USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY06 Final Performance Report (approx. May 06 – April 07)
July 16, 2007

Cover Page

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Fiscal Year:	2006
USDA-ARS Agreement ID:	59-0790-6-066
USDA-ARS Agreement Title:	Structural and Functional Studies of Trichothecene Biosynthetic Enzymes.
FY06 ARS Award Amount:	$ 53,547

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Award Amount
PGG	Structural and Functional Studies of Trichothecene Biosynthetic Enzymes.	$ 53,547
	Total Award Amount	**$ 53,547**

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Principal Investigator Date

* CBCC – Chemical, Biological & Cultural Control
EEDF – Etiology, Epidemiology & Disease Forecasting
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GET – Genetic Engineering & Transformation
HGR – Host Genetics Resources
HGG – Host Genetics & Genomics
PGG – Pathogen Genetics & Genomics
VDUN – Variety Development & Uniform Nurseries

Project 1: *Structural and Functional Studies of Trichothecene Biosynthetic Enzymes.*

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

The major question that was addressed by this project is whether understanding the structure and function of the trichothecene biosynthetic enzymes will lead to improved approaches to controlling FHB.

The initial strategy adopted was to clone and express all of the proteins in the trichothecene mycotoxin biosynthetic pathway seeking those that might be amenable to biochemical investigation. Thereafter it was planned to crystallize and determine the three-dimensional structure of the suitable candidates while simultaneously studying their biochemical properties. The trichothecene 3-O-acetyltransferase from *Fusarium sporotrichioides* and *Fusarium graminearum* (TRI101) were the first proteins to be successfully investigated, though progress has been made on other enzymes in the pathway. This was the most important target since it has been investigated intensively as a potential candidate for transgenic amelioration of trichothecene mycotoxins. Prior to this study the biochemical properties of this enzyme were not well understood the molecular level.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishment:

The three dimensional structures of trichothecene 3-O-acetyltransferase (TRI110) from *Fusarium sporotrichioides* and *Fusarium graminearum* have been determined complexed with coenzyme A and a variety of trichothecene mycotoxins. In addition, the kinetic properties of these enzymes towards DON, T-2, nivalenol, and isotrichodermal have been measured. These structures establish the molecular mechanism for acetyl transfer and provide insight into the structural basis for the differences in specificity. The kinetic studies reveal that there is a significant difference in the activity of TRI101 from *F. sporotrichioides* compared to the orthologous enzyme from *F. graminearum* towards DON. In particular the enzymatic ability of TRI101 from *F. sporotrichioides* to acetylate DON is 70 fold lower than the same enzyme from *F. graminearum.* The structures provide a molecular explanation for the differences in specificity between enzymes and establish the groundwork necessary to improve their efficiency.

Impact:

The structural and biochemical findings reported here have profound implications for the construction of transgenic resistant strains of wheat and barley. Incorporation of the *tri101* gene from *F. sporotrichioides* into wheat and barley does not provide resistance to DON and nivalenol in field trials. One explanation for this failure might be that the wrong enzyme was chosen since the enzyme from *F. graminearum* clearly has much better biochemical properties. This means that resistance to FHB might be obtained by incorporating a better enzyme.

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 scientific community now knows that it is important to consider the biochemical properties of the enzyme utilized to construct transgenic strains of wheat or barley. It is reasonable to assume that improved resistance to FHB in North America could be obtained by incorporating the *tri101* gene from *F. graminearum*.

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.

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