

**U.S. Wheat and Barley Scab Initiative
 FY00 Final Performance Report (approx. May 00 – April 01)
 July 30, 2001**

Cover Page

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Year:	FY2000 (approx. May 00 – April 01)
Grant Number:	
Grant Title:	Fusarium Head Blight Research
2000 ARS Award Amount:	\$32,195

Project

Program Area	Project Title	Requested Amount
Biotechnology	Study trichotheccene resistant genes from wheat.	\$35,000.00
	Requested Total	\$35,000.00¹

Principal Investigator

Date

¹ Note: The Requested Total and the Award Amount are not equal.

PI: Nancy Alexander

Grant:

Project 1: Study trichothecene resistant genes from wheat.

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

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 engineering fungal-resistant lines of wheat and barley. We are making cDNA libraries from infected/non-infected Frontana wheat heads as well as from wheat tissue culture exposed/not exposed to toxin. Toxin-degrading genes in these libraries will be isolated by transforming toxin-sensitive yeast, plating onto toxin-containing medium, and selecting colonies that grow. The cloned genes will then be sequenced and gene function analyzed.

We have started the search for toxin-resistance genes by using the moderately susceptible wheat variety Frontana. We have found that if plants that have been exposed to the fungus *Fusarium* are used for the development of cDNA libraries, then only fungal toxin resistance genes are isolated in our screening program. Therefore, we have used tissue cultures of Frontana that have been exposed to the trichothecene DON and have made cDNA libraries. We have developed a yeast system suitable for screening large numbers of yeast transformants to select out plasmids carrying toxin resistant genes. We are also working on developing a transformation system for the unicellular plant, *Chlamydomonas*, for use as a potential plant screening system.

2. What were the most significant accomplishments?

We have developed a sensitive system, using trichothecene-sensitive yeast, to screen large numbers of plasmids for the selection of toxin resistance genes. Using cDNA libraries made from tissue cultures of Frontana exposed to DON, we have identified two plasmids carrying genetic material capable of providing toxin resistance to yeast. One clone contains an entire gene and a BLAST search has identified a gene with 93% homology. The other clone contains the 3' end of a gene and this sequence is 92% homologous to the 3' end of a gene listed in GenBank. To ensure that the cloned DNA does indeed carry the resistance factor, the plasmids have been reisolated and re-introduced into the sensitive yeast and expression of toxin resistance has been confirmed.

We have also developed a model plant system, using *Chlamydomonas reinhardtii*, for screening toxicity of trichothecenes. We have found that hydroxylated trichothecenes are more toxic to *Chlamydomonas* than are acetylated compounds, suggesting that genes involved in acetylation might serve as useful detoxification mechanisms.

PI: Nancy Alexander

Grant:

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.

Ziegenhorn, S. 2000. Phytotoxicity of selected trichothecenes using *Chlamydomonas reinhardtii* as a model system and the isolation of trichothecene resistant genes from wheat. Presentation to Bradley University, Peoria, IL.

Alexander, N. J., McCormick, S.P., and Ziegenhorn, S.L. 2000. Phytotoxicity of selected trichothecenes using *Chlamydomonas reinhardtii* as a model system. *Natural Toxins*. 8:1-5.

Alexander, N. J., McCormick, S.P., and Ziegenhorn, S.L. 2000. Reduced virulence of *Fusarium graminearum* mutants deficient in TRI101 transacetylase activity. National Fusarium Head Blight Forum, Cincinnati, OH