USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY08 Final Performance Report (approx. May 08 – April 09)
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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:	2008
USDA-ARS Agreement ID:	59-0790-5-077
USDA-ARS Agreement Title:	Management of Fusarium Head Blight with Biological Control Agents.
FY08 USDA-ARS Award Amount:	$ 14,774

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
MGMT	Optimizing Parameters for Efficacy of Biological Control Agents of FHB.	$9,688
MGMT	Uniform Tests of Biocontrol Agents for Fusarium Head Blight.	$ 5,086
	Total Award Amount	**$ 14,774**

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Principal Investigator Date

* MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
BAR-CP – Barley Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Winter Wheat Region
SWW – Southern Sinter Wheat Region

(Form FPR08)

Project 1: *Optimizing Parameters for Efficacy of Biological Control Agents of FHB.*

1. **What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?**

   a) The optimization of a broth growth medium allowing dense growth of the *Bacillus* strains we are using as biological control agents (BCAs) is very important to successful spray application of the BCAs. In some broth formulations, cells of the BCAs tend to clump together forming macroscopic aggregates or flocs that settle to the bottom of the culture vessel, making spray application of the BCAs onto grain heads very difficult.
   --Resolution of the problem is being sought by our examining some new broth formulations that seem to deter widespread floc formation of BCA cells, and allow the BCAS to grow to high densities.

   b) Availability of a rapid estimate of production of lipopeptide biosurfactants by the BCAs is important to our broth formulation studies. 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. It is hypothesized that the more biosurfactant is produced in the culture broth, the more effective the BCAs will be in deterring FHB and/or reducing DON levels after spraying BCAs onto grain heads.
   --Resolution of the problem is being sought by our examining some quick and simple methods to semi-quantitatively estimate amount of biosurfactant produced by the BCAs in different broth formulations.

2. **List the most important accomplishment and its impact (i.e. how is it being used) to minimize the threat of Fusarium head blight or to reduce mycotoxins. Complete both sections (repeat sections for each major accomplishment):**

   a) **Accomplishment:** We have found that addition of some commercially available vegetable oils to broth media allows our BCAs to grow to high cell density (higher than several other broth formulations we have investigated) without much formation of aggregates or flocs by the bacterial cells. We have sprayed BCAs grown in these oil-amended media onto wheat heads in field plots in Brookings, SD, to see how efficacious they are in preventing development of FHB and/or reducing DON levels.

   Impact: If field plot application of these oil-containing broth formulations results in better control of FHB and/or reduction of DON than previous BCA applications in broth media formulations not containing oil, the oil-containing growth media could result in a commercial product that is affordable and easy to apply for grain producers.

   b) **Accomplishment:** We have found that a rapid and simple droplet collapse assay of culture broth supernatant from broth cultures of our BCAs gives a quick semi-quantitative indication of the amount of lipopeptide produced by our BCAs. The assay indicates that *Bacillus* strain 1BA produces less lipopeptide than *Bacillus* strain 1D3, for example.

Impact: The ability to semi-quantitatively estimate amounts of biosurfactant/lipopeptide produced by different BCAs in different broth formulations will enhance our ability to evaluate the effect of different carbon sources and other medium components on lipopeptide production. We will be able to correlate amounts of lipopeptide produced to the ability of different broth formulations to control FHB and/or reduce DON levels.

Project 2: *Uniform Tests of Biocontrol Agents for Fusarium Head Blight.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) 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. We have data from the 2006 through 2008 growing seasons. With different broth formulations being examined for optimizing BCA application, more than three years of BCA population data are needed to better understand how BCAs including strains 1BA and 1D3 behave in the field after spray application.
--Resolution: More data are sought by continuing to do similar BCA population counts via field plot work at Brookings, SD in the summer of 2009, 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.

b) There is still a need for evidence that strains 1BA and 1D3 produce metabolites such as lipopeptides (such as iturin and surfactin) on the grain heads. These lipopeptides are hypothesized to be the major mechanism whereby our 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. The droplet-collapse test we have started to use in our lab will allow us to more effectively collaborate with Dunlap.

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.

d) There is a continuing need to screen for the efficacy of our BCAs acting alone or in concert with fungicides, to control FHB and/or reduce DON levels in field plot trials.
--Resolution of this is sought by conducting further field plot trials, in South Dakota and elsewhere when the opportunity arises.

