**USDA-ARS/**
**U.S. Wheat and Barley Scab Initiative**
**FY09 Final Performance Report**
**July 15, 2010**

# Cover Page

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| **Fiscal Year:** | 2009 |
| **USDA-ARS Agreement ID:** | 59-0790-7-074 |
| **USDA-ARS Agreement Title:** | Heterogeneity & Toxigenic Potential of U.S. Fusarium Graminearum. |
| **FY09- USDA-ARS Award Amount:** | $ 49,363 |

**USWBSI Individual Project(s)**

| USWBSI Research Category* | Project Title | ARS Adjusted Award Amount |
|---|---|---|
| BAR-CP | Aggressiveness and Mycotoxin Potential of Fusarium graminearum in Field-grown Barley. | $ 13,551 |
| PBG | Traits of Biological Relevance in U.S. Populations of Fusarium graminearum. | $ 31,220 |
| PBG | Control of DON Production in Grain with Non-Toxigenic Strains of Fusarium. | $ 4,592 |
| | **Total Award Amount** | **$ 49,363** |

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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 Winter Wheat Region
SWW – Southern Sinter Wheat Region

**Project 1:** *Aggressiveness and Mycotoxin Potential of Fusarium graminearum in Field-grown Barley.*

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

Our long term objectives are to identify, and differentiate among, genetically coherent populations and species of Fusarium Head Blight (HB) pathogens in small grain cereals producing areas of the world with special focus on the U.S., to elucidate their genetic structure and molecular characteristics, to determine their distribution and prevalence in space and time, to determine their phenotypic characteristics, with special focus on aggressiveness and toxigenic potential, and to determine the impact and interaction of the various populations and species on host genotypes (both, deployed and in development), fungicides and other agricultural practices. Our USWBSI-funded research established that U.S. isolates of *Fusarium graminearum* do not belong simply in a single population as has been previously assumed, but that the pathogen population composition in the U.S. is complex and in flux. In addition to a widespread and predominant U.S. *F. graminearum* population (Midwestern (MW) 15ADON population), we have identified, molecularly and phenotypically characterized, and geographically and temporally mapped *F. graminearum* populations that are genetically distinct from the MW15ADON population. Genetically distinct populations have been identified in MN, ND, and more recently in SD [emergent (E) 3ADON and E15ADON populations] and in LA and AR [Southern Louisiana population (mainly NIV type, Gulf Coast population (mainly 3ADON and NIV types)]. We also detected a *Fusarium asiaticum* population (NIV type) to be present in Louisiana. Isolates can be placed into distinct populations by first using molecular markers to generate isolate-specific genotypes and then by analyzing the genotypic data by a Bayesian model-based clustering method.

We also determined that differences between populations are also present at a phenotypic level, affecting traits that are agriculturally and economically important, including aggressiveness, types of toxins produced and toxigenic potential. The recent identification and emergence of genetically distinct populations raises important questions for FHB management strategies. Though limited research on wheat to date has not provided evidence to support host genotype by pathogen strain interaction for FHB, if *F. graminearum* chemotypes/genotypes exhibit a differential response to barley host genotypes, then modifications need to be done to existing breeding procedures.

To detect potential differences in aggressiveness and toxigenic potential, we developed a novel approach for *F. graminearum* field research, in which we apply inoculum from synthetic populations to represent the population diversity by mixing conidia from 16 – 20 well-characterized isolates from specific populations. Isolates all have been characterized by molecular marker and evaluated for phenotypic characteristics (aggressiveness and toxigenic potential) 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):**

**Accomplishment:** In FY09, we completed the second year of a field experiment that was initially planned for two years in St. Paul, MN that in mist-irrigated nurseries evaluated the

aggressiveness and mycotoxin potential of emerging populations of *F. graminearum* from ND and MN in field-grown barley. Twelve barley varieties or lines with varying degree of resistance were selected and provided by collaborator Dr. Kevin Smith (barley breeder for the University of Minnesota). The host genotypes were inoculated with four genetically distinct *F. graminearum* populations and a water control in a replicated field experiment with a split plot design. HB severity was assessed according to previously established procedures. After harvest, samples from individual plots were measured for quantities/concentrations of different trichothecenes using GC/MS at the Mycotoxin Laboratory, University of Minnesota (Director: Dr. Yanhong Dong). Data was analyzed using JMP software with the assistance of Statistician Aaron Rendahl, University of Minnesota.

