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:	**Global Molecular Surveillance of FHB Species and Their Mycotoxin Potential.**
FY04 ARS Award Amount:	**$ 46,829**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
EDM	Global Molecular Surveillance of FHB Species and Their Mycotoxin Potential.	$ 46,829
	Total ARS Award Amount	**$ 46,829**

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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

(Form – FPR04)

Project 1: ***Global Molecular Surveillance of FHB Species and Their Mycotoxin Potential.***

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

Our phylogenetic analyses of DNA sequences from 11 nuclear genes totaling 13.6 kb have shown that the primary etiological agent of FHB, *Fusarium graminearum*, actually comprises 12 phylogenetically distinct and biogeographically structured species (hereafter referred to as the *Fg* clade) (O'Donnell *et al*. 2004; Starkey *et al*. unpubl.). In addition, our research has shown that the virulence-associated trichothecene mycotoxin genes appear to be under a novel form of balancing selection which may have important consequences for the fitness and aggressiveness of FHB pathogens on particular hosts or in particular environments (Ward *et al*. 2002).

Our ongoing molecular epidemiological studies indicate that only a fraction of this diversity is currently present within North America. However, due to the global nature of world trade, the inadvertent introduction of novel *Fg* clade pathogens or chemotypes has the very real potential to exacerbate the FHB problem within the U. S. To establish the first baseline on the global distribution of *Fg* clade species and their toxin potential, we screened 2200 Fusarium Head Blight strains from a global collection, totaling over 3.5 megabase pairs of unique DNA sequence data, in order to identify the FHB species and their host and geographic distributions. In addition, trichothecene toxin chemotype was determined for all 2200 strains using two sets of chemotype-specific primers that target the toxin genes TRI3 and TRI12 (Ward *et al*. 2002).

2. What were the most significant accomplishments?

We conducted a genetic screen to identify FHB species by sequencing parts of two phylogenetically informative genes (e.g. EF-1α and reductase totaling over 3.5 megabase pairs of unique DNA sequence data) from a global collection of 2200 strains, including over 1000 isolates from the U. S. and Canada. Results of this screen demonstrated that *Fusarium graminearum* accounts for ~99% of FHB within North America. However, two foreign FHB species were discovered within the U. S. (i.e., *F. asiaticum* and *F. meridionale*) together with an unnamed FHB species thought to be native to North America. In addition, trichothecene mycotoxin chemotypes were determined for all 2200 strains using two sets of chemotype-specific primers that target the toxin cluster genes TRI3 and TRI12 (Ward *et al*. 2002).

Our multilocus DNA sequence data identified three previously unknown FHB species, bringing the total number of phylogenetically distinct species within the B-trichothecene toxin-producing clade to 17 worldwide. DNA sequencing of the A-trichothecene FHB portion of the project has been completed, with portions of seven loci sequenced totaling 7 kb of DNA sequence. Preliminary analysis of these data indicates that 25 phylogenetically distinct species are represented in this collection of A-trichothecene FHB pathogens. This unique DNA sequence database is currently being interrogated to develop a high-throughput SNP-based platform for the accurate identification of FHB pathogens, host/geographic distributions and their mycotoxin potential.

To facilitate communication among scientists within the FHB community and quarantine specialists, eight unnamed species within the *Fusarium graminearum* (*Fg*) clade resolved by our multilocus phylogeny were formally described (O'Donnell *et al*. 2004). Development of robust PCR multiplex and SNP-based molecular tools for *Fg* clade species identification and chemotype determination have significantly improved global monitoring efforts and disease surveillance, thereby making available for the first time detailed information on the host and geographic distributions of FHB pathogens and their trichothecene chemotypes. Such knowledge is critical for enhancing our knowledge of the ecology, epidemiology and population biology of these mycotoxigenic cereal pathogens.