2. List the most important accomplishment and its impact (i.e. how is it being used) to minimize the threat of Fusarium head blight or to reduce mycotoxins. Complete both sections (repeat sections for each major accomplishment):

Accomplishment: Field plot trials were conducted in 2008 in both Langdon, North Dakota and Brookings, South Dakota to further assay the efficacy of *Bacillus* strain 1BA. The

Langdon trial involved spray application of 1BA at Feekes 10.51 to Howard hard red spring wheat; and the Brookings trial had spray application at Feekes 10.51 for Briggs spring wheat, and at full head emergence for Robust spring barley. All trials included application of Prosaro plus Induce NIS, alone and in combination with 1BA. In the Langdon hard red spring wheat trial, the co-application of 1BA with Prosaro and Induce NIS resulted in a deoxynivalenol (DON) level of 2.5 ppm, compared to 3.2 ppm for Prosaro and Induce alone; and 4.0 ppm for the untreated control. In the Brookings spring wheat trial, results from application of 1BA alone or with Prosaro plus Induce were not significantly different from untreated controls. However, in the barley trial, co-application of 1BA with Prosaro and Induce resulted in significantly lower DON levels and lower disease incidence in barley than for the untreated control.

Impact: The trials demonstrated that *Bacillus* strain 1BA when co-applied with Prosaro and Induce can sometimes reduce DON levels in grain more than can application of Prosaro and Induce alone.

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.L., and B.H. Bleakley. 2008. Physiologic profiling and carbon source utilization of four *Bacillus* strains used as biological control agents of FHB. Abstract, pg. 44. In: S.M. Canty, A. Clark, E. Walton, D. Ellis, J. Mundell, and D. Van Sanford (eds.), Proceedings of the 2008 National Fusarium Head Blight Forum, Dec. 2-4, 2007, Indianapolis, IN. East Lansing; Michigan State University.

Morgan, J.L., and B.H. Bleakley. 2008. Use of most probable number and PCR methods to estimate populations of *Bacillus* strain 1BA applied to wheat and barley for biological control of FHB. Abstract, pg. 45. In: S.M. Canty, A. Clark, E. Walton, D. Ellis, J. Mundell, and D. Van Sanford (eds.), Proceedings of the 2008 National Fusarium Head Blight Forum, Dec. 2-4, 2007, Indianapolis, IN. East Lansing; Michigan State University.

Ruden, K.R., L.E. Osborne, B.H. Bleakley, J. Morgan, and B.E. Ruden. 2008. 2008 Uniform trials for the performance of biological control agents in the suppression of Fusarium Head Blight in South Dakota. Abstract, pg. 58. In: S.M. Canty, A. Clark, E. Walton, D. Ellis, J. Mundell, and D. Van Sanford (eds.), Proceedings of the 2008 National Fusarium Head Blight Forum, Dec. 2-4, 2007, Indianapolis, IN. East Lansing; Michigan State University.

Jochum, C.C., G.Y. Yuen, G.Y., K.R. Ruden, B.H. Bleakley, J. Morgan, L. Osborne, L.E. Sweets, S. Halley, and K. Kinzer. 2008. 2008 Results from the uniform evaluation of biological agents for the control of Fusarium Head Blight on wheat and barley. Pp. 32-35. In: S.M. Canty, A. Clark, E. Walton, D. Ellis, J. Mundell, and D. Van Sanford (eds.), Proceedings of the 2008 National Fusarium Head Blight Forum, Dec. 2-4, 2007, Indianapolis, IN. 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. Abstract; in Proceedings of the 108th General Meeting of the American Society for Microbiology; Boston, MA. ASM Press.

Bleakley, B.H., and J. Morgan. 2008. Cellulase and xylanase activities of four *Bacillus amyloliquefaciens* strains used as biocontrol agents to antagonize plant pathogenic fungi. Abstract; in Proceedings of the Society for Industrial Microbiology Annual Meeting and Exhibition from August 10-14 in San Diego, CA

If your FY08 USDA-ARS Grant contained a VDHR-related project, include below a list all germplasm or cultivars released with full or partial support of the USWBSI. List the release notice or publication. Briefly describe the level of FHB resistance. If this is not applicable (i.e. no VDHR-related project) to your FY08 grant, please insert 'Not Applicable' below.

Not applicable.