U.S. Wheat and Barley Scab Initiative
FY01 Final Performance Report (approx. May 01 – April 02)
July 15, 2002

Cover Page

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Year:	**FY2001 (approx. May 01 – April 02)**
Grant Number:	**59-0790-9-071**
Grant Title:	**Fusarium Head Blight Research**
FY01 ARS Award Amount:	**$ 88,015**

Project

Program Area	Project Title	Requested Amount
Epid/Dis. Mgt.	Inoculum development in *Gibberella zeae*	$ 57,715
Epid/Dis. Mgt.	Genomics of *Gibberella zeae*, the head scab fungus	$ 68,115
	Total Amount Requested	**$ 125,830**

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Principal Investigator Date

(Form – FPR01)

Project 1: Inoculum development in *Gibberella zeae*

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

We have been studying the development of inoculum for the scab disease with the goal of using our findings to reduce or eliminate inoculum. The focus of our research has been on the development of perithecia, and their function to distribute ascospores. The specific objectives of the current proposal are: (A) To understand the process of colonization of vegetative tissue by *G. zeae* and how this colonization leads to perithecium production; (B) To characterize the perithecium developmental mutants obtained from insertional mutagenesis.

Objective A: Tissue from plants exhibiting head blight symptoms was collected at harvest, placed in mesh bags and returned to the field. Tissue was recovered monthly and examined microscopically for perithecium development and colonization as compared to tissue examined at harvest (t=0). Nodes, internodes and sheathes were colonized extensively in all samples with hyphae passing though all types of host cells. Ramification of hyphae through tissue was essentially complete at t=0, however, hyphal proliferation within cells and differentiation leading to perithecium development continued after harvest. Perithecia formed on nodes, internodes and sheathes, but not on leaf tissue. Perithecia emerged through stomates on the internodes and sheathes and the pith cavity contained dense hyphal growth adjacent to points of emergence. At the nodes and leaf bases, perithecia developed within epidermal cells and erupted through the epidermal walls, particularly at the sites of silica cells. We have investigated the use of the strain harboring the reporter gene GFP for infection studies. The strain is easily visible in the plant with the use of confocal microscopy and fluorescence microscopy. It colonizes similarly to the wildtype PH-1, but less aggressively. However, we have not been able to get this strain to generate perithecia. Therefore, we have found use of PH-1 more informative.

Objective B: We have completed analysis of one of the mutants through colonization of the wheat plant. This mutant, designated 123C-4, fully colonizes the wheat plant, but colonized plants are asymptomatic. Unlike the wild-type, the mutant does not produce wide, lipid-filled cells in the epidermis and under the stomates and does not produce perithecia. However, it colonizes with thin, undifferentiated hyphae which ramify throughout the host tissue. It produces "rhizomorph-like" hyphal bundles which run throughout the tissue. Genetic analysis of this mutant has shown the mutation segregates with the genetic tag. We are in the process of identifying the mutant gene.

2. What were the most significant accomplishments? Two observations regarding perithecium development may be important for breeding programs to consider. First, we do not know why perithecia develop at specific sites such as silica cells and stomata, however, the light transmitting qualities of the silica cells and open stomata may stimulate perithecium development. Perithecium development is known to require light. Would it be possible to reduce numbers of such cells particularly on the nodes, which take a long time to decompose? Second, although further studies will be needed for final conclusions, it appears that the fungus harnesses most of the nutrition from the plant and colonizes essentially the vegetative debris before the plant senesces. Therefore, preharvest colonization of plant tissue is more important than colonization from the soil and Type II resistance in vegetative tissues could contribute significantly to reduce inoculum production.

Project 2: Genomics of *Gibberella zeae*, the head scab fungus

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

Genomics processes offer the means to analyze gene expression patterns that can be used for rapid gene identification. Our goal is to establish genomics tools to facilitate the analysis of this important pathogen. Our first objective was to generate cDNA libraries from infected wheat plants (see H.C. Kistler), from young perithecia and from normal, nutrient balanced cultures and to generate EST sequences from these libraries. Our second objective was to establish microarrays and use these to identify genes important to pathogenesis and inoculum development. We have generated the library from the control culture and J-R Xu is generating the ESTs. We have had difficulty generating high-quality mRNA from the young perithecia. It appears RNases are very active in this tissue. We currently think we have solved the problem and will generate this library during the no-cost extension of this grant.

We have generated over 12000 ESTs in the past 3 years of funding. Analysis of these ESTs is described in a manuscript submitted to the journal Fungal Genetics and Biology. As part of this analysis, we generated a pool of nearly 2000 EST clones that represent a "single gene" set (ie, we have eliminated the redundant clones). The pool was selected by comparing sequences and organizing the ESTs into "contigs" based on those that are likely to represent the same gene. The 2000 EST clones have been amplified by PCR and individually examined on an agarose gel for single bands. Nearly 300 have more than a single band. The majority of these are representative ESTs from contigs. They will be replaced by other EST clones from the same contig. We anticipate putting the microarray together by the end of July and running preliminary experiments during August and September.

2. What were the most significant accomplishments?
   The pool of EST sequences has already yielded several genes important to fungal biology and pathogenicity. The cDNA for mannitol dehydrogenase (important for ascospore discharge) was readily isolated from the EST library after numerous difficulties with degenerate primers. We have identified other potential genes-- candidates for gene knock-out experiments-- and are pursuing these in this year's proposal.

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.

Publications

Guenther, J., and Trail, F. 200-. Development of Perithecia on Wheat Residues by *Gibberella zeae (*Anamorph *Fusarium graminearum*). Phytopathology, submitted.

Trail, F., Xu, J.-R., San Miguel, P., Halgren, R. G. and Kistler, H. C. 200-. Analysis of Expressed Sequence Tags from *Gibberella zeae* (anamorph *Fusarium graminearum*). Fungal Genetics and Biology, submitted.

Trail, F. and Xu, H. 200- Purification and characterization of mannitol dehydrogenase and isolation of the corresponding cDNA from the head blight fungus, *Gibberella zeae (Fusarium graminearum).* Phytochemistry, accepted.

Trail, F., Xu, H., Loranger, R. and Gadoury, D. 2002. Physiological and environmental aspects of ascospore discharge in *Gibberella zeae* (anamorph *Fusarium graminearum*). Mycologia 94(2):181-189.

Abstracts:
Trail, F., J-R Xu, P. San Miguel, I. Gaffoor, and C. Kistler. 2001. Expressed sequence tags from developmental stages of *Gibberella zeae*. Presented at the USDA Wheat and Barley Scab Forum, Cincinnati, Ohio, December.

Guenther, J. and F. Trail. 2001. Development of perithecia from Gibberella zeae on wheat residue. Presented at the USDA Wheat and Barley Scab Forum, Cincinnati, Ohio, December.

Oral presentations:
2002. Development and function of perithecia in *Gibberella zeae*. Invited talk to the Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Center. January 25.

2001. Understanding the biology of *Fusarium graminearum* through genetics and genomics. Invited talk presented at the 2001 Internationaler Kongress "Sustain Life- Secure Survival-Strategies for the Future", Vienna, Austria. Hosted by the Center of Applied Genetics, Universitat fur Bodenkultur Wien. November 18-21.

2001. Ascospore development and discharge in *Gibberella zeae*. Presented to the Department of Plant Pathology, Cornell University, Ithaca, N.Y. November 7.

2001. Ascospore development and discharge in *Gibberella zeae*. Presented to the Department of Plant Pathology, NYSAES, Cornell University, Geneva, N.Y. November 6.