U.S. Wheat and Barley Scab Initiative
FY02 Final Performance Report (approx. May 02 – April 03)
July 15, 2003

Cover Page

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Year:	**FY2002 (approx. May 02 – April 03)**
Grant Number:	**58-3640-2-138**
Grant Title:	**Fusarium Head Blight Research**
FY02 ARS Award Amount:	**$ 82,681**

Project

Program Area	Project Title	USWBSI Recommended Amount
EDM	Use of gene expression analysis to study pathogenicity in Gibberella zeae.	$40,750
EDM	Genetics and morphology of perithecium development in Gibberella zeae on wheat and in vitro.	$44,000
	Total Amount Recommended	**$84,750**

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Principal Investigator Date

(Form – FPR02)

Project 1: Use of gene expression analysis to study pathogenicity in *Gibberella zeae*.

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

Because model organisms for studying fungal pathogenesis have been primarily those that show a gene-for-gene relationship with their hosts, there is only very limited knowledge about fungal infection and toxin production in opportunistic pathogens such as *F. graminearum*. To date, the only known virulence factor in this fungus is the trichothecene toxin. In the past three years, we have generated over 10,000 ESTs (representing ~2,500 unigenes). We have generated microarrays with these ESTs to identify genes that are expressed during the initiation and devleiopment of perithecia. This year, we have succeeded in obtaining the sequence of the *F. graminearum* genome (www.genome.wi.mit.edu/annotation/fungi/fusarium). We have used the sequence information from the ESTs and the genomic sequence to generate gene knockout mutants that are important to fungal perithecium development. A more specific understanding of the interaction between the fungus and the plant is important towards designing more efficient control methods.

We had two main objectives:

I. To continue to search for new means of control by identifying genes essential for pathogenesis.

II. To use microarray analyses to identify genes which are important for inoculum development.

2. What were the most significant accomplishments?

Table 1 lists the genes we have identified by the sequence analysis, the putative function, based on homologues, and the progress that has been made this year. The effect of gene disruption on the phenotype is indicated for each gene. Note the importance of light detecting genes to perithecium development. We have also noted evidence for this during perithecium development in planta in last year's report.

A dye-swap experiment (using the microarrays) was designed to compare the expression profiles in mature undifferentiated hyphae and hyphae induced to form perithecia. This experiment is ongoing, and differential gene expression patterns have been observed, but as in all micrarray experiments, the data must be rigorously confirmed. We are in that process now.

Table 1. Genes selected from ESTs and genomic sequence for functional characterization

Description	No. Transformants	Predicted function in *F. graminearum*	Phenotype of knock-out mutants
Trehalose-6-phosphate synthase	>20	May be source of osmoticum (mannitol) involved in spore discharge.	Slow growing, Spore discharge and perithecia phenotype is similar to wild type.
WD/PAS1	~50	Involved in light sensing, may initiate perithecium production and maximal spore discharge	Formation of perithecia in the absence of induction. Perithecia embedded in agar (instead of on surface).
WD/PAS 2	In Progress	Involved in light sensing, may initiate perithecium production and maximal spore discharge	
Circadian clock-controlled protein 6 (a)	~50	Same as WD/PAS1	Perithecia shape affected, perithecia form in clusters.
Circadian clock-controlled protein 6 (b)	>20	Same as WD/PAS1	Spore discharge and perithecia phenotype is similar to wild type. No major differences have been observed.
1610 B01 Neutral Trehalase	In progress	Interrupts spore development or discharge	
1625_C05_E09ZT5 Opsin, light sensitive receptor	In progress	Light controlled development of perithecia.	

Project 2: Genetics and morphology of perithecium development in *Gibberella zeae* on wheat and in vitro.

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

Our goal is to understand the biology of *Gibberella zeae* especially with regard to spore production and to develop strategies for reduction or elimination of the inoculum of the scab disease. This year, we explored how the fungus colonizes vegetative tissue and produces perithecia. We have used both greenhouse inoculations and naturally inoculated plants to tease out the entire process from initial colonization through to perithecium production.
In addition, we are using strains we developed that have mutations in the perithecium developmental process to further clarify fungal differentiation in planta and to identify genes important to this process.

