USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY05 Final Performance Report (approx. May 05 – April 06)
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Cover Page

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Fiscal Year:	**2005**
FY05 ARS Agreement ID:	**59-0790-5-077**
Agreement Title:	**Management of Fusarium Head Blight with Biological Control Agents.**
FY05 ARS Award Amount:	**$ 10,732**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
CBC	Management of Fusarium Head Blight with Biological Control Agents.	$ 10,732
	Total Award Amount	**$ 10,732**

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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) Over the last couple years, we have studied lipopeptide production by one *Bacillus* strain (1BA) using HPLC, since the lipopeptides are hypothesized to be important in allowing the bacteria to antagonize FHB and/or suppress DON production. The HPLC analysis is useful, but sample analysis has been slow because of the long analysis times for each sample (about one hour).

During 2005-2006, we have started to collaborate with Dr. Christopher Dunlap of NCAUR-USDA-ARS, Peoria, IL. He uses different analytical equipment than we have had access to in the past. Initial results from Dunlap's analyses of broth culture samples from our *Bacillus* strains show that at least one lipopeptide is present. A plate bioassay conducted by Dunlap's laboratory (based on inhibition of growth of *F. graminearum* by bacterial broth) also showed that substances inhibitory to the fungus are present. However, only one lipopeptide appeared to be present in the analyses done by Dunlap, whereas our HPLC analysis indicated that two types of lipopeptide were present (iturin and surfactin). We will further study the lipopeptides produced by our *Bacillus* biocontrol agents (BCAs) in different culture media, to better understand what lipopeptides are produced by these bacteria, and what their chemical structures are. Dunlap's instrumentation will allow us to find what the lipopeptide structures are, and to compare them to known lipopeptides produced by other microorganisms. Our aim is to find culture conditions that optimize lipopeptide production by our BCAs in a predictable manner, so we can spray BCAs onto field plots at a bacterial growth stage where lipopeptides are present in significant amounts in the culture medium.

b) We have sought a methodology to enable study of the microbial ecology of *Bacillus* BCAs in field plots by viable count methods. This requires the ability to select for the BCA and distinguish it from native microbial flora found as natural inhabitants on wheat heads. Over the last year, our laboratory has found that elevated NaCl and temperature allows good growth of our *Bacillus* BCAs , including strain 1BA. We found that the elevated NaCl and temperature suppress most of the native microflora on wheat heads. We are currently conducting viable counts (by most probably number (MPN) technique) of bacteria on wheat heads before and after spraying *Bacillus* BCAs; to see how BCA numbers change with time after application, and if patterns of BCA survival are different when the BCA is grown in different broth culture media. The object is to optimize survival of the BCAs after field application, to give them a chance to antagonize FHB on wheat heads.

2. List the most important accomplishment and its impact (how is it being used?).

a) Accomplishment: We documented by more than one method (both HPLC and the methods of Dunlap) that our BCAs make at least one lipopeptide.

Impact: As well as continuing our collaborations with the laboratories of Drs. Schisler, Yuen, and Bergstrom, we initiated and will continue collaborative work with Dr. Chris Dunlap. We will work with him to establish how/when lipopeptides are produced by our BCAs, in what quantities, and what their molecular structures are. This will help establish culture conditions for optimizing lipopeptide production by our BCAs for field application.

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?: We have

verified by multiple methods that our BCAs produce lipopeptides that are likely important in allowing the BCAS to antagonize FHB and/or reduce DON levels under some field conditions.

b)Accomplishment: We have documented that high salt/high temperature conditions allow vigorous growth by our *Bacillus* BCAs. These strains are extremophiles, able to grow at elevated temperatures and salt concentrations that many other microorganisms on the wheat head cannot tolerate. We found that native microflora on wheat heads can be suppressed in large part or totally with extremophilic culture conditions (high temperature and high salt).

Impact: This information will allow initial microbial ecology studies to be done to get viable counts of BCA population on wheat heads over time after their spray application, to see how well these BCAs survive on wheat heads.

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?:

We established that our BCAs are extremophiles that can grow at high salt/high temperature. This will allow us to obtain viable count data on the population levels of our BCA on wheat heads over time after its spray application. We might be able to use environmental parameters to help promote growth and establishment of such extremophilic BCAs on wheat heads to help control FHB. For example, we hypothesize that elevated salt concentrations in the spray mix might allow the BCAs to grow and establish themselves on wheat heads, while antagonizing or suppressing growth of much of the native microflora and perhaps of *F. graminearum* as well.

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.

Dangel, A.L., and B.H. Bleakley. 2005. Characteristics, including tolerance to elevated heat and elevated salt concentration, of a *Bacillus* strain used as a biocontrol agent to control Fusarium Head Blight. *In* Canty, S.M., Boring, T., Wardwell, J., Siler, L., and Ward, R.W.(Eds.), Proceedings of the National Fusarium Head Blight Forum; 2005 Dec. 11-13; Milwaukee, WI. East Lansing, Michigan State University, p. 187.

Draper, M.A., B. Bleakley, K.R. Ruden, S.M. Thompson, and D.S. Wittmeier. 2005. 2005 Uniform trials for the performance of biological control agents in the suppression of Fusarium Head Blight in South Dakota. *In* Canty, S.M., Boring, T., Wardwell, J., Siler, L., and Ward, R.W.(Eds.), Proceedings of the National Fusarium Head Blight Forum; 2005 Dec. 11-13; Milwaukee, WI. East Lansing, Michigan State University, p. 189.

Yuen, G.Y., C.C. Jochum, B.H. Bleakley, M.A. Draper, K.R. Ruden, and L.E. Sweets. 2005. Standardized evaluation of biological agents for the control of Fusarium Head Blight: 2005 results. *In* Canty, S.M., Boring, T., Wardwell, J., Siler, L., and Ward, R.W.(Eds.), Proceedings of the National Fusarium Head Blight Forum; 2005 Dec. 11-13; Milwaukee, WI. East Lansing, Michigan State University, pp. 237-239.

Bleakley, B.H. 2006. Biological control of foliar and head diseases of wheat. AD-421 Progress Report (CRIS Report).