**Impact:** Our synthetic population inoculum approach enables us to detect differences in aggressiveness and toxigenic potential that are present at a population level and allows the scientific community to better judge the impact genetically diverse and novel *F. graminearum* populations may have on disease management. While the first year did not yield significant differences overall between *F. graminearum* populations in terms of FHB severity or DON accumulation, results from FY09 were quite different. Inoculations with the emergent 3ADON population resulted not only in the accumulation of significantly more DON than the other populations, but also caused significantly more disease (higher aggressiveness). Differences were substantial. While inoculation with the common MW15ADON population caused the accumulation of 9.34 ppm on average, inoculation with the E3ADON population lead on average to a more than two-fold higher DON concentration, 21.14 ppm. The other populations E15ADON and "emergent" (mix between E15ADON and E3ADON) also were significantly more toxigenic than the MW15ADON population. Aggressiveness, as measured as disease severity, in E3ADON-inoculated plots was more than 50% higher than in the MW15ADON inoculated plots (26.92 vs. 17.6). Two-way ANOVA did not reveal population*barley host genotype interactions in either year. Due to the inconsistent results between the two years, a final interpretation is currently not possible, and (at least) a third year of this field experiment is deemed necessary. It could well be that environmental conditions may play a major role in disease severity and toxin quantity outcomes in barley. This experiment and its results provide invaluable information for the barley community, as it allows for a knowledge-based judgment whether and how genetically distinct populations are of importance in breeding program. Knowledge of population composition is also useful for disease management decisions.

| | BARLEY 2008 | | | | BARLEY 2009 | | | |
|---|---|---|---|---|---|---|---|---|
| **POPULATION** | **FHB severity** | | **DON in ppm** | | **FHB severity** | | **DON in ppm** | |
| Emergent | 9.69 | a | 15.53 | a | 20.67 | b | 16.86 | b |
| E3ADON | 8.45 | a | 14.35 | a | 26.92 | a | 21.14 | a |
| E15ADON | not tested | | not tested | | 17.09 | b | 12.52 | c |
| MW15ADON | 10.17 | a | 13.80 | a | 17.60 | b | 9.34 | d |
| **CULTIVAR/LINE** | | | | | | | | |
| Stander | 20.82 | a | 24.47 | a | 22.62 | b c | 20.80 | a |
| Lacey | 19.84 | a | 21.48 | a b | 19.31 | b c d | 19.11 | a b |
| Stellar-ND | 11.00 | b | 20.98 | a b | 23.76 | b | 19.66 | a b |
| MNBrite | 11.28 | b | 11.45 | e f | 13.97 | e f | 10.45 | d e |
| GEN2-129 | 10.43 | b | 10.63 | e f | 16.01 | d e | 9.65 | e |
| Tradition | 7.53 | b c | 18.12 | b c | 17.97 | c d e | 15.11 | b c |
| Robust | 9.03 | b c | 15.71 | c d | 14.32 | d e f | 15.68 | b c |
| Conlon | 9.53 | b c | 9.17 | f g | 63.58 | a | 21.62 | a |
| ND20448 | 6.01 | c d | 10.78 | e f | 15.78 | d e | 11.17 | c d e |
| FEG8-14-083-F(29) | 2.39 | d | 12.35 | d e f | 15.60 | d e | 14.41 | c d |
| FEG8-14-083-S(1) | 2.42 | d | 13.41 | d e | 13.77 | e f | 12.18 | c d e |
| M122 | 2.97 | d | 6.18 | g | 10.12 | f | 9.74 | e |

