USDA-ARS / USWBSI
FY03 Final Performance Report (approx. May 03 – April 04)
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Cover Page

PI:	**Frances Trail**
Institution:	**Michigan State University**
Address:	**Department of Botany & Plant Pathology** **342 Plant Biology Lab** **East Lansing, MI 48824**
E-mail:	**trail@msu.edu**
Phone:	**517-432-2939**
Fax:	**517-353-1926**
Year:	**FY2003 (approx. May 03 – April 04)**
FY03 ARS SCA ID:	**58-3640-2-138**
FY03 ARS Agreement Title:	**Studies of inoculum formation and genomics in Gibberella zeae.**
FY03 ARS Award Amount:	**$ 86,275**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
EDM	Genomics of Gibberella zeae, the head scab fungus.	$ 42,925
EDM	Colonization of wheat plants by Gibberella zeae and the genetics of perithecium development.	$ 43,900
	Total Amount Recommended	**$ 86,275**

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Principal Investigator Date

* BIO – Biotechnology
CBC – Chemical & Biological Control
EDM – Epidemiology & Disease Management
FSTU – Food Safety, Toxicology, & Utilization
GIE – Germplasm Introduction & Enhancement
VDUN – Variety Development & Uniform Nurseries

Project 1: ***Genomics of Gibberella zeae, the head scab fungus.***

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

Specific Objective I. To continue to search for new means of control by identifying processes essential for pathogenesis by identification of specific genes that affect life the life cycle of the fungus. Since the completion of the sequencing of the fungal genome, we turn our attention to functional analysis of *F. graminearum* genes. Systematic disruption of individual genes identified by genomics studies is one of the most powerful tools to characterize their biological function and confirm gene identities beyond a simple search for matches to a database. Genes important to the disease cycle are identified, and specifically mutated, then tested for effects on the life cycle.

Specific Objective II. To use microarray analyses to identify genes that are important to inoculum development. The goal of this proposal was to conduct experiments that would generate preliminary data and test the feasibility for (high-throughput) functional analysis of *Fusarium* genes. We planned to use these preliminary data to apply for further funding for a larger functional genomic project. Our approach in this proposal is to continue to emphasize the functional aspects of genomics that will lead to new means of control, and to use existing ESTs to identify genes importance for *F. graminearum* pathogenesis.

2. What were the most significant accomplishments?

Specific Objective I. We have completed disruption and pathogenicity testing for 2 genes for trehalose biosynthesis. From testing of these disruptants, disruptants of 16 PKS genes and several path- insertional mutants (the latter two from other funding sources), we comclude that pathogenicity testing can be greatly affected by cultivar. All of our pathogenicity tests have been performed using the cultivar Norm. Preliminary experiments with other varieties indicate that some distinctions can be made between path+ and path – phenotypes using slightly more resistant cultivars. This should be tested more rigorously.

Specific Objective II. Last funding cycle, my laboratory generated a microarray based on the ESTs USWBSI funded in previous cycles (generated by Trail, Kistler and Xu labs). This cycle my laboratory completed experiments using these arrays to identify genes showing differential patterns of expression during development. The array represents about 18% of the genes in the genome. Compared with vegetative mycelia, 493, 450, and 326 cDNAs were differentially expressed in 4-day, 5-day and 6-day perithecia, respectively. 109 cDNAs were up-regulated in all 3 perithecial samples compared to vegetative mycelia, in which 70% were specific to the perithecia library. Based on their putative identities, cDNAs that were up-regulated in all three perithecia samples represented genes involved in lipid catabolism, amino acid metabolism and transportation, protein transportation, post-translationally modification, and genes encoding cell wall proteins. Up-regulated genes involved in lipid catabolism included those important for fatty acid elongation, fatty acid oxidation, and metabolisms of membrane phospholipids. These studies give us a handle on genes used for nutritional support for perithecium development (see report for Project 2). Finally, as you are aware, Kistler, Xu and Trail were able to obtain the money to generate a genomic Affymetrix microarray chip and to initiate studies for the life cycle.

Project 2: ***Colonization of wheat plants by Gibberella zeae and the genetics of perithecium development.***

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

The major source of inoculum for scab epidemics is arguably the ascospore. My laboratory has been investigating the initiation and development of the sexual stage of the fungus to identify help identify potential "Achille's heels" as targets for control. Our most recent efforts have focused on the pattern of colonization of vegetative tissues, as stem tissue is a major component of field debris. Towards this end, we have characterized the early stages of colonization, followed developmental changes in the fungus and, after finding that the fungus stores large reserved of lipid in mycelia before the plant senesces, we have begun to explore the nutritional relationship between host and fungus. It is doubtful that the fungus could survive the winter and develop perithecia without these lipid reserves.

