USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY06 Final Performance Report (April 06 – April 08)
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Cover Page

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Fiscal Year:	2006
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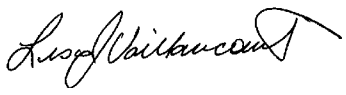
USDA-ARS Agreement Title:	The Relationship Between Fungal Colonization and DON Contamination in Wheat Seeds.
FY06 ARS Award Amount:	$ 9,562

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
	Total Award Amount	**$ 9,562**



7/15/08

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?

Reduction of mycotoxins is a primary goal of the Fusarium head blight (FHB)-resistance breeding program in Kentucky and elsewhere, but in the past correlations between our assessments of FHB disease severity and deoxynivalenol (DON) contamination of the wheat at harvest have often been relatively poor. Previous studies had reported that *F. graminearum* biomass in the seed is highly correlated with levels of mycotoxin contamination, and thus we considered the possibility that measurement of fungal biomass may provide a useful new tool for screening of our breeding populations. **Our objectives** included 1) Develop and optimize a protocol for quantification of *F. graminearum* fungal biomass in developing and mature wheat kernels based on the *tri5* primers and SYBR-Green quantification using real-time (RT) quantitative (Q) PCR 2) Correlate fungal biomass with DON levels in kernels of three different breeding lines from the Kentucky breeding program reported to be highly resistant, moderately resistant, and susceptible to FHB. The long-term goal was to develop an RT-QPCR protocol that could be used to assist our FHB-resistance breeding program here.

We used two highly characterized laboratory strains of *Fusarium graminearum* for most of our studies, and we did our inoculations in the greenhouse, due to the necessity for controlled conditions for optimization of our protocols. We were interested to find that these genetically highly similar strains differed significantly in quantitative traits affecting pathogenicity and fecundity. This demonstrated to us that the strains that are utilized for breeding programs could have a very significant effect on the result. It also established for us the limits of currently available population genetic tools in predicting the extent of strain variation. These findings have moved us toward a new goal of investigating the genetic basis for the quantitative variation in pathologically significant traits in these two highly characterized laboratory strains.

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:

PH-1 (NRRL 31084) originally isolated from corn in Michigan, and Gz3639, isolated from wheat in Kansas, both have sequenced genomes. Both belong to lineage 7 and are genetically highly similar. We tested the strains for pathogenicity on three wheat varieties carrying different sources of resistance to FHB. Both strains had similar levels of pathogenicity when inoculum levels were high, but PH-1 was more aggressive at lower inoculum concentrations or when plants were light-stressed. A ½ kernel DNA extraction protocol was modified from McDonald et al. (1994) to yield sufficient DNA for PCR analysis. Once we discovered the degree of variability in pathogenicity, that existed between these two lab strains, as well as among several other field strains that we tested, we modified our original plan to develop a protocol for the Smartcycler due to its lack of capacity for large numbers of samples. Instead we have developed a high-throughput assay for amplification of the trichothecene synthase (Tri5) *Fusarium* genes using an Applied Biosystems 7500 apparatus. We have also developed protocols for crossing the strains, and we have identified CAPs from among a large set of published SNPs that can be used as molecular markers for the crosses of the two laboratory strains.

Impact:

We have developed the necessary tools to study the relationship of mycotoxins with fungal biomass in infected wheat kernels colonized by different strains and by progeny of various crosses. If we can show a correlation between fungal mass and trichothecene levels in these experiments, this will aid our Kentucky breeding effort.

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?

These findings are a starting-point for studies of the genetic basis for quantitative variation in pathogenicity, toxigenicity, and fecundity among different closely related laboratory and field strains of *F. graminearum*. In particular it may help us to identify new genetic markers that will be more predictive of specific traits in these populations that are relevant to pathogenicity. This in turn could give us important new insights into how natural selection can act on strain variants to produce population shifts of the type recently observed in the upper Midwest. This may help us to improve the durability of our breeding effort.

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.

Bec, S., Vaillancourt, L.J., Van Sanford, D. 2008. How different are the two model *Fusarium graminearum* strains PH-1 and Gz3639 from one another? Phytopathology 98:S20

Bec, S., Van Sanford, D., Vaillancourt L.J. 2007. Comparisons of the morphology and pathogenicity of *Fusarium graminearum* strains PH1 and Gz3639. Fungal Genetics Newsletter 54: abstract #484