**Project 2:** *Traits of Biological Relevance in U.S. Populations of Fusarium graminearum.*

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

Our long term objectives are to identify and differentiate among genetically coherent populations and species of Fusarium Head Blight (HB) pathogens in small grain cereals producing areas of the world with special focus on the U.S., to elucidate their genetic structure and molecular characteristics, to determine their distribution and prevalence in space and time, to determine their phenotypic characteristics, with special focus on aggressiveness and toxigenic potential, and to determine the significance of the various populations and species on host genotypes (both, deployed and in development), fungicides and other agricultural practices. Our USWBSI-funded research established that U.S. isolates of *Fusarium graminearum* do not belong simply in a single population as has been previously assumed, but that the pathogen population composition in the U.S. is complex and in flux. In addition to a widespread and predominant U.S. *F. graminearum* population (Midwestern (MW) 15ADON population), we have identified, molecularly and phenotypically characterized, and geographically and temporally mapped *F. graminearum* populations that are genetically distinct from the MW15ADON population. Genetically distinct populations have been identified in the MN, ND, and more recently in SD [emergent (E) 3ADON and E15ADON populations] and in LA and AR [Southern Louisiana population (mainly NIV type, Gulf Coast population (mainly 3ADON and NIV types)]. Isolates can be placed into distinct populations by first using molecular markers to generate isolate-specific genotypes and then by analyzing the genotypic data by a Bayesian model-based clustering method.

We also determined that differences between populations are also present at a phenotypic level, affecting traits that are agriculturally and economically important, including aggressiveness, types of toxins produced and toxigenic potential. The recent identification and emergence of genetically distinct populations raises important questions for FHB management strategies.

To detect potential differences in aggressiveness and toxigenic potential, we developed a novel approach for *F. graminearum* field research, in which we apply inoculum from synthetic populations to represent the population diversity by mixing conidia from 16 – 20 well-characterized isolates from specific populations. Isolates all have been characterized by molecular marker and evaluated for phenotypic characteristics (aggressiveness and toxigenic potential) 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):**

**Accomplishment 1:** In FY09, we completed the second year of a two-year field experiment in St. Paul, MN that in mist-irrigated nurseries evaluates the aggressiveness and mycotoxin potential of emerging populations of *Fusarium graminearum* in field-grown wheat. Twelve wheat varieties or lines with varying degree of resistance were selected and provided by collaborator Dr. James Anderson (wheat breeder for the University of Minnesota). The host

genotypes were inoculated with four genetically distinct *F. graminearum* populations and a water control in a replicated field experiment with a split plot design. FHB severity was assessed according to previously established procedures. After harvest, samples from individual plots were measured for quantities/concentrations of different trichothecenes using GC/MS at the Mycotoxin Laboratory, University of Minnesota (Director: Dr. Yanhong Dong). Data was analyzed using JMP software with the assistance of Statistician Aaron Rendahl, University of Minnesota.

**Impact:** Our synthetic population inoculum approach enables us to detect differences in aggressiveness and toxigenic potential that are present at a population level and allows the scientific community to better judge the impact genetically diverse and novel *F. graminearum* populations may have on disease management. While in the first year the synthetic "emergent population" (consisting of a mix of E3ADON and E15ADON isolates) overall averaged 30% more DON across all cultivars than the other two synthetic populations (MW15ADON and E3ADON), no differential response of wheat host genotypes to inoculation with the different populations was identified and also no significant differences were observed in VSK or FHB severity values. In 2009, an extra population treatment (E15ADON) was added. The "emergent" population again generated a higher DON level than MW15ADON. This result was due to a higher toxigenic potential of the E15ADON component, as this newly added population treatment (E15ADON) resulted in the highest DON levels, producing on average 70% more DON than the MW15ADON population. This experiment demonstrates that differences of phenotypic characteristics exist at a population level, which need to be taken into account for any disease management decisions.

**Accomplishment 2:** We have identified a new population of *F. graminearum* in MN (Northland population). Many isolates in this population (up to 40%) do not produce NIV or DON, but are pathogenic. Efforts are underway to determine whether these strains produce new toxins.

**Impact 2:** The Northland population appears to become more frequent and also may expand its geographic range. Any new toxins this population may produce need to be identified for consumer protection.

**Accomplishment 3**: From 2008 and 2009 collections, provided to us by our collaborator Dr. Gene Milus, University of Arkansas, we have established ca. 1,500 strains from AR.

**Impact 3:** From historical collection we have previously observed that AR harbors the most diverse pathogen population. Significantly, AR isolates that were classified as belonging to the MW15ADON population, were diverse for trichothecene type (NIV, 3ADON and 15ADON). By analyzing the contemporary Arkansas population, we will be better able to evaluate the risk of NIV or 3ADON types moving further north into other states.

