USDA-ARS / USWBSI
FY04 Final Performance Report
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Cover Page

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Year:	**FY2004**
FY04 ARS Agreement ID:	**NA**
FY04 ARS Agreement Title:	**Genomics, Population Genetics and Development of *Gibberella zeae*.**
FY04 ARS Award Amount:	**$ 84,878**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
EDM	Distribution, Survival and Discovery of New Populations of *Fusarium graminearum* in the U.S.	$ 50,732
EDM	Genomics of *Gibberella zeae*, the Head Scab Fungus.	$ 31,146
	Total ARS Award Amount	**$ 84,878**

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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: ***Distribution, Survival and Discovery of New Populations of Fusarium graminearum in the U.S.***

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

The re-emergence of FHB in past decade is likely due to a combination of factors including unfavorable climatic conditions, changes in agronomic practices and the lack of high levels of genetic resistance in currently planted wheat and barley. Another unknown but potentially important factor for the disease is the level of genetic variation in the pathogen. In order to assist effective plant breeding and disease management programs, it is essential to understand the sources and extent of genetic variation in the head blight pathogen both in the U.S. and worldwide.

Genetic diversity of populations from pathogen surveys the U.S. have been inferred based on allele differences at polymorphic, single copy loci defined by RFLPs and multilocus haplotypes have been constructed for each strain. Genetic data on strains have been arranged into geographic populations corresponding defined regions within the U.S. (e.g. state, county, field etc.) and analyzed according to geographic source. In order to determine the degree of outcrossing in the fungus, the extent of linkage disequilibrium between pairs of loci were calculated.

2. What were the most significant accomplishments?

From a previous survey of diversity in *Fusarium graminearum* collected from 86 fields in 53 counties in 9 Midwestern states in 1999 and 2000, we identified a small population in ND and MN (7%) that produced 3-acetyl, deoxynivalenol (3ADON) that were genetically distinct (Nm = 0.5) from the predominant 15-acetyl, deoxynivalenol (15ADON) producing *F. graminearum*. Collections in 2003 from 40 wheat fields in 24 counties in ND, and 9 fields in 7 counties in MN resulted in 2,133 isolates. The 3ADON type was widespread and at high frequency (21% in ND, 24% in MN). Further analysis with primers targeting three VNTR loci demonstrated that recombination in *F. graminearum*, although occurring, may be an infrequent event, as only 70 potential recombinants between the two populations were identified. Chemotyping in collections from 2001-2003 indicates that 15ADON is still the only type in other Midwestern states, though the nivalenol type was the most frequent in isolates from LA. The predominance of the nivalenol type in LA and the build-up of the 3ADON type in MN and ND suggest that selection is a principle evolutionary force acting on populations of *F. graminearum*. At the same time this study demonstrates the need for continual monitoring of the population composition, as *F. graminearum* in the U.S. is certainly not as homogeneous as previously believed.

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?

There is a lack of knowledge concerning the way in which the head blight pathogen, *Fusarium graminearum* causes disease in plants. This basic knowledge will be required to develop novel strategies for the control of the disease and the mycotoxins produced by the fungus. Genomics technology makes it possible to study the expression of potentially all of the genes in an organism. Agricultural scientists have begun using this technology to improve crops and study pathogenicity. A genome project for the scab fungus provides a unique opportunity to harness this technology for the study of the disease cycle of this important fungus.

2. What were the most significant accomplishments?

To better understand the molecular mechanisms of plant infection and virulence of *F. graminearum*, we used the REMI (Restriction-Enzyme Mediated Integration) approach to generate random insertional mutants. Eleven pathogenicity mutants were identified by screening 6,500 hygromycin-resistant transformants. In mutant M8, the transforming plasmid was integrated 110-bp upstream from the start codon of the cystathionine beta-lyase gene (CBL1). Gene replacement mutants deleted for CBL1 was also obtained. The cbl1 mutants were methionine autotrophic and significantly reduced in virulence, indicating that the methionine synthesis pathway is important for pathogenesis in F. graminearum. We also have identified genes disrupted by the transforming DNA in three other REMI mutants. In mutants M68, the transforming vectors were inserted in the NADH: ubiquinone oxidoreductase. The putative b-ZIP transcription factor gene ZIF1 and the transducin beta-subunit-like gene TBL1 disrupted in mutants M7 and M75, respectively, had no known homologs in filamentous fungi and were likely to be novel fungal virulence factors. Further characterization of ZIF1 and TBL1 genes are under the way.

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.

Seong, S., Hou, Z., Tracy, M., Kistler, H.C. and Xu, J.-R. 2005. Random insertional mutagenesis identifies genes associated with virulence in the wheat scab fungus *Fusarium graminearum*. Phytopathology 95: 744-750.

Goswami, R.S. and Kistler, H.C. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology 5: 515-525.

Suga, H., Gale, L.R. and Kistler, H.C. 2004. Development of VNTR markers for two *Fusarium graminearum* clade species. Molecular Ecology Notes 4: 468-470.

Gale, L.R., Bryant, J.D., Ochocki, G.E., Ward, T.J., and Kistler, H.C. *Fusarium graminearum* in the U.S.: heterogeneous and in flux. Fungal Genetics Newsletter 52 (Suppl): 63. 2005.

Cuomo, C., Ma, L.-J., Butler, J., Calvo, S., DeCaprio, D., Elkins, T., Galagan, J., Xu, J.-R., Trail, F., Kistler, C., and Birren, B. Sequencing and analysis of the *Fusarium graminearum* genome. Fungal Genetics Newsletter 52 (Suppl): 81. 2005.

Gale, L.R., Bryant, J.D., Giese, H., Katan, T., O'Donnell, K., Suga, H., Usgaard, T.R., Ward, T.J. and Kistler, H.C. A genetic map of *Gibberella zeae* using sequence-tagged sites and AFLPs. Fungal Genetics Newsletter 52 (Suppl): 82. 2005.

Seong, K.-Y., Yao, J., Kistler, H.C., Xu, J.-R. REMI mutagenesis and identification of infection defective mutants in Wheat Scab Fungus *Fusarium graminearum*. Fungal Genetics Newsletter 52 (Suppl): 106. 2005.

Birren, B., Kistler, H.C., Xu, J. R. and Trail, F., Genomics of *Fusarium graminearum*. NSF/USDA Microbial Genome Sequencing Program Awardee Workshop. San Diego, CA. Final Program. p. 52-53, 2005.

Chang, Y.-L., Cho, S., Kistler, C., Sheng, H.-C., and Muehlbauer, G. Bacterial artificial chromosome-based physical map of *Gibberella zeae* (*Fusarium graminearum*). Plant and Animal Genome XII Final Abstract Guide p. 94, 2005.

Goswami, R.S., Xu J-R., and Kistler, H.C. Genomic analysis of host–pathogen interactions between *Fusarium graminearum* and its gramineous hosts. Phytopathology 94:S35, 2004.

Kistler, H.C., Trail, F., Birren, B., Ma, L., Galagan, J., Gale, L. R., O'Donnell, K., Seong, K., and Xu, J.-R. Genomics of the wheat and barley pathogen, *Fusarium graminearum*. Phytopathology 94:S122, 2004.