment:

- Experiments revealed ***inoculation with Tox- WG-9 can reduce DON concentrations in the seed*** (Tables 1 and 2). The Tox- isolate, however, did not suppress scab symptom development.
- PCR analysis of fungi from individual seed revealed preinoculation with WG-9 reduced the frequency of seed infection by PH-1. In one experiment for example, less than 50% of the seeds from heads preinoculated with WG-9 prior to inoculation with PH-1 were infected with PH-1, as compared to 100% infection of seeds by PH-1 in heads with no WG-9 preinoculation.

Table 1. Results from 4 representative experiments testing co-inoculation of wheat heads with Tox- WG-9 and Tox+ PH-1, both at 10^4 spores/ml. Experiments 1&2: WG-9 spray inoculated 1 day before spray inoculation with PH-1. Experiments 3&4: WG-9 and PH-1 simultaneously inoculated into center floret of each head.

Experiment & cultivar	Treatment	% infected spikelets	% FDK	DON (ppm) in diseased seed	DON (ppm) in asymptomatic seed
1 'Bobwhite'	WG-9/PH-1	70.7 A	79.3 AB	**24.9 B**	0.8 B
	Water/PH-1	34.4 B	65.6 B	**176.0 A**	3.1 A
	WG-9/water	78.0 A	93.2 A	0#	0#
	Water/water	3.4 C	4.6 C	Not tested	Not tested
2 'Wheaton'	WG-9/PH-1	64.8 A	91.7 A	**12.1 B**	2.8 B
	Water/PH-1	12.0 B	86.4 A	**97.9 A**	16.5 A
	WG-9/water	78.5 A	96.5 A	1.7 C	0#
	Water/water	8.0 B	7.5 B	Not tested	Not tested
3 'Bobwhite'	PH-1 only	38.4 A	32.9 A	**202.4 A**	2.5 A
	WG-9 + PH-1	53.9 A	46.7 A	**119.8 A**	0.5 B
	WG-9 only	4.6 B	4.5 B	0#	0#
	Water	0.4 B	0.3 B	Not tested	Not tested
4 'Wheaton'	PH-1 only	68.0 A	75.1 A	**48.4 A**	1.0 A
	WG-9 + PH-1	56.7 A	81.1 A	**18.7 B**	0.3 B
	WG-9 only	23.3 B	46.5 B	0#	0#
	Water	2.8 C	9.4 C	Not tested	Not tested

Letters indicate significant differences at P=0.05.
Below detection level of 0.5 ppm. Values were not used in statistical analysis.

Impact:

This study has demonstrated a novel strategy to manage DON worthy of further research. The results support the hypothesis that a Tox- isolate of *Fusarium graminearum* might be used as a biological control agent to compete with or exclude Tox+ pathogens strains and, thus, reduce DON levels in the harvest grain. The high disease severity and expected yield loss caused by the Tox- strain alone is an obvious drawback to immediate use of the strategy. But we anticipate this problem could be overcome by 1) applying WG-9 at much lower spore concentrations; 2) using scab resistant cultivars; or 3) using Tox- strains of *F. graminearum* with lower virulence than WG-9. Another alternative would be to identify the mechanisms by which WG9 exerts its competitive/exclusive effects.

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.

Gary Y. Yuen, C. Christy Jochum, Liangcheng Du, Isis Arreguin, and Liane R. Gale. 2009. Inhibition of deoxynivalenol accumulation by preinoculation with nontoxigenic *Fusarium graminearum* - Preliminary tests of a novel strategy. Proceedings of the 2009 National Fusarium Head Blight Forum, page 100.

Inhibition of Deoxynivalenol Accumulation by Preinoculation with Nontoxigenic *Fusarium graminearum* - Preliminary Tests of a Novel Strategy. Gary Y. Yuen, C. Christy Jochum, Liangcheng Du, Isis Arreguin, and Liane R. Gale. Poster presented at 2009 National Fusarium Head Blight Forum, Dec. 7-9, Orlando, FL.