**Accomplishment 4**: Baseline studies have been completed to determine fungicide sensitivity in isolates from different populations for seven fungicides (Topguard, Folicur, Caramba, Proline, Multiva, Prosaro and Tilt).

**Impact 4:** It will be important to determine whether differences in fungicide sensitivities exist.

**Project 3:** *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 L. R. Gale was tested against the standard Tox+ strain PH-1. Seven greenhouse experiments were completed by G. 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 was spray inoculating WG-9 first and PH-1 1 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 1 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 L. Du. A multiplex PCR system with primers based on TRI3 and TRI12 gene sequences was used for the identification procedure (Figure 1).

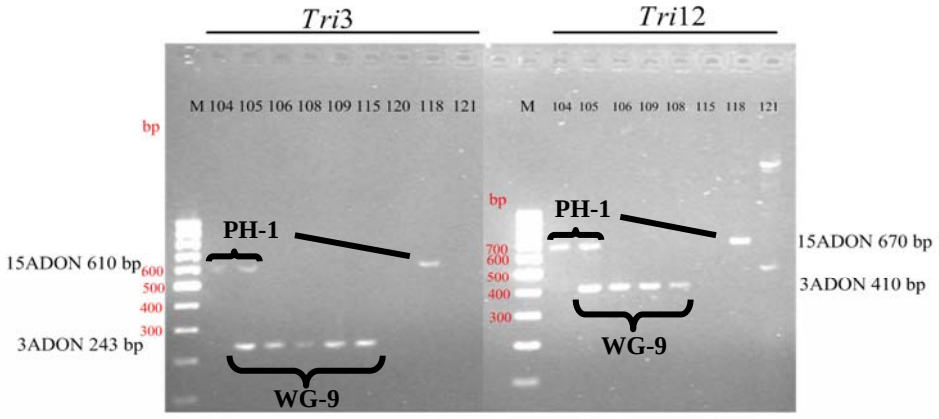


**Figure 1.** Representative electrophoresis results from *TRI3* (left) and *TRI12* (right) multiplex PCR of DNA extracted from plated seed and mycelia. *Fg* PH-1 (TOX+) and *Fg* WG-9 (TOX-) are indicated by amplicons of different size in each multiplex**.**

**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 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 coinoculation 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.**

Gale, L. R., Harrison, S. A., Ward, T. J., O'Donnell, K., Milus, E. A., Gale, S. W., and Kistler, H. C. (2010). Nivalenol-producing *Fusarium graminearum* and *F. asiaticum* are prevalent on wheat in Southern Louisiana. Phytopathology. Accepted, pending revisions.

Horevaj, P., Gale, L. R., and Milus, E. A. (2010). Resistance in winter wheat lines to initial infection and spread within spikes by deoxynvalenol and nivalenol chemotypes of *Fusarium graminearum*. Plant Disease. Accepted, pending revisions: 03/10

Karugia, G. W., Suga, H., Gale, L. R., Nakajima, T., Ueda, A., and Hyakumachi, M. (2009). Population structure of *Fusarium asiaticum* from two Japanese regions and eastern China. *Journal of General Plant Pathology* 75:110-118.

Karugia, G. W., Suga, H., Gale, L. R. Nakajima, T., Tomimura, K., and Hyakumachi, M. (2009). Population structure of the *Fusarium graminearum* species complex from a single Japanese wheat field sampled in two consecutive years. *Plant Disease* 93:170-174.

Yuen, G. Y., Jochum, C. C., Du, L., Arreguin, I., and Gale, L. R. (2009). Inhibition of deoxynivalenol accumulation by preinoculation with nontoxigenic *Fusarium graminearum* - preliminary tests of a novel strategy. Page 100 in 2009 National Fusarium Head Blight Forum Proceedings.

Gale, L. R., Dill-Macky-R., Anderson, A. A Smith, K. P., and Kistler, H. C. (2009). Aggressiveness and mycotoxin potential in field-grown wheat and barley. Page 173 in 2009 National Fusarium Head Blight Forum Proceedings.

Gale, L. R., and Kistler, H.C. (2009). Does toxic synergy explain the co-existence of two emergent populations of *Fusarium graminearum* in the Upper Midwest. *Fungal Genet. Newsl.* 56 (Suppl.):224.