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

PI:	**John Sherwood**
Institution:	**Montana State University**
Address:	**Dept. of Plant Sciences and Plant Pathology** **305 AgBioscience Bldg.** **Bozeman, MT 59717-3500**
E-mail:	**sherwood@montana.edu**
Phone:	**406-994-5153**
Fax:	**406-994-7600**
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Grant Number:	**58-5442-2-314**
Grant Title:	**Fusarium Head Blight Research**
FY02 ARS Award Amount:	**$ 53,659**

Project

Program Area	Project Title	USWBSI Recommended Amount
BIO	Control of Scab with Puroindoline-Containing Transgenic Wheat and Barley.	$55,000
	Total Amount Recommended	**$55,000**

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Principal Investigator Date

Project 1: Control of Scab with Puroindoline-Containing Transgenic Wheat and Barley.

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

The long term goal of this research is to apply the use of the anti-microbial puroindoline proteins (PINs) to plant disease resistance. The goal of the research proposal was to confirm and strengthen the evidence that the puroindolines can provide control of wheat and barley scab caused by *Fusarium graminearum* and *F. culmorum*. The specific aims included:

a. Confirm of field and greenhouse results using wheat transformed with the *pin* genes with *F. culmorum* and extend those experiments to *F. graminearum.*
b. Determine the effect of puroindolines on *in vitro* growth of *F. graminearum*.
c. Start the process to transform barley with the wheat *pin* genes.

2. What were the most significant accomplishments?

Objective a. During the funding period, we performed experiments in which *Fusarium culmorum* and *F. graminearum* were used to inoculate wild-type HiLine spring wheat, a transgenic control with which HiLine was transformed with the Bar resistance gene, and Line 82, which is HiLine transformed with *pinB*. The *F. culmorum* experiment was performed twice and the *F. graminearum* experiment was performed 3 times. In each case, the level of severity was reduced when compared to either HiLine or the transgenic control with *pinB* present. This was manifested primarily in a reduction of the most sever infections (40-100% of spikelets infected) with a concomitant increase in spikelets with less severe infections.

Objective b. Using an in vitro plate growth assay in which extracted puroindoline protein is added to the culture medium, *F. graminearum* growth was inhibited by approximately 30%. This is greater than the growth inhibition observed with *F. culmorum* (about 18%) under similar conditions.

Objective c. We initiated a program to transform barley with either *pinA* or *pinB*. There is little known natural tolerance to head scab in barley, so if the puroindolines were proven to be useful components of a scab control program, it would be wise to begin the process immediately. First, the transformation conditions optimal of transformation of the barley variety Harrington were determined. During that process, 582 barley calli derived from embryos were bombarded with *pin A*. From that sample, 14 hygromycin-resistant plants were regenerated. Two of those were determined to contain the *pinA* gene by PCR of leaf tissue. One of those plants produced seed. The T1 generation plants have been shown by PCR to contain *pinA*.

An additional 4,000 barley calli have been bombarded with either *pin A* or *pin B*. From the earlier bombardments, (approximately 2,000 calli), 24 hygromycin-resistant plantlets have been regenerated. They are not sufficiently grown at this time to determine whether they contain the *pin* genes

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. Gerhardt, S.A., C. Balconi and J.E. Sherwood. 2002. Control of *Fusarium* Scab with Puroindoline-Containing Transgenic Wheat. Annual Meeting of the American Phytopathological Society, Milwaukee, July 27-31 (Abstract).

2. Giroux, M., J. Sherwood and B. Beecher. 2002. Puroindolines confer grain texture changes and anti-fungal properties in transgenic cereals. American Association of Cereal Chemists. Montreal, Oct 13-17 (Abstract).

Gerhardt, S.A., C. Balconi and J.E. Sherwood. 2002. Control of *Fusarium* Scab with Puroindoline-Containing Transgenic Wheat. National Fusarium Head Blight Forum. Erlanger Ky. Dec 7-9. (Abstract).

Giroux, M.J., T. Sripo, S. Gerhardt, and J. Sherwood. 2003. Puroindolines: their role in grain hardness and plant defense. Biotech. Genet. Eng. Rev. Vol 20: in press.