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

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USDA-ARS Agreement ID:	59-0206-9-087
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**

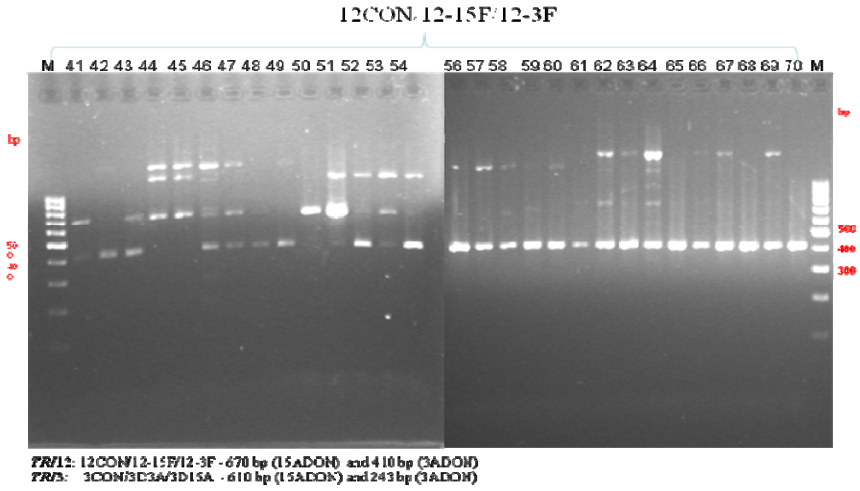
Liangcheng Du June 17, 2011
Principal Investigator Date

* 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: *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. The identity of the *Fusarium* strain infesting individual seed were determined by Du. A multiplex PCR system with primers based on *TRI3* and *TRI12* gene sequences was used for the identification procedure (Figure 1).



TRI12: 12CON/12-15F/12-3F - 670 bp (15aDON) and 410 bp (3aDON)
TRI3: 3CON/3D3A/3D15A - 610 bp (15aDON) and 243 bp (3aDON)

The figure above shows samples from #41-60, which were treated with both WG-9 and PH-1 (P2), and samples #61-70, which were treated with WG-9 only. As expected, samples #61-70 should amplify 3ADON, and a band of 410 bp for 3ADON was clearly present when the *Tri*12 primer complex was used. Samples #41-60 were expected to show a combination of amplification for 15ADON and 3 ADON. The pattern of the 670 bp and 410 bp is indicative of the competition of the two strains in each of the treatments. For example, samples #54-60 showed mainly the band for 3ADON, suggesting that the Tox^- strain was favorably competing with Tox^+ under these conditions. Approximately 218 samples had been evaluated using this multiplex PCR.

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:

- 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 pre-inoculation 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 pre-inoculated 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 pre-inoculation.

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.

Justin Huffman, Ryan Gerber, and Liangcheng Du. 2010. Recent advancements in the biosynthetic mechanisms for polyketide-derived mycotoxins. *Biopolymers* 93: 764-776.

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.

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. Poster presented at 2009 National Fusarium Head Blight Forum, Dec. 7-9, Orlando, FL.