USDA-ARS / USWBSI
FY04 Final Performance Report
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Cover Page

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Year:	**FY2004** (approx. May 04 – April 05)
FY04 ARS Agreement ID:	**58-3640-2-138**
FY04 ARS Agreement Title:	**Genomics, Population Genetics, and Development of *Gibberella zeae*.**
FY04 ARS Award Amount:	**$ 75,563**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
EDM	Genomics of *Gibberella zeae*, the Head Scab Fungus.	$ 41,438
EDM	Colonization of Wheat Plants by *Gibberella zeae* and the Genetics of Perithecium Development.	$ 34,125
	Total ARS Award Amount	**$ 75,563**

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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?

One of the major projects in our laboratory is to understand the development and discharge of ascospores, the primary inoculum for the head blight disease. Towards this end, this year we have worked towards analyzing the role of cellular molecular motors in the forcible discharge of ascospores. In the functioning of the asci in active spore discharge, the cell must stretch up through the ostiole (neck) of the flask shaped perithecium, and fire its spores into the air. The stretching action is estimated to approximately double the length of the ascus (Trail et al., 2005). Normal membrane function does not permit this type of stretching. There are several hypotheses to explain ascus expansion. Ascus membrane extension may be due to rapid and simultaneous fusion of small vescicles to the membrane surface. Many vescicles are visible at the ends of the developing ascus with light microscopy. Fewer vescicles appear after the ascus is mature, but TEM studies have shown the presence on some (Trail, unpublished). Another possibility is that the plasma membrane is stretched by the action of actin filaments beneath the surface. Myosin motors move the actin filaments. We have performed expression analysis on each of 4 myosin genes to determine their expression during sexual development. We are disrupting 4 of the myosin genes to determine if they are responsible for ascus movement.

2. What were the most significant accomplishments?

Of the 4 myosin genes, expression analysis has shown that all appear to be expressed during all stages of development. Therefore, we have disrupted each of them individually to determine their effect on discharge of ascopores. The disruption of the Class I myosin resulted in transformants with an extremely slow growth phenotype. Considering the findings concerning host colonization and sequestering of fats for overwinter survival, disruption of normal mycelial growth would make this myosin a desirable target for fungicide. There are myosin inhibitors which have been used to study human muscle function. Obviously, some work needs to be done on the possibility of designing inhibitors that would be specific to fungi. Since myosins function much differently in their different roles, design of effective and specific fungal inhibitors is a distinct possibility. We have determined that the mutant generates increased amounts of aurofusarin, but tests for accumulation of other mycotoxins are underway. Three other myosin genes have been disrupted. None of the others appears to affect hyphal growth as dramatically as the one mentioned above. However, we have just initiated analysis of the effect on sexual differentiation.

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?
We have found that perithecium development in the plant is associated with light transmitting cells, stomates and silica cells (Guenther and Trail, 2005). It is not understood what signals in the plant are perceived by the fungus to stimulate perithecium initials to form in association with these special cells. Furthermore, it is not known what stimulates perithecium maturation following a potential overwintering period. We had previously identified a UV induced mutant which, in culture, produced ectopic perithecia; that is, perithecia appear on the tips of aerial hyphae, beneath the agar, everywhere they normally are not formed. They also are formed in the absence of light. We believe understanding the gene which is responsible for this mutation would be a key to understanding what triggers perithecium formation in the field. This year we asked 2 questions: How does this UV mutant produce perithecia in the wheat plant and can we identify the gene that is mutated? We were interested in examining the first question with regard to this UV mutant and several other light-gene mutants we have generated. Unfortunately, our assay to produce perithecia in the greenhouse and lab were not successful. After several attempts, we decided it to examine only the UV mutant because we knew it had profound effects of perithecium development (the others did not appear to) in culture, and because, since it was generated by UV irradiation and not genetic manipulation (as are all of the others), we could place colonized plant material in the field to stimulate natural perithecium development conditions. After overwintering inoculated and senesced whole plants from the greenhouse, we examined both UV mutant and control plants. Interestingly, the UV mutant generated perithecia most prominently on the inside of the leaf sheath, between the sheath and stalk of the wheat. Otherwise, they appeared normal, and were produced in association with the stomates. In contrast, wild-type isolates do not normally produce perithecia in this region (Guenther and Trail, 1995). To identify the gene associated with this mutation, we used a cosmid library to transform the mutant in an attempt to complement the mutation. We have identified a pool of approximately 40 cosmids that contains the gene necessary to complement the mutant. We are in the process of using these 40 cosmids to identify the single cosmid necessary for the complementation. We hope to have this cosmid identified by the end of the summer and the gene in hand this fall.

Since the last progress report, we worked several months of last summer to complete our 2003 proposed of the study of transfer of carbon from plant to fungus. The completion of these studies reveals that sucrose is used optimally by the fungus as opposed to fructose or glucose. Note that sucrose is the primary photosynthate transported through the host. Furthermore, the fungus uses an invertase to break down the sucrose before it can uptake it. We finished characterizing the lipid profile in culture and *in planta* (not reported last time). As dikaryotic hyphae (fat storing hyphae) form from haploid hyphae (see Guenther and Trail,2005), the trend is to convert saturated fatty acids back to unsaturated fatty acids as the major storage form. *In planta,* we see a change in the quantity and quality of fatty acids as the fungus progressively colonizes. This indicates that the fungus is breaking down lipids in the plant and absorbing and storing them internally as it moves through the plant. It appears that one can follow the development of the hyphae with the trend of what fats are stored. This tells us how the fungus sequesters energy for the intensive process of perithecium development.