2. What were the most significant accomplishments?

We have found that the stems are extensively colonized forming head infections prior to becoming debris. This implies that colonization is occurring due to primary infection and not due to colonization of debris as a saprophytic process. This reflects previous studies that the fungus is not a good saprophyte and needs to establish itself in the tissue before harvest. The implications of this are that if breeding can be done to minimize colonization and therefore later perithecium development, the disease cycle may be interrupted. A focus on Type II resistance would therefore be recommended.
We see that this is a vascular pathogen, with colonization through this tissue first. Vascular occlusions are formed in the xylem vessels in the susceptible cultivar Norm in 25 % of the inoculated plants in the greenhouse. It is significant to note that there was a direct correlation between those plants where the fungus did not leave the head to move down the stem and plants where these vascular occlusions were observed. In this “resistant” population, symptom development did not occur beyond the inoculated spikelet in 11 % of the total plants inoculated. Could this type of defense, which appears to be very effective, be used in development of resistant cultivars?

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.

Papers:

Trail, F., Mott, E., Andries, C., Farley, T., Gaffoor, I, Urban, M., Phillips, W., Pitkin, J., and Hammond-Kosack, K. 200-. Isolation of *Fusarium graminearum* insertional mutants compromised for mycotoxin production and pathogenesis on wheat. Molecular Plant-Microbe Interactions, submitted.

Trail, F., Xu, J.-R., San Miguel, P., Halgren, R. G. and Kistler, H. C. 2003. Analysis of Expressed Sequence Tags from *Gibberella zeae* (anamorph *Fusarium graminearum*). Fungal Genetics and Biology 38:187-197.

Trail, F. and Xu, H. 2002. Purification and characterization of mannitol dehydrogenase and isolation of the corresponding cDNA from the head blight fungus, *Gibberella zeae (Fusarium graminearum)*. Phytochemistry 61:791-796.

Abstracts:

Qi, W., Kwon, C. and F. Trail. 2003. Identification of genes expressed during hyphal differentiation in the head scab fungus, Fusarium graminearum. North Central Division Meeting of APS. June 26-27. East Lansing, MI.

Goswami, R.S., F. Trail, J.R. Xu , H.C. Kistler. 2003. Fungal genes expressed during plant disease development in the *Fusarium graminearum*/wheat interaction. Presented at the Fungal Genetics Conference, Asilomar, Monterey, CA

Trail , F. ,Martin Urban, Iffa Gaffoor, Ellie Mott , Corrie Andries and Kim Hammond-Kosack. 2003. Isolation and characterization of *Fusarium graminearum* mutants compromised in mycotoxin production and virulence. Presented at the Fungal Genetics Conference, Asilomar, Monterey, CA

Trail, F., Chil Kwon, Iffa Gaffoor, and Luis Velasquez. 2002. How is the force generated for ascospore discharge? Invited presentation, US-Israel BARD workshop on Molecular perspectives on fungal biology and pathology: Current status/future directions. October, Lake Tahoe, CA.

C. Kwon and F. Trail. 2002. The mechanism of forcible ascospore discharge in *Gibberella zeae*. Phytopathology 92 (6) S 43.

Goswami, R. S., F. Trail, J.R. Xu and H.C. Kistler. 2002. Differential expression of genes in *Fusarium graminearum* strains with low and high virulence for wheat. Phytopathology 92 (6) S31.

Trail, F., J.R. Xu, P. San Miguel, and H.C. Kistler. 2002. Genomics of the mycotoxin producing fungus, *Fusarium graminearum* (*Gibberella zeae*). Mycopathologia 155:8.

Talks:
Trail, F. 2002. Genomics of *Fusarium graminearum*. Invited address at the 2002 Scab Forum. Cincinnati, Ohio, Dec. 7-9.