U.S. Wheat and Barley Scab Initiative
FY01 Final Performance Report (approx. May 01 – April 02)
July 15, 2002

Cover Page

PI:	**Nancy Alexander**
Institution:	**USDA-ARS**
Address:	**1815 N. University St.** **Peoria, IL 61604**
Email:	**alexannj@mail.ncaur.usda.gov**
Phone:	**309-681-6295**
Fax:	**309-681-6686**
Year:	**FY2001 (approx. May 01 – April 02)**
Grant Number:	**N/A**
Grant Title:	**Fusarium Head Blight Research**
FY01 ARS Award Amount:	**$ 32,124**

Project

Program Area	Project Title	Requested Amount
Biotech	Identification and Isolation of Trichothecene Resistance Genes	$ 33,000
	Total Amount Requested	**$ 33,000**

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Principal Investigator Date

(Form – FPR01)

Project 1: Identification and Isolation of Trichothecene Resistance Genes

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

The primary objective of this research project is to identify and isolate genes that would be available for researchers to use to produce *Fusarium*-resistant lines of wheat and barley. Since toxins are virulence factors in wheat head blight, we are looking for wheat genes that are able to provide resistance to the fungal toxin DON with the ultimate goal of finding genes that may be effective in reducing fungal pathogenesis.

We have continued our search for toxin-resistance genes by making cDNA libraries from a mutant of *Fusarium* and from *Trichothecium*. The *Fusarium* mutant lacks a functioning *Tri101*, the gene responsible for acetylating the hydroxyl at the C-3 position and therefore making the trichothecene molecule less toxic to the fungus. However, the mutant fungus is still able to tolerate trichothecenes, suggesting there are other genes involved in toxin resistance. We are searching for these genes by making a cDNA library from the mutant. *Trichothecium* produces trichothecin and this fungus is able to grow in the present of *Fusarium* toxins, however, the reverse is not true. Therefore, *Trichothecium* must have some additional genes involved in toxin resistance, and we are interested in isolating these gene(s).

We are also developing a model plant transformation system to be used as a rapid screening mechanism for the identification of toxin-resistant genes. Genes of interest identified by our yeast screening system will be moved into the model plant system to determine if they are able to be expressed *in planta* before moving to the complex higher plant transformation system.

2. What were the most significant accomplishments?

We identified the best time of the growth cycle of *Trichothecium* for making a cDNA library. This was done by identifying the time frame during which *Trichothecium* makes toxin, isolating the RNA, and making a cDNA library in a yeast vector. Due to manufacturing problems of the ligase contained with the cDNA kit, we had low numbers of transformants initially. However, we were able to determine that the ligase was the problem and therefore, were able to make a cDNA library containing a large number of transformants. We also completed making a library for the *Fusarium Tri101* disruptant.

Yeast transformations, with subsequent testing of the transformants on toxin-containing medium, identified a number of toxin-resistant colonies. These colonies are being tested now for the gene which they contain.

In order to get high expression of microbial genes in *Chlamydomonas*, we designed a promoter and plasmid carrying the *hsp* gene and *nit* genes from *Chlamydomonas*. We then ligated *Tri101* into the construct. Therefore, we have a plasmid to test in the *Chlamydomonas* transformation system.

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.

Alexander, N.J., McCormick, S.P. 2001. *Tri8* encodes a trichothecene C-3 esterase. National Fusarium Head Blight Forum, Cincinnati, OH.

Ziegenhorn, S.L. 2000. Phytotoxicity of selected trichothecenes using *Chlamydomonas reinhardtii* as a model system/ Isolation of trichothecene resistant genes from wheat. Thesis, Bradley University.

Brown, D.W., McCormick, S.P. Alexander, N.J., Proctor, R.H., and Desjardins, A.E. 2001. A genetic and biochemical approach to study trichothecene diversity in *Fusarium sporotrichioides* and *Fusarium graminearum*. Fungal Genetics and Biology 32:121-133.

McCormick, S.P. and Alexander, N.J. 2002. *Fusarium Tri8* encodes a trichothecene C-3 esterase. Appl. Environ. Microbiol. 68:2959-2964.

Alexander, N.J., McCormick, S.P., and Hohn, T.M. The identification of the *Saccharomyces cerevisiae* gene *AYT1* (ORF-YLL063c) encoding an acetyltransferase. Accepted, Yeast.