Accomplishment: We have used multilocus DNA sequence data and multiplex PCR assays to establish a baseline of FHB species and trichothecene toxin chemotype diversity globally. These studies have shown for the first time that the primary etiological agent of FHB, *Fusarium graminearum*, comprises at least 12 phylogenetically distinct and biogeographically structured species worldwide. Although *F. graminearum* accounts for over 99% of FHB within North America, three additional *Fg* clade species, including two recently introduced foreign FHB pathogens, were detected in our survey of FHB within the U. S. To facilitate communication among scientists within the FHB community and quarantine specialists, eight unnamed species within the *Fusarium graminearum* (*Fg*) clade resolved by our multilocus phylogeny were formally described (O'Donnell *et al*. 2004). Development of robust PCR multiplex and SNP-based molecular tools for *Fg* clade species identification and chemotype determination have significantly improved global monitoring efforts and disease surveillance, thereby making available for the first time detailed information on the host and geographic distributions of FHB pathogens and their trichothecene chemotypes. Such knowledge is critical for enhancing our knowledge of the ecology, epidemiology and population biology of these mycotoxigenic cereal pathogens.

Impact: Our studies are the first to alert plant breeders charged with developing wheat and barley cultivars with broad-based resistance to FHB that the morphospecies *Fusarium graminearum* comprises at least 12 phylogenetically distinct and biogeographically structured species and that half of these species are still segregating for trichothecene toxin chemotype. Furthermore, our studies alert plant disease specialists and quarantine officials that only a fraction of the FHB pathogen and toxin chemotype diversity is currently represented within North America. Providing names for the newly discovered FHB pathogens should greatly facilitate communication among scientists within the FHB community, including quarantine specialists. Moreover, the unique multilocus DNA sequence database we have developed is currently being interrogated to develop the first SNP microsphere array for the high-throughput indentification of all known B-trichothecene toxin-producing FHB species and their toxin chemotypes in order to improve disease surveillance efforts and to facilitate a greater understanding of the ecology, epidemiology and population dynamics of these FHB pathogens.

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?:
The published SNP data, including the DNA sequence data submitted to NCBI, represent important technology transfer because plant disease specialists now have direct access to the first set of FHB pathogen SNP markers.

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 articles:

Gale, L. R., Bryant, J., Giese, H., Katan, T., O'Donnell, K., Suga, H., Taga, M., Usgaard, T. R., Ward, T. J. and Kistler, H. C. Chromosome maps of the head blight pathogen *Fusarium graminearum* based on genetic and physical mapping and cytological observations. Genetics: (In review).

Geiser, D.M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.J., Zhang, N., Kuldau, G.A. and O'Donnell, K. FUSARIUM-ID v.1.0: A DNA sequence database for identifying *Fusarium*. Europ. J. Pl. Pathol. 110:473-479. 2004.

O'Donnell, K., Ward, T. J., Geiser, D. M., Kistler, H. C. and Aoki, T. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genetics and Biology 41:600-623. 2004.

Non-peer-reviewed articles:

Comis, D. A First for *Fusarium*: Wheat and barley disease fungus is fully mapped and on the Web. Agricultural Res. 53:6-7. 2005.

O'Donnell, K., Geiser, D. M. and Aoki, T. Species recognition and identification of agriculturally important Fusaria: Current status and future prospects. Proceedings of the 10th International Congress for Culture Collections, p. 233-238. Tsukuba, Japan. 2004.

Presentations:

Poster/Published abstract:

Gale, L. R., Bryant, J., 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. XIII Fungal Genetics Conference, p. 82. 2005.

Gale, L. R., Bryant, J., Ochocki, G. E., Ward, T. J. and Kistler, H. C. *Fusarium graminearum* in the U.S.: heterogeneous and in flux. XXIII Fungal Genetics Conference, p. 63. 2005.

Kistler, H. C., Birren, B., Ma, L.-J., Calvo, S., Galagan, J., Gale, L. R., O'Donnell, K., Trail, F., Ward, T. J. and Xu, J.-R. The whole genome sequence of the wheat and barley pathogen *Fusarium graminearum.* Abstracts of the 7th European Conference on Fungal Genetics, p. 210. 2004.

