U.S. Wheat and Barley Scab Initiative
FY02 Final Performance Report (approx. May 02 – April 03)
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Cover Page

PI:	**Ronald W. Skadsen**
Institution:	**USDA-ARS**
Address:	**Cereal Crops Research Unit** **501 N. Walnut St.** **Madison, WI 53705**
E-mail:	**rskadsen@facstaff.wisc.edu**
Phone:	**608-262-3672**
Fax:	**608-264-5528**
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Grant Number:	**NA**
Grant Title:	**Fusarium Head Blight Research**
FY02 ARS Award Amount:	**$ 47,486**

Project

Program Area	Project Title	USWBSI Recommended Amount
BIO	Genetic transformation of barley with an altered hordothionin gene.	$48,673
	Total Amount Recommended	**$48,673**

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Principal Investigator Date

Project 1: Genetic transformation of barley with an altered hordothionin gene.

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 affected 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 is first necessary to learn the requirements for strong redirected expression of antifungal genes, such as the endosperm-specific *Hth* gene encoding hordothionin. 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. To do this most effectively, it is also necessary to understand the process of *Fusarium* infection. The proposed research incorporates subcellular targeting research and research on *F. graminearum* infection. 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 long-range goal is to produce an antifungal gene/targeting vector that can be used in both barley and wheat.

2. What were the most significant accomplishments?

-Progress has been made toward the expression of *Hth* in non-endosperm tissue. Previously, *Hth* mRNA accumulated in transformants, but the corresponding HTH protein did not. Rather than to await the results of stable transformation with new *Hth* vectors, a test vector was constructed in which components of *Hth* were ligated in translational fusion with a hexa-alanine swivel, followed by a green fluorescent protein (*gfp*) reporter gene. This allows the translatability of the *Hth* sequences to be tested by transient particle bombardment of the target organ (lemmas, in this case). By removing the signal sequence and altering several less frequently used codons, strong *gfp* expression was seen. Transformants must now be produced to determine whether HTH protein accumulates.
-Two tissue-specific promoters were produced. *Lem2* should direct antifungal gene expression to the lemma/palea, and *Ltp6* should direct expression to the pericarp epithelium. Both sites are normally colonized by Fusarium. A previous lemma-specific promoter, *Lem1*, was used successfully by Ann Blechl's lab to get lemma-specific expression in wheat. This indicates that *Lem2* and *Ltp6* may also have utility in wheat.
-Metabolic profiling studies of pericarp tissue were conducted in collaboration with Cynthia Henson's lab. Initial results indicate that low MW carbohydrates, necessary for developing turgor pressure during hyphal penetration, are produced in the first 24 h after infection.
-H_2O_2 production was co-localized with the early growth of F. graminearum (transformed with *gfp*) on barley pericarp. This demonstated that the pericarp produces one of the early components of antifungal response. Further studies may reveal whether barley lacks an additional critical downstream response needed for resistance.

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.

1. Fu, J., Abebe, T., Federico, M., Kaeppler, H.F., Skadsen, R.W. Transformation and expression of an altered antifungal protein hordothionin gene in transgenic barley and oat. Abs. S-82. 10th International Assoc. of Plant Tissue Culture and Cell Biology. Orlando, FL, June 2002.

2. Skadsen, R.W., Abebe, T., Federico, M.L., Kaeppler, H.F., Henson, C.A. Strategies for combating Fusarium through gene expression targeting, metabolic profiling and signaling analysis. Proc. of 2002 National Fusarium Head Blight Forum. Erlanger, KY, Dec. 7-9, 2002.

3. Fu, J., Abebe, T., Federico, M.L., Kaeppler, H.F., Skadsen, R.W. Expression of a seed-specific antifungal protein hordothionin gene is inhibited in the leaves of transgenic barley and oat at the pre- and post-translational levels. Abs. 155. Ann. Mtg. Am. Soc. of Plant Biology, Denver, Aug. 2002.

4. Abebe, T., Fu, J., Federico, M.L., Skadsen, R.W., Kaeppler, H.F. Cloning the lemma- and palea-specific Lem2 gene in barley. Abs. 61. Ann. Mtg. Am. Soc. of Plant Biology, Denver, Aug. 2002.