U.S. Wheat and Barley Scab Initiative
Annual Progress Report
September 18, 2000

Cover Page

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Year:	**FY2000**
Grant Number:	
Grant Title:	**Fusarium Head Blight Research**
Amount Granted:	**$33,000.00**

Project

Program Area	Objective	Requested Amount
Biotechnology	Study trichothecene resistant genes from wheat.	$35,000.00
	Requested Total	$35,000.00[1]

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Principal Investigator Date

[1] Note: The Requested Total and the Amount Granted are not equal.

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. 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.

2. Please provide a comparison of the actual accomplishments with the objectives established.
We have established a yeast system suitable for screening for plasmids carrying toxin resistance genes. This has enabled us to screen large numbers of transformants in a relatively short period of time. We are also working on developing a model plant system, using the unicellular plant *Chlamydomonas reinhardtii*, for transformation studies for screening toxin resistant genes. The first phase of development has shown that *Chlamydomonas* is sensitive to trichothecenes and should serve as a system for screening plasmids carrying toxin resistant genes. From our screening of wheat cDNA libraries, we have two clones that contain genetic material that confer toxin resistance to our toxin-sensitive yeast strain. 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. Attempts to isolate the entire gene have, as yet, been unsuccessful. 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. The yeast strain, carrying the plasmid with one entire gene, is presently being used in toxin intermediate feeding studies in an attempt to determine function. The cloned intact gene is also being inserted into a plasmid behind the maize ubiquitin gene. These accomplishments cover all of the objectives established in our proposal. Due to the exciting potential of these two genes, we have chosen to keep their identity anonymous at this time while we pursue patent protection.

3. What were the reasons established objectives were not met? If applicable.
The entire sequence of the second gene is not known due to the recalcitrance of obtaining a 5' end using a 5' RACE kit. Perhaps we are not using the best primers, perhaps the RNA pool does not contain the full length message, perhaps the gene is too large for us to pull it out using the 5' RACE methodology. We are presently trying other methods to obtain the full length gene.

4. What were the most significant accomplishments this past year?
We have developed a sensitive system, using trichothecene-sensitive yeast, to screen large numbers of plasmids for the selection of toxin resistance genes. We have also developed a model plant system, using *C. reinhardtii*, for screening toxicity of trichothecenes. And, we have isolated, cloned, and identified two wheat genes that confer toxin resistance to a toxin sensitive yeast.

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., Ziegenhorn, S., Muehlbauer, G., McCormick, S., and Kurtz, B. 1999. Isolation of trichothecene resistant genes from the wheat cultivar Frontana. National Fusarium Head Blight Forum, Sioux Falls, SD.

Wood, M., Comis, D., Hardin, B., McGraw, L., and Stelljes, K. 1999. "Fighting Fusarium", Agricultural Research (USDA/ARS). June.

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.

We will be evaluating the wheat genes for effectiveness in our model system and are pursuing the possibility of filing for patent protection on their toxin resistance functionality.