U.S. Wheat and Barley Scab Initiative
Annual Progress Report
September 18, 2000

Cover Page

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Year:	**FY2000**
Grant Number:	**59-0790-9-071**
Grant Title:	**Fusarium Head Blight Research**
Amount Granted:	**$71,000.00**

Project

Program Area	Objective	Requested Amount
Epidemiology	To study ascospores and methods of dispersal.	$46,000.00
Biotechnology	Study the disease cycle of the head scab fungus Gibberella zeae using genomics technology.	$25,000.00
	Requested Total	$71,000.00[1]

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Principal Investigator Date

[1] Note: The Requested Total and the Amount Granted are not equal.

Project 1: To study ascospores and methods of dispersal.

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

Our approach is two-fold: To study perithecium development in the field, and to identify the physiological and genetic components that allow these propagules to be effectively dispersed. Toward this end, we have monitored formation of perithecia in up to 10 fields since 1997 and documented the timing of their appearance throughout the year. To characterize the mechanism of discharge, we have screened 5000 insertional mutants of *G. zeae* and found one that is ascospore-discharge minus but morphologically normal. Finally, we are currently identifying the components involved in this pressure buildup inside the ascus.

2. Please provide a comparison of the actual accomplishments with the objectives established.

Objective 1: To continue to characterize the timing and appearance of mature perithecia on field debris. In brief, the results indicate that temperatures below 9ºC inhibit perithecium formation.

Objective 2: To characterize discharge minus mutants by Mendelian genetics and by morphological analysis. After screening 5005 insertional mutants, we located one mutant that fails to discharge its ascospores, one developmental mutant that is arrested in perithecium development just before asci form, and several other mutants do not produce perithecia at all.

Objective 3: To identify the source of osmotic pressure in the ascus. To screen for compounds inhibitory to perithecium formation and discharge. We have purified an enzyme, mannitol dehydrogenase (MTD), involved in biosynthesis of mannitol. We have sent the protein out to be sequenced so we can make degenerative primers to the protein sequence and pull out the MTD gene. We have determined that K+ ion influx into the ascus also initiates discharge. ICP-Mass Spectrometry analysis of the ascus fluid discharged along with the ascospores showed that K+ ion levels are high, and Ca+ is undetected. Several ion channel inhibitors inhibit spore discharge from exposed perithecia.

3. What were the reasons established objectives were not met? If applicable.

We should be able to complete our objectives within the proposed timeframe.

4. What were the most significant accomplishments this past year?

A. Identification of a mutant that is morphologically normal but does not discharge its ascospores gives us a significant foothold into determining the mechanism of discharge.

B. The 9°C cutoff for temperatures under which no perithecia will form is an important observation. We do not have the facilities to pursue further studies of environmental effects on peritehcium development under field conditions, but hope that others will use this information in other areas of the country.

C. Identification of Eosin B as a nontoxic compound that inhibits spore discharge.
This illustrates the possibility of finding nontoxic, active compounds to inhibit discharge in the field.

Project 2: Study the disease cycle of the head scab fungus *Gibberella zeae* using genomics technology.

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

Little is known of the biology of *G.zeae*. Important aspects of the disease cycle include development, metabolism and pathogenicity. This project initiates a genomics project for *G. zeae* to facilitate an understanding of the biology of this fungus. This understanding will lead to novel approaches to control of the pathogen. We (Trail, Kistler and Xu) will sequence 10,000 expressed sequence tags (ESTs) from 3 different developmental/ pathogenic stages of the fungus. This is the first step towards identifying genes important to the biology of this pathogen.

2. Please provide a comparison of the actual accomplishments with the objectives established.

Objectives:

1.To make three cDNA libraries of *G. zeae* of the following stages: A) Nitrogen-starved culture B) near-mature perithecia C) infected wheat heads

All 3 cDNA libraries have been made. I was responsible for library (B).

2.To sequence 10,000 ESTs and to make the sequences broadly accessible by posting them on the web. To analyze these sequences for homology to known sequences, and predict functions.

The sequencing for library B was done by Olin Anderson. After initially sequencing 557 sequences, we found a very few sequences to be highly abundant. After the cDNA library was sent to be sequenced, further work in my lab revealed that this stage, 5 days after induction, was perhaps a little old in the developmental sequence. We have halted sequencing to decide if a better range of sequences will be obtained by sequencing a library made from 4 day old perithecia. We will decide within the next couple of weeks and then continue sequencing the original library, or make a second library and sequence it.

3. To initiate genomic analysis, including formation of a BAC library and use the microarrays to identify genes essential for developmental process associated with the damaging effects of the fungus.

We have not begun this work as yet. We will initiate it after more sequencing has been done.

3. What were the reasons established objectives were not met? If applicable.

We anticipate completing the work in the time proposed.

4. What were the most significant accomplishments this past year?

By performing a search (BLAST) of known sequences against the ESTs from *G. zeae*, we have been able to locate several ESTs that may be important to our studies of ascospore discharge. These include genes for ion channels. Without these sequences, it would be difficult to clone the ion channel genes.

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.

H. Xu, C. Andries, and F. Trail. 2000. Studies on the mechanism of forcible discharge of ascospores in *Gibberella zeae*. Phytopathology 90:S86

F. Trail, C. Andries, and H. Xu. 2000. The mechanism of forcible discharge of ascospores in *Gibberella zeae*. Presented at the Gordon Conferences on Cellular and Molecular Mycology, Phymouth, N.H.