

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY07 Final Performance Report (approx. May 07 – April 08)
July 15, 2008**

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
Fiscal Year:	2007
USDA-ARS Agreement ID:	59-0790-5-077
USDA-ARS Agreement Title:	Management of Fusarium Head Blight with Biological Control Agents.
FY07 ARS Award Amount:	\$ 9,756

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
CBCC	Management of Fusarium Head Blight With Biological Control Agents.	\$9,756
	Total Award Amount	\$ 9,756

Principal Investigator

Date

* CBCC – Chemical, Biological & Cultural Control
EEDF – Etiology, Epidemiology & Disease Forecasting
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GET – Genetic Engineering & Transformation
HGR – Host Genetics Resources
HGG – Host Genetics & Genomics
IIR – Integrated/Interdisciplinary Research
PGG – Pathogen Genetics & Genomics
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) A major part of the project is to quantify numbers of bacterial biocontrol agents (BCAs) (for our project, selected *Bacillus spp.*) after they are sprayed onto heads of wheat and barley. For the 2006 and 2007 growing seasons, we have counts of the BCA *Bacillus* 1BA. More than two years of BCA population data are needed to better understand how 1BA behaves in the field after spray application.
--More data are sought by continuing to do similar BCA population counts via field plot work at Brookings, SD in the summer of 2008, to provide a larger data set to better gauge population fluctuations of 1BA after it is sprayed onto wheat and barley heads in the field. We are also involved in the Uniform Biological Control tests that are part of the Scab Initiative.
- b) There is still a need for evidence that 1BA and related BCAs produce metabolites such as lipopeptides (such as iturin and surfactin) on the grain heads. These lipopeptides are thought to be the major mechanism whereby 1BA and several other *Bacillus spp.* used as BCAs inhibit growth of *F. graminearum*, reduce FHB, and/or reduce DON levels.
--Resolution of the problem is being sought by studies done over the last year, and also in progress with Chris Dunlap of USDA-ARS-Peoria, analyzing methanol extracts from grain heads via mass spectrometry (MALDI-TOF) to semi-quantitatively assay the amount of lipopeptide present.
- c) There is also a need for evidence of lipopeptide genes on treated grain heads using PCR.
--Resolution of this is sought by ongoing studies that extract DNA from inoculated grain heads, then use PCR to verify presence of lipopeptide genes in the sample.

**2. List the most important accomplishment and its impact (how is it being used?).
Complete all three sections (repeat sections for each major accomplishment):**

Accomplishment: a) Field plot population studies of 1BA:

Populations of *Bacillus* strain 1BA were studied during 2006 and 2007 in field plots at Brookings, SD after spray application at anthesis. Field plot treatments in 2006 used cells of 1BA grown in several different broth media; and including the fungicide Folicur with a spreader or sticker in some treatments. Field plot treatments in 2007 used only tryptic soy broth cultures of 1BA by itself; and 1BA + Prosaro (a fungicide mix) + Induce NIS (nonionic surfactant). Population counts were done over a 20 day period via MPN technique. Controls not receiving 1BA inoculation gave low cell counts in both years, no higher than about 3.5×10^2 CFU/g fresh plant weight. Numbers on barley heads were low, comparable to control treatments, throughout the 2007 study. In both years, there was a rapid decline in numbers of 1BA on wheat heads in the first few days after spraying, followed by an increase in numbers in some treatments to levels around 10^4 CFU/g fresh plant weight. Treatments using 1BA grown in defined broth yielded lower numbers of 1BA on wheat heads over time than treatments using 1BA grown in complex broth. In both years, some treatments showed a major increase in 1BA numbers around 10 days after spraying. In 2007 in the treatment with 1BA alone, vegetative cell counts peaked around day 10, then declined sharply, followed by a second increase in numbers by day 20, with an increase in endospore numbers. Compared to the treatment with 1BA alone, in the treatment combining 1BA with Prosaro and Induce NIS, population peaks shifted in time. Peak numbers of vegetative cells occurred at about

PI: Bleakley, Bruce

USDA-ARS Agreement #: 59-0790-5-077

day 6 then declined. As vegetative numbers of 1BA in this treatment declined, endospore numbers increased.