2. What were the most significant accomplishments?

Our work demonstates that *Fusarium graminearum* colonizes the vegetative portions of the plant extensively before senescence occurs. The fungus cannot begin with late season infections and store sufficient material to overwinter and reproduce in the spring. By the time the wheat is bleached, there are no lipids in the vegetative material. This information targets, more than ever, the importance of addressing the infections process early and not later in the season. It also emphasizes the importance of Type II resistance, which reduces spread.

We have identified a gene which likely controls the signals for perithecium development. If we can manipulate the fungus to produce perithecia in a place which will not allow the ascospores to become airborne, inoculum may be reduced. Work is underway to complete the characterization of this gene.

Accomplishments:
1. We have found that perithecium development in the plant is associated with light transmitting cells, stomates and silica cells (Guenther and Trail, 2005). The fungus is able to determine the surface of the plant and develop perithecia in association with that surface.

2. *In planta,* we see a change in the quantity and quality of fatty acids as the fungus progressively colonizes. This indicates that the fungus is breaking down lipids in the plant and absorbing and storing them internally as it moves through the plant. The quality of the lipids is characteristic of the developmental stage of the fungus.

Impact:
This work shows that secondary colonization of plant tissue, after the plant has senesced is of limited importance to inoculum production. Therefore, it highlights the importance and urgency of developing Type II resistance mechanisms, as these will be most effective in limiting inoculum.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:

We have identified the process whereby the fungus procured energy to complete its life cycle. We also have shown that some sensing occurs between host and fungus to stimulate differentiation. Knowledge of these 2 processes will assist other researchers in identifying fungicide and resistance targets.

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.

Since much of my work is related to the USWBSI mission, I have put an * next to those that were funded by USWBSI, but I include related work funded by other sources.

Peer-Reviewed publications related to USWBSI funding:

*Trail, F., Gaffoor, I., and Vogel, S. 2005. Ejection mechanics and trajectory of the ascospores of *Gibberella zeae* (anamorph *Fusarium graminearum*). Fungal Genetics and Biology: *42:528-533*

*Guenther, J. and Trail, F. 2005. The development and differentiation of *Gibberella zeae* (anamorph: *Fusarium graminearum*) during colonization of wheat. Mycologia 97 (1): 232-240. Our photo of asci is on the cover!

Presentations since June 2004:

*Sexual Development in *Gibberella zeae.* Frances Trail, John Guenther and Weihong Qi. Poster presented at the 2nd International Symposium on Fusarium Head Blight. Orlando, Ca. Dec. 2004.

Function of Asci in *Gibberella zeae.*Luis Velasquez, Yvonne Letourneau and Frances Trail. Poster presented at the 2nd International Symposium on Fusarium Head Blight. Orlando, Ca. Dec. 2004.

Polyketide function in *Gibberella zeae.* Iffa Gaffoor, Daren Brown, Weihong Qi, Robert Proctor, Ron Plattner and Frances Trail. Poster presented at the 2nd International Symposium on Fusarium Head Blight. Orlando, Ca. Dec. 2004.

Sequencing and analysis of the *Fusarium graminearum* genome. 2005. Christina Cuomo, Li-Jun Ma, Jonathan Butler, Sarah Calvo, Dave DeCaprio, Tim Elkins, James Galagan, Jin Rong Xu, Frances Trail, Corby Kistler, and Bruce Birren. Fungal Genet. Newsl. 50 (Suppl): 147.

Defining the Secretome of *Fusarium graminearum*. John S. Scott-Craig, Kohhei Otani, Frances Trail, Heather E. Hallen, Brett Phinney, Janet M. Paper, Neil Adhikari, and Jonathan D. Walton. Fungal Genet. Newsl. 50 (Suppl): 202

Differential gene expression during perithecial development in *Gibberella zeae* (anamorph *Fusarium graminearum*). Heather E Hallen, Weihong Qi, Frances Trail. Fungal Genet. Newsl. 50 (Suppl): 399

The ascospore discharge mechanism of *Gibberella zeae*. Luis Velasquez, Y. LeTourneau, C. Platt, H. Hallen and F. Trail. Fungal Genet. Newsl. 50 (Suppl): 450

Seminars and Invited Talks:

2005. Functional analysis of the polyketide synthase genes in *Gibberella zeae*. Iffa Gaffoor, Daren W. Brown, Ron Plattner, Robert Proctor, Weihong Qi, and Frances Trail. Presented at the Fungal Genetics Conference, Asilomar, CA. April. Fungal Genet. Newsl. 50 (Suppl): 523.

*2005. Sexual development and pathogenicity in *Gibberella zeae*. Invited talk at Department of Plant Pathology, Kansas State University, January, 2005.

*2004. Using genomics to understand the life cycle of *Gibberella zeae*. Plenary talk at the 2nd International Symposium of Fusarium Head Blight. Dec. 11-15. Orlando, Fla.

2004. Functional analysis of the polyketide synthase genes of *Gibberella zeae*. Invited talk presented at the Midwest American Chemical Society Meeting, Peoria Illinois. October.

*2004. 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. Presented by L. Velasquez.

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