

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY09 Final Performance Report
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Cover Page

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USDA-ARS Agreement ID:	59-0790-5-077
USDA-ARS Agreement Title:	Management of Fusarium Head Blight with Biological Control Agents.
FY09- USDA-ARS Award Amount:	\$ 12,683

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
MGMT	Optimizing Parameters for Efficacy of Biological Control Agents of FHB.	\$ 12,683
	Total Award Amount	\$ 12,683

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
 DUR-CP – Durum 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

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) **Problem:** The optimization of a broth growth medium fostering dense growth of the *Bacillus* strains we use as biological control agents (BCAs) is very important to successful spray application of the BCAs. High cell density, little or no clumping or flocking of cells, and detectable high levels of lipopeptide biosurfactants are desired, to facilitate spray application of the BCAs onto grain heads. We started in 2009 to examine the use of lipid amendments (plant oils) in growth media to promote these properties in BCA broth cultures.

--**Resolution:** We are continuing to optimize composition of broth formulations that encourage the above qualities. Also, physical parameters (including shaking speed and temperature) of broth incubation are being examined to find optimal parameters.

- b) **Problem:** 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. We hypothesize 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: Over the past year, we have begun using a qualitative droplet collapse assay for estimating the amount of biosurfactant produced by the BCAs in different broth formulations. It has demonstrated there are differences in biosurfactant production between the four BCA strains we have used; and that the lipid-amended broth results in increased amounts of biosurfactant production by the BCAs.

--In addition, we continue to collaborate with Dr. Chris Dunlap to quantify the amount of lipopeptides produced by our BCAs in broth; and to determine the identities and structures of the lipopeptides. Dr. Dunlap's work has verified that the lipid-amended broth results in greatly increased amounts of lipopeptides compared to previous broth formulations without lipid amendment.

- c) **Problem:** 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. For the 2009 growing season, it appeared that bacterial counts were extremely low. It was not initially clear if this was due to our use of lipid-amended culture broth, or to some operator error. With our current use of a much better growth medium than we previously used to culture the BCAs, we need more BCA population data 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 on spring wheat at Brookings, SD in the summer of 2010. Preliminary data suggests that the low counts from 2009 work were due to operator error, so that the 2010 data will be important in providing a comparison of BCA population counts with inocula from the new lipid amended media, to compare to the previous years where the inoculums was not grown with lipid amendment.

- d) **Problem:** There is still a need for evidence that strains 1BA and 1D3 produce metabolites such as lipopeptides (iturin and surfactin) on the grain heads. These lipopeptides are

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hypothesized to be the major mechanism whereby our BCAs inhibit growth of *F. graminearum*, reduce FHB, and/or reduce DON levels. Preliminary work we have done with Chris Dunlap using BCA inocula grown without lipid amendment did not detect lipopeptides on grain heads.

--**Resolution:** We will continue to work 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. We suspect the new broth formulation for BCA inocula will improve the chance of detecting lipopeptides on inoculated grain heads.

- e) **Problem:** There is a need for evidence of lipopeptide genes on treated grain heads using PCR. Attempts we have made to date have not revealed this.

Resolution: We will increase the number of inoculated grain heads used for DNA extraction; then use PCR to verify presence of lipopeptide genes in the sample.

- f) **Problem:** 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: We will conduct further field plot trials, in South Dakota and elsewhere including Langdon, ND with Scott Halley when the opportunity arises.

- g) **Problem:** A commercial formulation of one or more of our BCAs is not presently available.

Resolution: We are working with the SDSU Foundation/Technology Transfer Office to make a commercial BCA product available.

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 compared results of the rapid and simple droplet collapse assay of culture broth supernatant from broth cultures of our BCAs that gives a quick semi-quantitative indication of the amount of lipopeptide produced by our BCAs, to results obtained by Chris Dunlap using much more elaborate analysis including MALDI-TOF instrumentation. Both the droplet collapse assay and Dunlap's analyses indicated that amending broth media with plant oils greatly increased the amounts of biosurfactant produced by the BCAs compared to what we obtained with previous broth media. Both the droplet collapse assay and Dunlap's analyses also indicate that the different *Bacillus* strains used as BCAs produce different amounts of biosurfactant; and that the amount of biosurfactant produced increases over time and reaches a maximum from one to two weeks after broth inoculation.

Impact: Similar results concerning biosurfactant production were obtained using both low technology simple assay and state of the art biochemical instrumentation. This gives us confidence that our semi-quantitative droplet collapse assay is reliable and agrees with more elaborate analyses. 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

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produced to the ability of different broth formulations to control FHB and/or reduce DON levels.

b) Accomplishment: Field plot trials were conducted in 2009 in Brookings, South Dakota to further assay the efficacy of *Bacillus* strains 1BA and 1D3. The Brookings trial had spray application at Feekes 10.51 for Briggs spring wheat. Some trials included application of Prosaro plus Induce NIS, alone and in combination with 1BA and/or 1D3. Desirable effect of BCA application included the following. Disease incidence was significantly lower for the following treatments compared to untreated control: 1D3 + canola oil; 1BA + canola oil + Induce NIS; 1D3 + Prosaro + canola oil; and 1BA + 1D3 + Prosaro + canola oil. Disease severity was significantly lower for the following treatments compared to untreated control: 1BA + 1D3 + Prosaro + canola oil; and Prosaro + Induce NIS. Disease index was significantly lower for the following treatments compared to untreated control: 1D3 + Prosaro + canola oil; 1BA + 1D3 + Prosaro + canola oil; and 1D3 + Prosaro + elevated trace elements. FDK was significantly lower for the following treatments compared to untreated control: 1D3 + Prosaro + canola oil; 1BA + 1D3 + Prosaro + canola oil; and 1D3 + Prosaro + elevated trace elements. DON was significantly lower for the following treatments compared to untreated control: 1BA + Prosaro + canola oil; 1BA + 1D3 + Prosaro + canola oil; and 1D3 + Prosaro + elevated trace elements.

Impact: The trials demonstrated that *Bacillus* strains 1BA and 1D3 when co-applied with Prosaro 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.

Bleakley, B.H., J. Morgan, and J. Vahrenkamp. 2009. Populations of *Bacillus* strains applied to wheat heads for biological control of FHB: Results of Brookings, SD 2009 field plots. Abstract, pg. 25. In: S.M. Canty, A. Clark, J. Mundell, E. Walton, D. Ellis, and D. Van Sanford (eds.), Proceedings of the 2009 National Fusarium Head Blight Forum, Dec. 7-9, 2009, Orlando, FL. ASAP Printing, Inc., Okemos, MI.

Ruden, K.R., L.E. Osborne, and B.H. Bleakley. 2009. 2009 Trial for the performance of biological control agents for the suppression of Fusarium Head Blight in South Dakota. Abstract, pg. 74. In: S.M. Canty, A. Clark, J. Mundell, E. Walton, D. Ellis, and D. Van Sanford (eds.), Proceedings of the 2009 National Fusarium Head Blight Forum, Dec. 7-9, 2009, Orlando, FL. ASAP Printing, Inc., Okemos, MI.