Kuldau, G. A., Ward, T. J., O'Donnell, K., Archibald, D. D., Jimenez-Gasco, M., Zitomer, N. Geiser D. M. Reassessment of type-A trichothecene-producing *Fusarium* using molecular phylogenetics and HPLC-MS toxin profiles. Chemical and Biological Terrorism Defense Gordon Conference. Buelton, CA, 2004.

O'Donnell, K. The importance of species biology in a genomics era: Examples from *Fusarium*. XXIII Fungal Genetics Conference, p. 3. 2005.

Starkey, D. E., Ward, T. J., O'Donnell, K., Geiser, D. M., Kuldau, G., Clear, R. M., Gale, L. R., Kistler, H. C. and Aoki, T. Delineation of species boundaries within *Fusarium graminearum*, the causative agent of Fusarium Head Blight. XXIII Fungal Genetics Conference, p. 71. 2005.

Suga, H., Karugia, G. W., Ward. T. J., Gale, L. R., Tomimura, K., Nakajima, T., Kageyama, K. and Hyakumachi, M. Development of a PCR-RFLP-based identification system for *Fusarium asiaticum* and genetic characterization of western Japanese isolates. XXIII Fungal Genetics Conference, p. 72. 2005.

Ward, T. J., Starkey, D. E., Geiser, D. M., Kuldau, G., Kistler, H. C., Aoki, T., Gale, L. R. and O'Donnell, K. An evolutionary framework for tackling Fusarium Head Blight: Species recognition, toxin evolution and biogeography of the *Fusarium gramienarum* species complex. National Fusarium Head Blight Forum Proceedings, p. 582. 2004.

Ward, T. J., Starkey, D. Page, B. and O'Donnell, K. A multilocus SNP microsphere array for identification of Fusarium Head Blight species and chemotypes. XXIII Fungal Genetics Conference, p. 75. 2005.

Zitomer, N. C., Geiser, D. M., Ward, T. J., Jiminez-Gasco, M., Archibald, D. D., O'Donnell, K. Kuldau, G. A. The use of HPLC-MS to characterize toxin-production from fusaria based on new phylogenetic concepts within the genus. American Phytopathological Society Meeting. Anaheim, CA, 2004.

Invited Lectures:

O'Donnell, K. The importance of species biology in a genomics era: Examples from *Fusarium*. XXIII Fungal Genetics Conference, 2005.

O'Donnell, K. Discordant evolution of Fusarium Head Blight species and their toxins: Evidence from multigene genealogies. Connecticut Agricultural Experiment Station. 2005.

O'Donnell, K. Species recognition and Identification of Agriculturally Important Fusaria: Current Status and Future Prospects. 10th International Congress for Culture Collections, Tsukuba, Japan, 2004.

Starkey, D. E. A multilocus genotyping array for identification of Fusarium head blight species and chemotypes. Satellite Workshop to the Gordon Research Conference on Mycotoxins and

Phycotoxins, "Applications of Emerging Technologies to Mycotoxin and Phycotoxin Research," Salisbury Cove, Maine, June, 2005.

Ward, T.J. An evolutionary framework for the comparative and functional genomics of the *Fusarium graminearum* species complex. 2nd International *Fusarium* Genomics Workshop held in conjunction with the 7th European Conference on Fungal Genetics, Copenhagen, Denmark, 2004.

Ward, T. J. An evolutionary framework for tackling Fusarium Head Blight: Species recognition, toxin evolution and biogeography of the *Fusarium gramienarum* species complex. National Fusarium Head Blight Forum Proceedings, p. 582. 2004.

Ward, T. J. Evolution and functional significance of trichothecene mycotoxin diversity in plant-pathogenic *Fusarium*. General Meeting of the American Society for Microbiology, Atlanta, Georgia, June, 2005.