Impact: Two years of field plot data showed similar behavior of 1BA after spray application onto wheat heads at anthesis. This work documents that our *Bacillus* strain 1BA persists and grows on wheat heads after application, which is considered to be an important trait for a BCA that controls a pathogen by producing antibiotics such as lipopeptides.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:

About 10 days after wheat anthesis, there appears to be a predictable increase in numbers of 1BA sprayed onto heads at anthesis. Survival and growth of the BCA on wheat heads is greatly affected by the medium used to grow BCA inoculum, and by fungicides and adjuvants in spray. Population fluctuations of 1BA on barley heads are different from that on wheat heads.

Accomplishment: b) Evidence of lipopeptide in wheat heads after spraying:

Methanol extracts of wheat heads inoculated with 1BA were processed and sent to Chris Dunlap's laboratory. Initial results were negative for presence of lipopeptide. Dunlap's analytical protocol works well with *Bacillus* broth populations of 10^7 CFU/ml or higher; whereas our samples from inoculated wheat heads were shown by MPN counts to have 1BA numbers of about 10^4 CFU/g. We are working with Dunlap to establish the lower limit of detection for numbers of lipopeptide-producing *Bacillus* his technique can detect.

Impact: Establishing a lower limit of detection for number of lipopeptide-producing *Bacillus* that can be detected by MALDI-TOF analysis is being sought, to see if cell numbers lower than that in dense broth cultures can be detected.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:

Lower limit of detection for numbers of lipopeptide-producing BCAs on plant material is not certain, but will become more certain after this work proceeds further.

Accomplishment: c) Evidence of lipopeptide genes on inoculated grain heads using PCR: DNA extracted from pure cultures of 1BA in the laboratory show amplified bands after PCR that indicate presence of lipopeptide genes. Work later this year will focus on using the same methodology to look for similar bands in amplified DNA extracted from inoculated grain heads.

Impact: Pure culture work with strain 1BA verifies that it has the expected bands indicative of lipopeptide genes.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:

Detection of lipopeptide genes in DNA extracted from grain heads treated with lipopeptide-producing BCAs should be possible, once PCR parameters are optimized.

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.

- Morgan, J., B.H. Bleakley, and C.A. Dunlap. 2007. Field and laboratory studies to monitor cell populations, lipopeptides and lipopeptide genes of *Bacillus* 1BA, a biocontrol agent active against Fusarium Head Blight. Abstract, pg. 106. In: S.M. Canty, A. Clark, D. Ellis, and D. Van Sanford (eds.), Proceedings of the 2007 National Fusarium Head Blight Forum, Dec. 2-4, 2007, Kansas City, Missouri. East Lansing; Michigan State University.
- Ruden, K.R., B.H. Bleakley, and B.E. Ruden. 2007. 2007 Uniform trials for the performance of biological control agents in the suppression of Fusarium Head Blight in South Dakota. Abstract, pg. 136. In: S.M. Canty, A. Clark, D. Ellis, and D. Van Sanford (eds.), Proceedings of the 2007 National Fusarium Head Blight Forum, Dec. 2-4, 2007, Kansas City, Missouri. East Lansing; Michigan State University.
- Yuen, G.Y., C.C. Jochum, S. Halley, G. Van Ee, V. Hoffman, and B.H. Bleakley. 2007. Effects of spray application methods on biocontrol agent viability. Pp. 149-152. In: S.M. Canty, A. Clark, D. Ellis, and D. Van Sanford (eds.), Proceedings of the 2007 National Fusarium Head Blight Forum, Dec. 2-4, 2007, Kansas City, Missouri. East Lansing; Michigan State University.
- Yuen, G.Y., C.C. Jochum, K.R. Ruden, J. Morgan, B.H. Bleakley, and L.E. Sweets. 2007. Results from the 2007 standardized evaluation of biological agents for the control of Fusarium Head Blight on wheat and barley. Pp. 153-157. In: S.M. Canty, A. Clark, D. Ellis, and D. Van Sanford (eds.), Proceedings of the 2007 National Fusarium Head Blight Forum, Dec. 2-4, 2007, Kansas City, Missouri. East Lansing; Michigan State University.
- Bleakley, B.H., and J. Morgan. 2008. Field Plot Studies of Survival and Growth of the Biocontrol Agent *Bacillus* Strain 1BA Applied To Wheat Heads. Poster abstract: *In* Abstracts of the 108th General Meeting of the American Society for Microbiology; June 1-5, 2008; Boston, MA.
- Baye, N.L. 2007. *Bacillus* strains used for biological control of Fusarium Head Blight: identification, growth studies, and lipopeptide production. Master' thesis, South Dakota State University, Brookings, SD.