USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY06 Preliminary Final Performance Report (approx. May 06 – April 07)
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Cover Page

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FY06 ARS Award Amount:	$ 9,562

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Award Amount
EEDF	The Relationship Between Fungal Colonization and DON Contamination in Wheat Seeds.	$ 9,562
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Principal Investigator Date

* CBCC – Chemical, Biological & Cultural Control
EEDF – Etiology, Epidemiology & Disease Forecasting
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GET – Genetic Engineering & Transformation
HGR – Host Genetics Resources
HGG – Host Genetics & Genomics
PGG – Pathogen Genetics & Genomics
VDUN – Variety Development & Uniform Nurseries

Project 1: *The Relationship Between Fungal Colonization and DON Contamination in Wheat Seeds.*

1. What major problem or issue is being resolved and how are you resolving it?

Fusarium head blight (FHB) resistance-breeding programs for wheat varieties adapted to Kentucky and the southeastern U.S. are underway at the University of Kentucky. Screening of populations for FHB resistance is expensive and time consuming. Screening tools that have been used in Kentucky include (i) visual assessment of disease severity, (ii) counts of infected ears or spikelets postanthesis, and (iii) counts of *Fusarium*-damaged or -infected kernels at harvest. Reduction of mycotoxins is a primary goal of our breeding program, but the correlation between our assessments of FHB disease severity and DON contamination of the wheat at harvest turned out to be relatively poor. Although DON levels in the grain can be measured directly, different methods often result in different estimates of contamination, and the most accurate techniques are too costly, inconvenient, or time-consuming for routine use in a breeding program. **Our hypothesis** was that a direct, quantitative measurement of *F. graminearum* biomass in the seed will be highly correlated with levels of mycotoxin contamination, and thus will provide a useful new tool for screening of our breeding populations. Quantitative polymerase chain reaction (PCR) protocols for fungal pathogens, including *Fusarium spp*., offer a convenient and cost-effective means for rapid analyses of fungal biomass. For example, a quantitative assay for trichothecene-producing *Fusarium spp*. was developed based on real time (RT) PCR was used to demonstrate that the fungal biomass in scab-infected wheat seeds was highly correlated with levels of DON in Europe. Our objectives were 1) Develop and optimize a protocol for quantification of *F. graminearum* fungal biomass in developing and mature wheat kernels using RT PCR. 2) Correlate fungal biomass with DON levels in kernels of three different breeding lines that are highly resistant, moderately resistant, and susceptible to FHB 3) Relate fungal development and localization over time to levels of DON in infected seeds from resistant, moderately resistant, and susceptible wheat varieties, infected with a diverse group of *F. graminearum* genotypes.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishment: We have characterized fungal colonization and development of FHB symptoms in three diverse breeding lines inoculated with several different *F. graminearum* strains. We can produce a range of disease severities using different host-pathogen combinations and different levels of inoculum, and we will use to develop our correlation analysis. We have collected individual wheat seeds adjacent to the infection points, divided them in halves, freeze dried and stored them for further PCR and toxicology analysis. We have developed, and continue to refine, our techniques for estimating fungal biomass and DON levels in single wheat kernels and half-kernels. The half-kernel DNA extraction protocol has been modified from McDonald et al. (1994) to yield sufficient DNA for the PCR analysis. PCR protocols for amplification of the trichothecene synthase (Tri5) and β-tubulin *Fusarium* genes, and wheat actin gene, have been developed and tested on DNA extracted from healthy and FHB symptomatic wheat kernels. We have modified the GC/MS protocol from Mirocha et al, 1989 for the identification and quantification of deoxynivalenol and it's derivatives in half-kernels.

Impact: We have developed some new tools that will allow us to study the relationship between the accumulation of DON and its derivates and fungal biomass present in the infected wheat kernels. Our observations confirm that the development of symptoms and

production of DON and other trichothecenes is highly dependent on host and pathogen genotypes, and on environmental factors including inoculum load and light intensity. We have been able to manipulate these factors to control the disease outcome, and this will help us to understand and predict more about the expression of resistance both to colonization and disease, and to mycotoxin accumulation under a variety of conditions in these important wheat breeding lines.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before? We have refined and characterized protocols for the analysis of DON and fungal biomass in single wheat kernels and even half-kernels. Because there appears to be significant variation from kernel to kernel within a single wheat head, a correlation analysis using single kernels will provide a more stringent test of the relationship between fungal biomass and mycotoxin production. Single- and half-kernel assays will also help us to develop a better understanding of the effect of factors including position on the spike in relation to the original infection; host and pathogen genotypes; and inoculum load; on fungal colonization, symptom development, and mycotoxin production. This information will ultimately help us to develop better, more predictive, methods for screening wheat germplasm in breeding programs.

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.

S. Bec, D. Van Sanford, L. Vaillancourt, 2007. **Comparisons of the morphology and pathogenicity of *Fusarium graminearum* strains PH1 and Gz3639**. Fungal Genetics Newsletter #54, abstract 484. http://www.fgsc.net/asil2007/xxivFGCposterAbs.htm#path