2. What were the most significant accomplishments?

Objective 1. This year we submitted a manuscript which is a significant contribution to our understanding of fungal development *in planta,* particularly in regard to function of dikaryotic hyphae. Staining with acridine orange revealed that wide (dikaryotic) hyphae develop in two different paths, depending on their location in the plant. They may give rise to perithecia or give rise to monokaryotic hyphae that continue the colonization process. This result supports our previous finding that wide hyphae are a stage of development unique from thin-infecting hyphae and fungal fruiting bodies. We proposed to identify the major fungal lipids that accumulate prior to perithecium development. This objective has been completed in culture for both wild type parent (PH-1) and the developmental mutant 123C-44, which does not accumulate lipids. *In planta*, the objective has been completed for PH-1 with results pending for 123C-44. The major fatty acids stored in the wide hpyae in culture were C18:2, C18:1 and C:18 saturated, mainly as triacylglycerides. Cultures induced to produce wide hyphae had nearly twice the fatty acids per dry weight mycelium that uninduced cultures had. The same profile was found with hyphae grown in the wheat stems. No fatty acids were produced in planta by the mutant.

Objective 2 We proposed to determine the carbon source taken up by the fungus and used to synthesize the lipids identified in Objective 1. While this project has not been completed, the progress to date should make the remainder of the work to be completed fast and straightforward. Sugar uptake studies show that glucose and fructose are readily taken up by the fungus, but sucrose uptake is delayed possibly by necessity of enzyme action. *In planta* labeling studies are in progress.

Sadly, this year, progress on characterization of the insertional mutants was interrupted by a serious illness of my postdoctoral fellow, who is doing this work as a secondary project. He was unable to function for nearly 6 months, but is back with us and appears to be completely recovered.

In addition, this year we completed a project on the mechanism of ascospore discharge begun under the USWBSI funding, and transferred to USDA NRI funding after preliminary data were obtained. We have shown that the source for generation of turgor pressure during active discharge of ascospores is potassium and chloride ion flux. At the second meeting of the USWBSI, I presented data showing that Eosin B, a component of diaper rash medicine, and ion channel inhibitor, inhibited discharge.

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 you grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Peer-reviewed Publications:

Trail, F., Gaffoor, I. and Vogel, S. Ejection mechanics and trajectory of a ballistic fungal spore. Submitted to *Nature*. Passed editorial review and sent out for peer review.

Guenther, J. and Trail, F. The development and differentiation of *Gibberella zeae* (anamorph: *Fusarium graminearum*) during colonization of wheat. Mycologia, Submitted.

Posters and Oral Presentations:

Velasquez, L, and F. Trail. Using genomics to study development and pathogenicity of the *Gibberella zeae,* the head blight fungus. Invited talk to the *World Conference on In vitro Biology*. San Francisco, CA, May 2004. Presented by L. Velasquez.

Trail, F., Qi, W., Vogel, S., Letourneau, Y. and Velasquez, L. Sexual development and function in *Gibberella zeae.* Poster Presented at the 7th annual European Conference on Fungal Genetics, Copenhagen, Denmark. April, 2004.

Kistler, H.C., Bruce Birren, Sarah Calvo, James Galagan, Liane R. Gale, Li-Jun Ma , Kerry O'Donnell, Frances Trail, Todd Ward, Jin-Rong Xu and the *Gibberella zeae* International Genomics Initiative. The whole genome sequence of the wheat and barley pathogen, *Fusarium graminearum.* Poster Presented at the 7th annual European Conference on Fungal Genetics, Copenhagen, Denmark. April, 2004.

Kistler, H.C., Birren, B., Calvo, C., Galagan, J., Gale, L.R., Ma, L.-J., Trail, F. and Xu, J.-R. 2004. The whole genome sequence of the wheat and barley scab fungus, Fusarium graminearum. Poster Presented at the Plant and Animal Genome XII Conference. January, San Diego, CA.

Trail, F. 2004. Form and function during development of *Gibberella zeae.* Rosie Perez Memorial Seminar Distinguished Speaker. Department of Plant Pathology, North Carolina State University, March.

Trail, F. 2003.Radio interview: "Fungi, Guns and Spores". Interviewed for the *MicrobeWorld* radio series, produced in cooperation with the American Society for Microbiology, aired on 57 radio stations (mainly NPR) throughout the United States. At http://www.microbeworld.org/home.htm, click on the MicrobeWorld radio tower icon on the lower left hand of the screen (November 11th piece).

Trail, F. 2003. Form follows function in sexual development in *Gibberella zeae*. To the MSU Genetics Program at the Annual Retreat. Michigan, August. Oral Presentation

Trail, F. 2003. Form follows function in sexual development in *Gibberella zeae*. Invited symposium talk at the Phytochemistry Society of North America, Peoria, Illinois, August.

Trail, F. 2003. Elucidating the role of turgor pressure in forcible ascospore discharge. The International Biomechanics Conference, East Lansing, MI, July. Oral Presentation.

Trail, F. 2003. Mechanism of firing of ascospores from fungal fruits. Symposium presentation to honor the retirement of Dr. Dallice Mills. Corvallis Oregon, July.

Trail, F., Guenther, J., Letourneau, Y., Kwon, C. Qi, W., Velasquez, L. 2003. Sexual development and function in *Gibberella zeae.* Poster presented at the Wheat and Barley Scab Initiative annual Fusarium Forum, Minneapolis, MN, December.