USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY06 Final Performance Report (approx. May 06 – April 07)
July 16, 2007

Cover Page

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Fiscal Year:	2006
USDA-ARS Agreement ID:	59-0790-5-077
USDA-ARS Agreement Title:	Management of Fusarium Head Blight with Biological Control Agents.
FY06 ARS Award Amount:	$ 12,139

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Award Amount
CBCC	Management of Fusarium Head Blight with Biological Control Agents.	$ 12,139
	Total Award Amount	**$ 12,139**

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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
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) One major part of 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. The first attempt to do this work was in summer of 2006. Counts of *Bacillus* 1BA were done, using growth media with elevated NaCl concentration (8.5%) and incubation at elevated temperature (47^{0} ℂ), which 1BA tolerates but which inhibits growth of most bacteria native to the grain heads. The results from 2006 showed that BCA numbers varied over time depending on the broth medium the BCAs were grown in, and on the other materials that were co-applied with the BCAs onto grain heads (including Folicur, spreaders/binders, and surfactants). However, variability in the replicates for each treatment was high.
--Resolution of this problem is being sought in 2007 by conducting similar field plot estimates of 1BA numbers, with the difference that incubation conditions are somewhat more selective (50^{o} C incubation, and 10% NaCl in the enumeration medium), to see if these more stringent growth conditions help to reduce the variation between replicate counts of 1BA applied in different treatments. Also, more thorough BCA population counts will be obtained for barley this year than in 2006; and heat pasteurization of grain head material will be conducted throughout the experiment, to better assess if 1BA is in the form of vegetative cells or endospores after it is sprayed onto grain heads. Additionally, several spontaneous rifampicin-resistant mutants of 1BA have been obtained for future studies. The availability of this antibiotic resistance marker for the 1BA mutants will provide an additional tag on the BCA to help allow its recovery from grain heads after application in BCA population studies.

b) Although there is now good evidence that 1BA grows on grain heads after it is sprayed, there is not yet good 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 being done this summer in collaboration with Chris Dunlap of USDA-ARS-Peoria. Grain heads from field plots inoculated with 1BA and other *Bacillus* BCAs are being harvested, then extracted with methanol. Methanol extracts will be sent to Dunlap for analysis by mass spectrometry (MALDI-TOF) to semi-quantitatively assay the amount of lipopeptide present in the different field plot treatments. Studies will be carried out later in 2007 to see if there is evidence for lipopeptide genes on treated grain heads using PCR. Use of real time PCR is planned for early next year, to see if expression of BCA genes coding for lipopeptide can be detected on grain heads inoculated with 1BA and/or other *Bacillus* BCAs.

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: We documented that after it was sprayed onto grain heads, numbers of *Bacillus* strain 1BA varied over time, with growth occurring at certain times and population declines occurring at other times. Different treatments (1BA applied with or without Folicur, spreaders/binders, and surfactants) displayed different BCA population growth patterns over time. Some treatments seemed to foster growth of 1BA in the field more than others.

Impact: Documentation of growth of BCAs after their application is not always available. This work provided documentation that our *Bacillus* strain 1BA does persist and grow on grain 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?
The documented growth of the BCA after its application onto grain heads provides evidence to other biocontrol researchers and other people in the Scab Initiative with an interest in using BCAs that these biological agents can colonize and grow on grain heads after they are applied.

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.

Gill, A. 2006. Development of an isolation method that uses tolerance to salt and heat, to select for *Bacillus* strain 1BA, a biocontrol agent used to control Fusarium Head Blight. M.S. thesis, South Dakota State University, Brookings, SD.

Gill, A.L., and B.H. Bleakley. 2006. The use of chemical and physical stressors, 8.5% NaCl and 47^{o} C, to assay populations of a *Bacillus* strain used to control Fusarium Head Blight on wheat heads in field plots. Abstract, p. 9. *In* S.M. Canty, A. Clark, and D. Van Sanford (Eds.), Proceedings of the 2006 National Fusarium Head Blight Forum. 2006 Dec. 10-12; Research Triangle Park, NC. University of Kentucky.

Ruden, K.R., B. Bleakley, S.M. Thompson, K. Maxson-Stein, and M.A. Draper. 2006. 2006 Uniform trials for the performance of biological control agents in the suppression of Fusarium Head Blight in South Dakota. Abstract, p. 20. *In* S.M. Canty, A. Clark, and D. Van Sanford (Eds.), Proceedings of the 2006 National Fusarium Head Blight Forum. 2006 Dec. 10-12; Research Triangle Park, NC. University of Kentucky.

Yuen, G.Y., C.C. Jochum, K.R. Ruden, L.E. Sweets, B.H. Bleakley, and M.A. Draper. 2006. 2006 Results from the standardized evaluation of biological agents for the control of Fusarium Head Blight on wheat and barley. Pp. 27-30. *In* S.M. Canty, A. Clark, and D. Van Sanford (Eds.), Proceedings of the 2006 National Fusarium Head Blight Forum. 2006 Dec. 10-12; Research Triangle Park, NC. University of Kentucky.

Bleakley, B.H. 2007. Biological control of foliar and head diseases of wheat. AD-421 Progress Report (CRIS Report).