USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY10 Final Performance Report
One-Year No Cost Extension through FY11
July 13, 2012

Cover Page

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Fiscal Year:	FY10
USDA-ARS Agreement ID:	59-0206-0-058
USDA-ARS Agreement Title:	Optimizing Parameters for Efficacy of Biological Control Agents of FHB.
FY10 USDA-ARS Award Amount:	$ 13,554

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
MGMT	Optimizing Parameters for Efficacy of Biological Control Agents of FHB.	$ 13,554
	Total ARS Award Amount	**$ 13,554**

7-13-12

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 Soft Winter Wheat Region
SWW – Southern Soft Red Winter 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:** 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 a 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: We are continuing to examine lipopeptide production by our BCAs, evaluated by different methods. We are also assaying extracellular lipase and protease activities, to see if there is correlation between these enzyme activities and lipopeptide protection.

   b) **Problem:** Effect of manganese on lipopeptide production, and perhaps production of other antibiotics, by our *Bacillus* BCAs is still unclear. We hypothesize that at the correct concentration, manganese can stimulate production of these compounds.
   Resolution: We continue to examine effect of manganese on lipopeptide production in both pure cultures in the lab, and for pure cultures or commercial BCA *Bacillus* products applied in the field.

   c) **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 when the opportunity arises.

   d) **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) **<u>Accomplishment:</u>** The growth medium used for producing microbial BCAs can be critical to optimizing their effectiveness in controlling FHB. We hypothesize that including plant oil in the broth medium used to grow several of our *Bacillus* BCA strains promotes biosurfactant production via production of extracellular lipase(s). The *Bacillus* biological control agents (*Bacillus amyloliquefaciens* strains 1-BA, 1-BC, 1-BE and 1-D3) were inoculated into a broth medium containing Tryptic soy broth + yeast extract + manganese with or without plant oil. Later, the broth culture medium was subjected to biosurfactant

analysis through three assays (droplet collapse, oil spreading, and turbidometric analysis). Also, assays were done to examine the strains for extracellular lipase production. Oil amendment and shaking of broth cultures had a significant impact on biosurfactant production. All the strains experienced increased biosurfactant production, with differences observed among the strains. Strain 1D3 showed the best biosurfactant production, in comparison to all other strains. Lipase production was observed for all the strains. Further work will examine the effect of different oils on the production of extracellular lipase and its influence on biosurfactant production.

Impact:
How the lipase activity of our *Bacillus* BCAs correlates to lipopeptide production and antifungal activity needs to be further examined. That knowledge will be used to optimize production of the BCAs for optimal antifungal activity in the field on grain heads.

b) **Accomplishment:** A multi-location research project was conducted on various wheat market classes at six locations; North Dakota (hard red spring-HRSW), Nebraska (2 locations hard red winter), South Dakota (HRSW), New York (soft red winter-SRWW) and Missouri [2 cultivars-SRWW with different susceptibility to Fusarium head blight (FHB)]. Treatments applied as a single application at Feeke's Growth Stage (FGS) 10.51 included i) Taegro™; ii)Taegro + Prosaro™ iii) Taegro + Prosaro + chelated manganese (Mn) (Pro-manganese 5 chelated manganese solution; Tetra Micronutrients; Woodlands, TX); and iv) Prosaro. Treatments applied sequentially at FGS 10.51 and 5 to 7 days later included v) Taegro and Taegro; vi) Taegro + Prosaro and Taegro; and viii) Taegro + Prosaro + Mn and Taegro. A vii) nontreated was included as a control. All fungicides and biological fungicides were tank mixed with the adjuvant Induce (Helena Chemical Co.) at 0.125% v/v. Taegro (Novozymes Biologicals, Inc.) is a *Bacillus subtilis var. amyloliquefacians* Strain FZB24 containing 5.0 x 1010 colony forming units per gram and was include at 3.5 oz. / acre. Prosaro (Bayer CropScience) is a 50:50 blend of tebuconazole and prothioconazole and was applied at 6.5 fl. oz. /acre. Manganese is known to stimulate antibiotic production by some *Bacillus* species. The Mn was added at a rate of 0.01% v/v. Out of seven trials, significant ($P \leq 0.05$) F-tests for FHB incidence were reported at NY (five of seven treatments less than the nontreated) and MO-Roane (five of seven treatments less than the nontreated), severity at ND (four of seven treatments less than the nontreated), and index at NY (five of seven treatments less than the nontreated), at Mead, NE (three of seven treatments less than the nontreated), and at ND (four of seven treatments less than the nontreated). Significant differences for yield were reported from three of seven reporting locations at NY, ND and MO-Roane (one of seven treatments increased yield over the nontreated). Significant differences for test weight were reported at MO-Elkhart (five of seven treatments different from the untreated). Of four reporting trials significant differences in deoxynivalenol accumulation in the seed (DON) were reported at NY (seven of seven treatments less than the nontreated), Mead, NE (three of seven treatments less than the nontreated) and MO-Elkhart (six of seven treatments less than the nontreated). Significant differences for % *Fusarium* damaged kernels were reported at Mead, NE (two of seven treatments less than the nontreated) and MO-Elkhart (four of seven treatments less than the untreated) from two of four reported trials. Significant differences for foliar disease levels were reported from three of three reporting trials, NY (six of seven treatments less than the nontreated), MO-Elkhart

