USDA-ARS / USWBSI
FY03 Final Performance Report (approx. May 03 – April 04)
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Cover Page

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Year:	**FY2003 (approx. May 03 – April 04)**
FY03 ARS Agreement ID:	**58-5442-2-314**
FY03 ARS Agreement Title:	**Control of scab with puroindoline-containing transgenic barley.**
FY03 ARS Award Amount:	**$ 53,883**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	Control of scab with puroindoline-containing transgenic barley.	$ 53,883
	Total Amount Recommended	**$ 53,883**

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Principal Investigator Date

* BIO – Biotechnology
CBC – Chemical & Biological Control
EDM – Epidemiology & Disease Management
FSTU – Food Safety, Toxicology, & Utilization
GIE – Germplasm Introduction & Enhancement
VDUN – Variety Development & Uniform Nurseries

Project 1: ***Control of scab with puroindoline-containing transgenic 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 described in this proposal is to confirm and strengthen the evidence that the puroindolines can provide control of wheat and barley scab caused by *Fusarium graminearum* (F.g.) and *F. culmorum* (F.c.). The specific objectives include:

1. Repeat greenhouse and field experiments to confirm increased tolerance of puroindoline-transformed wheat inoculated with *F. culmorum* and *F. graminearum*.
2. Generate and begin evaluation of transgenic plants.

2. What were the most significant accomplishments?

Objective 1. Field experiments were performed in the summer of 2003 with the following variables: 2 organisms *(*F.g. and F.c.), 2 inoculation levels and two planting dates. The percentage of infected florets was determined for 20 plants with combinations of variables. With 100,000 F.g. spores/ml inoculum, the percentage of infected florets was reduced by 27% (12.9% in *pinb*-containing line 82 vs. 17.7 % in wild-type HiLine) and 80% with F.c. (8.0% in line 82 vs 39.6% in HiLine). With 50,000 spores/ml inoculum, infection was reduced by 86% (3.7% vs 28.0%) with F.g. and by 82% (5.2% vs 29.3%) with F.c. A second planting two weeks after the first using 100,000 spores/ml inoculum had infected florets reduced by 73% with F.g. (6.2% vs 23.3%) and 69% with F.c. (6.5% vs 20.8%). Field samples were tested for toxin levels (DON), which were directly proportional to disease level. Greenhouse trials showed reductions of 48% and 34% with F.g. (32.9% vs 63.3%) and F.c. 12.0% vs 18.1%) respectively. We also tested several *pinA* or *pinB* transformants of the wheat variety Bob White. Seed from the T1 generation showed 30% and 52 % reductions in percent floret infection with F.g and F.c, respectively. However, seed from the T5 generation lost tolerance, indicating that the transformation events were unstable.

Objective 2. Of 4,500 calli of the barley variety Harrington bombarded with the *pinA* gene driven by the maize ubiquitin promoter and the hygromycin-resistance gene (*hph*), thirty hygromycin-resistant barley plants regenerated and were transplanted successfully to soil. Initial analyses by PCR performed on the regenerated To plants indicated several positives for both *pinA* and *hph*. Stable integration will be confirmed on T1-generation plants.

Four seed lots from line 82 (*pin B*- transformed HiLine), which exhibits increased resistance to *Fusarium* head scab, were back-crossed to the wild-type parent and then allowed to self. The back-cross was performed to select for the positive characteristics of growth, seed set, yield, seed size and hardness seen in HiLine, while retaining the *pin B* gene. Selfed plants from the F-1 seed are currently almost ready to harvest. A random seed lot from each plant will be planted in the greenhouse and leaves from each subsequent seedling will be PCR-assayed for the presence of the *pin B* gene. Those testing positive will be allowed to mature and will be compared to HiLine for the other characteristics described above. Seed from the best progeny will be grown and re-challenged with *F. graminearum* and *F. culmorum.*

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 you grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Sherwood and Giroux. 2003. Controlling scab with puroindoline-expressing wheat and barley. In:Canty, S.M., Lewis, J., Siler, L. and Ward, R.W. (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2003 Dec13-15; Bloomington MN. East Lansing: Michigan State University. pp. 40.

Giroux, M.J., T. Sripo, S. Gerhardt, and J. Sherwood. 2003. Puroindolines: their role in grain hardness and plant defense. Biotech. Genet. Eng. Rev. 20:265-278.