USDA-ARS / USWBSI
FY04 Preliminary Final Performance Report
July 15, 2005

Cover Page

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Year:	**FY2004** (June 04 – June 05)
FY04 ARS Agreement ID:	**59-0790-3-081**
Agreement Title:	**Genetic Mechanisms to Control Head Scab.**
FY04 ARS Award Amount:	**$ 88,849**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	RNAi Control of Deoxynivalenol Contamination of Barley.	$ 41,044
EDM	Role of Dioxygenases in Fusarium graminearum Sporulation.	$ 47,805
	Total ARS Award Amount	**$ 88,849**

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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: ***RNAi Control of Deoxynivalenol Contamination of Barley.***

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

One of the most severe mycotoxin problems in the U.S. is trichothecene contamination of small grains by *Gibberella zeae* (anamorph *Fusarium graminearum*) in a disease called scab or Fusarium head blight (FHB). Here we propose a novel method to control trichothecene contamination in barley and/or wheat using RNA interference (RNAi) technology to block mycotoxin production. Through use of RNAi technology we will:

1. Identify the parameters required for uptake and spread of siRNA in fungi
2. Demonstrate RNAi control of trichothecene production in barley and/or wheat

RNAi is a conserved eukaryotic gene regulatory mechanism often referred to as gene silencing. We propose to silence expression of a key transcription factor gene (*tri6*) for the control of tricothecene production in *Fusarium* by transforming barley and wheat with an inverted repeat sequence of *tri6* (we propose two crops as one may work better than the other). It is hypothesized that the inverted repeat transcript will be fragmented into small RNA species known as siRNAs as a part of a conserved eukaryotic silencing mechanism. These siRNAs will then be taken up by hyphae and trigger a silencing mechanism in *Fusarium*. This research is designed to quickly control mycotoxin contamination of barley and wheat.

2. What were the most significant accomplishments?

A. We have successfully shown that we can silence trichothecene production in *Gibberella zeae* using inverted repeat (IRT) constructs of *tri6* , the trichothecene cluster gene encoding the transcription factor required for expression of the trichothecene biosynthetic genes. This work was recently published and in collaboration with Dr. Daren Brown, USDA, Peoria.

McDonald T., Brown D., Keller N. P., Hammond T. (2005) Inverted Repeat Transgenes Silence Mycotoxin Production in *Aspergillus* and *Fusarium* species. Mol Plant Microbe Interactions 18:539-545.

B. We are collaborating with Dr. Heidi Kaeppler and Dr. Ron Skadsen to put an IRT of *tri6* into wheat and barley respectively. Jointly we have created a plant transformation of the tri6 IRT in one of Dr. Kaepplers' wheat vectors. Her student has transformed wheat embryos and is in the midst of culturing wheat callus of putative transformants. We hope to have transformed wheat in fall/winter 2005 with mature wheat to test in 2006. Efforts will focus on wheat for the moment.

Project 2: ***Role of Dioxygenases in Fusarium graminearum Sporulation.***

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

Fusarium head blight (scab) is one of the most devastating diseases of wheat and barley. It is caused by a number of mycotoxin producing *Fusarium* spp. including *F. graminearum, F. culmorum,* and *F. sporotrichioides.* The latter two spp. infect primarily by asexual spores (conidia) whereas *F. graminearum,* the principle scab causing fungus infects host plants with both sexual (ascospores) and asexual spores. Impediments to spore production would be useful in controlling this disease. Biochemical and genetic studies suggest that oxylipins, oxygenated derivatives of unsaturated fatty acids, are conserved signaling and structural molecules modulating fungal asexual and sexual spore development. *F. sporotrichioides* mutants *(Δppo1* strains) lacking an oxygenase responsible for oxylipin production, are severely impaired in asexual spore production compared to that of wild type. Additionally, these mutants are do not produce the mycotoxin T-2. T-2 toxin inhibition, aberrations in spore production and reduced aerial growth suggests that this *ppo* gene and/or its products could be a target for control strategies of scab. Our goal is to test this hypothesis by inactivating the *F. graminearum* oxylipin genes and examine such strains for asexual and sexual spore production, pathogenicity and mycotoxin biosynthesis. These efforts will help in developing efficient control measures to minimize this persistent disease problem and spread in the USA and other parts of the world.

2. What were the most significant accomplishments?

A. As described above, we have been able to inactivate an oxygenase in *F. sporotrichiodes* and show that loss of this gene affects sporulation and toxin formation. This is published.

McDonald T, Devi T, Shimizu K, Sim S-C, Keller NP (2004) Signaling events connecting mycotoxin biosynthesis and sporulation in *Aspergillus* and *Fusarium spp.* In New Horizon of Mycotoxicology for Assuring Food Safety, Proceedings of the International Symposium of Mycotoxicology (Editor: Takumi Yoshizawa) pp 139-147.

B. We have initiated a collaboration with Drs. Daren Brown and Robert Butchko at USDA, Peoria, to investigate the role of oxygenases in *F. graminearum* sporulation and trichothecene development. We have constructed a lipoxygenase deletion vector and Dr. Butchko has transformed *F. graminearum* with this construct. He is currently examining transformants for deletion of *lox.* Dr. Brown will analyze *lox* mutants for pathogenicity and toxin production and our lab will analyze the mutants for ascospore and conidia production.

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.

McDonald T., Brown D., Keller N. P., Hammond T. (2005) Inverted Repeat Transgenes Silence Mycotoxin Production in *Aspergillus* and *Fusarium* species. Mol Plant Microbe Interactions 18:539-545.

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