(five of seven treatments less than the nontreated) and MO-Roane (five of seven treatments less than the untreated). In summary, treatments with significant improvement over the nontreated included ii, iii, iv, vi, and viii for visual symptoms of FHB. Treatments with reduction in DON compared to the nontreated included ii, iii, and vi and in two studies iv, vi and viii. Deoxynivalenol accumulations were low in the reported trials. Treatments that increased yield over the nontreated included iii, vii, and viii. Prosaro was effective in reducing the visual symptoms of FHB at NY and Mead, NE but not ND. However, there were examples where the addition of a biological in a tank mix or as a sequential treatment reduced FHB symptoms or yield over Prosaro including treatments vi, and viii for severity, iii, vi and viii for index and iii for yield in ND, viii for yield in NY and MO-Roane, and ii, vi and viii for DON in MO-Elkhart.

Impact:
This year's results lend further evidence for combining a biological with a fungicide to maximize control of FHB and DON

c) **Accomplishment:** The objective of this study was to continue evaluating the efficacy of selected biological control agents (BCAs), alone or in combination with fungicide, that can suppress different measures of FHB under field conditions in both North Dakota and South Dakota. Briggs hard red spring wheat was planted at Brookings, SD. Trial treatments included an untreated check; the fungicide premix Prosaro®; *Bacillus* strain 1BA; *Bacillus* strain 1D3; a combination of *Bacillus* strain 1BA and *Bacillus* strain 1D3; and combinations of Prosaro with one or more of the *Bacillus* BCAs. Chelated manganese was added to the spray mix for some treatments. All treatments were applied at anthesis. Plots were treated with pathogen by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field, and applying overhead mist irrigation each day for 10 days following anthesis. Following the treatments, plots were evaluated for FHB incidence, FHB head severity, and FHB field severity. Plots were harvested for yield and test weight and samples were collected for *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON). In Brookings, SD, grain yield was about 20 bushel/acre or less, much less than for average years, probably due in large part to high moisture, as well as other diseases than FHB being present. Damage due to Bacterial Blight and Black Chaff was widespread, and may have caused excessive damage to the vascular and photosynthetic apparatus of the wheat. Readings of FHB effects in the wheat were difficult to make due to the excessive bacterial disease. No DON was detected in the grain.

Impact
The damage and drain on plant resources caused by bacterial disease clearly was so extreme in Brookings that it precluded development of FHB. Management and varietal choices in future trials will try to limit the extent of this bacterial disease on the wheat.

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., K.R. Ruden, N. Srinivasa-Murthy and S. Halley. 2011. "2011 Trial of the Performance of Selected Biological Control Agents for the Suppression of Fusarium Head Blight in South Dakota and North Dakota. In: S. Canty, A. Clark, A. Anderson-Scully, D. Ellis and D. Van Sanford (EDS.). *Proceedings of the 2011 National Fusarium Head Blight Forum* (p.122). East Lansing, MI/Lexington, KY: U.S. Wheat and Barley Scab Initiative.

Halley, S., G. Yuen, C. Jochum, B.H. Bleakley, N.K.S. Murthy, K.R. Ruden, K.D. Waxman, G.C. Bergstrom and L.E. Sweets. 2011. "Uniform Biological Fungicide Evaluations for Control of Fusarium Head Blight and Deoxynivalenol in Wheat." In: S. Canty, A. Clark, A. Anderson-Scully, D. Ellis and D. Van Sanford (EDS.). *Proceedings of the 2011 National Fusarium Head Blight Forum* (pp.140-141). East Lansing, MI/Lexington, KY: U.S. Wheat and Barley Scab Initiative.

Srinivasa Murthy, N. and B. H. Bleakley. 2011. "Lipase Activity of *Bacillus* Strains used for Biological Control of Fusarium Head Blight. In: S. Canty, A. Clark, A. Anderson-Scully, D. Ellis and D. Van Sanford (EDS.). *Proceedings of the 2011 National Fusarium Head Blight Forum* (pp.151). East Lansing, MI/Lexington, KY: U.S. Wheat and Barley Scab Initiative.