U.S. Wheat and Barley Scab Initiative
FY00 Final Performance Report (approx. May 00 – April 01)
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Cover Page

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Grant Number:	
Grant Title:	**Fusarium Head Blight Research**
2000 ARS Award Amount:	**$48,780**

Project

Program Area	Project Title	Requested Amount
Biotechnology	Targeting of antifungal proteins to inhibit growth of Fusarium graminearum in barley.	$50,000.00
	Requested Total	$50,000.00[1]

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Principal Investigator Date

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

Project 1: Targeting of antifungal proteins to inhibit growth of Fusarium graminearum in barley.

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

Currently, there are no known barley lines with biochemical resistance to *Fusarium*. In order to save barley as a crop in certain growing regions, it may be necessary to introduce *Fusarium* resistance through genetic transformation. Many technical obstacles must be overcome before stable pathogen-resistant transgenic cereals can be introduced into the field. It will be futile to cultivate *Fusarium*-resistant lines in which the resistance gene(s) is expressed constitutively. Antifungal proteins must be expressed in the most appropriate tissue and subcellular compartment to avoid placing a metabolic burden on the plant and to minimize pressures which select for resistant pathogen strains. Until the biology of Fusarium is better understood, we assume that an antifungal protein must be expressed in the path of the initial Fusarium growth. This would therefore be the intracellular space between the plasma membranes and cell walls of epidermal cells, most likely of the lemma/palea and pericarp epithelium. We have made several reporter gene constructs containing possible thionin targeting sequences, delivered these into barley tissue by particle bombardment and analyzed expression and secretion of the reporter gene protein (GUS).

To target antifungal gene products effectively, it is necessary to understand the process of *Fusarium* infection. We are continuing studies with a strain of Fusarium expressing the green fluorescent protein. With this, we are developing a method whereby the earliest events in the infection process can be viewed by confocal microscopy.

Results from these studies will be incorporated with ongoing research, which has produced a lemma-specific promoter and several other floret-specific candidate genes. The planned product is a precise antifungal gene-targeting vector that can be used in both barley and wheat.

2. What were the most significant accomplishments?

Synthesized gene constructs containing several possible subcellular targeting signals in which the targeting signal is adjoined to a GUS reporter gene -
HTH (thionin) components for these constructs were subcloned from our full-length α-hordothionin clone, Thio12. GUS was subcloned from the GUS gene in pAHC25. An oligonucleotide primer encoding KDEL (vacuolar targeting signal) has been synthesized and placed in an expression vector. The completed constructs were inserted into pAHC25 behind the Ubi promoter and intron.

Delivered the constructs into barley pericarp tissue through particle bombardment and assessed the effectiveness of targeting signals through GUS staining -
The targeting vectors were delivered into doughy stage lemmas by particle bombardment. However, it was too difficult to analyze targeting to the extracellular space in this tissue. We therefore switched to the use of etiolated seedling coleoptiles. Subcellular targeting constructs were tested by transient expression assays. Although weak, all secreted GUS activity (MUG assay) into the apoplast, whereas no MDH activity was secreted (cell leakage control). The unaltered GUS control did not secrete, and had only localized GUS staining spots. In all of the non-control bombardments, the entire coleoptile turned blue after GUS staining. This indicated that any expressed protein containing the thionin signal peptide at the 5' end would be rapidly secreted out of the cell. Although this is an encouraging result, more studies are needed to determine whether artifacts could have caused these results.

Conduct confocal microscopy studies with Gfp/Fusarium strain to determine the subcellular route of Fusarium invasion -
Several methods were explored for visualizing the initial Fusarium penetration events. Standard paraformaldehyde fixing and paraffin embedding techniques failed because they destroyed GFP fluorescence. Cryostat sectioning preserved fluorescence, but the spore attachments and cell structure were apparently disrupted during sectioning. A much simpler technique has been devised whereby lemmas are infected for 6 h and then manually peeled into fine tissue strips. This results in a high frequency of conidiospore attachments. These were viewed by confocal microscopy, but excessive "flare" prevented viewing at the epidermis. We are now experimenting with the use of tissue strips set into enclosed deep well slide chambers. The images will allow a determination of the growth and penetration pattern of *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.

Sathish P, Federico ML, Fu J, Skadsen RW, Kaeppler HF (2000) Targeted expression of antifungal protein genes in barley. 6th Internatl. Cong. on Plant Mol. Biol., Quebec, Canada. June 2000. Plant Mol. Biol. Rep. 18:2 (suppl), Abs. S03-104.

Skadsen RW, Sathish P, Kaeppler HF (2000) Expression of thaumatin-like permatin PR-5 genes switches from the ovary wall to the aleurone in developing barley and oat seeds. Plant Sci 156:11-22.

Skadsen RW, Sathish P, Fu J, Federico ML, Kaeppler HF (2000) Targeted expression of a thionin gene to inhibit growth of Fusarium graminearum in barley. Proc. of the 2000 National Head Blight Forum. pp 46-49.