

USDA-ARS / USWBSI
FY03 Final Performance Report (approx. May 03 – April 04)
July 15, 2004

Cover Page

PI:	Bruce Bleakley
Institution:	South Dakota State University
Address:	Department of Biology & Microbiology NPBL-Box 2140D, Room 252 Brookings, SD 57007
E-mail:	Bruce Bleakley@sdstate.edu
Phone:	605-688-5498
Fax:	605-688-5624
Year:	FY2003 (approx. May 03 – April 04)
FY03 ARS Agreement ID:	59-0790-0-061
FY03 ARS Agreement Title:	Management of Fusarium Head Blight with Biological Control Agents.
FY03 ARS Award Amount:	\$ 6,829

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
CBC	Management of Fusarium Head Blight with Biological Control Agents.	\$ 6,829
	Total Amount Recommended	\$ 6,829

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: Management of Fusarium Head Blight with Biological Control Agents.

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

- a) We conducted studies to see whether one of our *Bacillus* strains, strain 1BA, produces iturin, a cyclic lipopeptide antibiotic that has antifungal properties. Using an established procedure, we acid-precipitated broth culture supernatant and, using reversed phase HPLC, injected the sample onto a Phenomenex column to see if iturin peaks could be observed/resolved. Using an iturin A standard, we know that the HPLC system can detect iturin A. Samples from culture supernatant of strain 1BA produced peaks, but not at the same exact retention time as for the iturin A standard. Strain 1BA may produce iturin A, but our extraction procedure might alter the antibiotic's structure so that its retention time is changed. Or strain 1BA might produce an iturin that is structurally different enough from iturin A so that its retention time is different from the iturin A standard. To help resolve this, we will modify the extraction procedure (using a lower concentration of acid to precipitate material from the supernatant). Also, we will confer with chemists on our university campus such as Dr. Duane Matthees to make sure our procedure is optimal.
- b) Our bacterial strain 1BA may produce an iturin other than iturin A. The only standard iturin we can find commercially is iturin A. There are other iturin class antibiotics that would be useful for use in our study as standards for HPLC, but there is no readily available source to obtain them from. To help resolve this, we will obtain one or more *Bacillus* strains that are known to produce iturin class antibiotics, grow them in the same manner as our strain 1BA, and analyze their culture supernatant in the same manner by HPLC. These bacterial strains will serve as further positive controls (in addition to the commercial iturin A), to verify that our procedure is able to detect iturin A from culture supernatants.
- c) Further trials will be conducted in the greenhouse and field plots with the *Bacillus* strains we have isolated and characterized in the laboratory, to evaluate their efficacy in controlling FHB and/or reducing DON levels.

2. What were the most significant accomplishments?

- a) We verified that iturin A from a commercial supplier (Sigma) can antagonize growth of *Fusarium graminearum* in laboratory plate assays.
- b) We modified our defined culture medium for growth of our *Bacillus* biocontrol strains (reducing the levels of iron and copper, and adjusting the pH after autoclaving) so that the sterile defined broth is now very clear (transparent), making assay of bacterial growth easier than with the previous defined broth formulation that developed precipitate after autoclaving. Growth of bacteria in this clear defined broth formulation is as good or better than growth of bacteria in the original defined broth formulation.
- c) We verified that application of our biocontrol bacteria resulted in reduction of DON levels in South Dakota field plots in some situations.
- d) We continued to work in cooperation with D. Schisler, G. Yuen, M. Draper, and G. Bergstrom on the biocontrol of FHB. In addition to our own *Bacillus* biocontrol strains, we grew broth cultures of biocontrol bacteria from Schisler, Yuen, and Bergstrom for field application. We are working with Yuen to see if his bacterium can be applied in combination with one of ours to provide better control of FHB and/or reduce DON. We are enumerating populations of all applied biocontrol bacteria in South Dakota field plot trials to see if they survive and/or grow after application to wheat and barley..

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.

Baye, N.L., Bleakley, B.H., Draper, M.A., and Calli, E.. 2003. Laboratory studies with purified iturin A, and with *Bacillus spp.* grown in complex and defined growth media, to ascertain the identity and ability of compounds that inhibit *Fusarium graminearum*. In Canty, S.M., Lewis, J., and Ward, R.W. (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2003 Dec. 13-15; Bloomington, MN. East Lansing: Michigan State University. p. 60.

Draper, M.A., Bleakley, B.H., Baye, N.L., Ruden, K.R., Calli, E., and Schilling, S.M. 2003. 2003 Uniform trials for the performance of biological control agents in the suppression of Fusarium Head Blight in South Dakota. In Canty, S.M., Lewis, J., and Ward, R.W. (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2003 Dec. 13-15; Bloomington, MN. East Lansing: Michigan State University. pp. 67-68.

Yuen, G.Y., Jochum, C.C., Bleakley, B.H., Ruden, K.R., Draper, M.A., Schisler, D.A., Zhang, S., Boehm, M.J., Lipps, P.E., and Bergstrom, G.C. 2003. Cooperative multistate field tests of biological agents for control of Fusarium Head Blight in wheat and barley. In Canty, S.M., Lewis, J., and Ward, R.W. (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2003 Dec. 13-15; Bloomington, MN. East Lansing: Michigan State University. pp. 113-115.

Bleakley, B.H. 2004. Biological control of foliar and head diseases of wheat. AD-421 Progress Report (CRIS Report).