

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

**Cover Page**

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<b>Grant Title:</b>	<b>Fusarium Head Blight Research</b>
<b>FY02 ARS Award Amount:</b>	<b>\$ 6,413</b>

**Project**

<b>Program Area</b>	<b>Project Title</b>	<b>USWBSI Recommended Amount</b>
EDM	Role of a Colletotrichum graminicola pathogenicity gene homologue in F. graminearum.	\$6,413
	<b>Total Amount Recommended</b>	<b>\$6,413</b>

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Principal Investigator

\_\_\_\_\_  
Date

## **Project 1: Role of a *Colletotrichum graminicola* pathogenicity gene homologue in *F. graminearum*.**

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

The short-term goal of this pilot project was to identify and characterize a *Fusarium graminearum* homologue of a gene that is known to be essential for pathogenicity of a related fungus, *Colletotrichum graminicola*. This gene is called CPR1, and it appears to encode a component of the eukaryotic signal peptidase enzyme. A mutation in the CPR1 gene in *C. graminicola* destroys the ability of this pathogen to cause necrotic symptoms in its host, corn. The long-term goal of this project is to attempt to create a mutation in the homologue of the CPR1 gene in *F. graminearum*, and to study the effect of this mutation on the pathogenicity of *F. graminearum* to wheat. We began by using degenerate polymerase chain reaction to amplify a small fragment of the putative CPR1 homologue from a field isolate of *F. graminearum*. Our collaborators Dr. Daren Brown (at the USDA research lab in Peoria) and Dr. Jin-Rong Xu (at Purdue University) used this fragment to probe genomic cosmid libraries of two well-characterized laboratory strains of *F. graminearum*. Dr. Brown identified a single hybridizing cosmid from strain GZ3639, and Dr. Xu identified several hybridizing cosmids from strain PH-1. Dr. Brown sequenced the cosmid from GZ3639 in its entirety. The PH-1 strain has recently had its whole genome sequenced. Thus, we are able to compare this entire region, of approximately 40 Kb, between the two strains. In our laboratory we went on to generate very high-quality sequence in the region of the CPR1 homologue gene itself by producing at least three reads in each direction across every base for the gene copies from each strain. This was necessary because sequence resulting from high-throughput genomics projects incorporates frequent errors. The structure of the CPR1 homologue gene was predicted on the basis of sequence motifs known to indicate transcription and translation start and stop sites, and intron splice sites. The CPR1 homologue was identical in both strains, and is approximately 54% identical at the protein level to CPR1 from *C. graminicola*. We are presently working to obtain evidence that this gene is expressed in both strains, and to confirm the position of predicted introns. We have preliminary evidence suggesting that the gene is indeed expressed in *F. graminearum*. In addition to the work described above, we also produced several green-fluorescent isolates of *F. graminearum* to use for future production of strains that are mutated in the CPR1 homologue gene. The presence of green fluorescence will make it possible for us to directly visualize and compare the mutant versus wild-type fungus as it colonizes wheat tissues.

### **2. What were the most significant accomplishments?**

A homologue of a known fungal pathogenicity gene was identified and fully characterized from two different standard lab strains of *F. graminearum*. Green fluorescent strains have been produced by transformation. We are now in a position to produce a mutation in this putative pathogenicity gene, and to investigate the effect of this mutation on pathogenicity of *F. graminearum* to wheat tissues. If we are successful, this gene and its pathway (protein transport and secretion) will be identified as legitimate targets for antifungal therapies designed to combat the wheat scab disease.

**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.**

Because this research is still in its preliminary stages, no publications have resulted yet. However, I anticipate that a publication will appear in a major research journal when we have completed our studies.

I want to point out that I was not familiar with the *F. graminearum* fungus prior to receiving this funding from the scab initiative, although I have worked for many years with other plant-pathogenic fungi. The scab initiative made it possible for me to develop new and valuable collaborations and to learn the methodologies and background I need if I am to make a contribution to our understanding of this very important disease.

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