USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY09 Final Performance Report
July 15, 2010

Cover Page

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Fiscal Year:	2009
USDA-ARS Agreement ID:	59-0206-9-056
USDA-ARS Agreement Title:	FHB Management Research in New York.
FY09- USDA-ARS Award Amount:	$ 74,620

USWBSI Individual Project(s)

USWBSI Research Category*	**Project Title**	**ARS Adjusted Award Amount**
MGMT	Enhancement of Biocontrol of FHB/DON Through an Understanding of Microbial Ecology.	$ 31,220
MGMT	Within-Field Inoculum from Corn Debris and the Management of FHB/DON.	$ 28,766
MGMT	Integrated Management Strategies for FHB and DON in New York.	$ 14,634
	Total Award Amount	**$ 74,620**

Gary C. Bergstrom July 13, 2010
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: *Enhancement of Biocontrol of FHB/DON Through an Understanding of Microbial Ecology.*

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

The goal of this research is to identify strategies for enhancement of FHB biocontrol by elucidating the ecology of interactions between *F. graminearum* and the biocontrol agent *Bacillus subtilis* strain TrigoCor on wheat florets. Like other biocontrol agents assessed by USWBSI researchers, TrigoCor gives excellent and consistent biological control of FHB in the greenhouse but not in the field; we aim to identify the factors leading to this disparity by describing the dynamics of microbial populations and of *Bacillus*-generated antifungal metabolites relative to biological control. We examined populations of *Bacillus* on wheat heads over critical infection periods in the greenhouse and in two field locations in New York. Using dilution plating, we quantified *Bacillus* populations on wheat heads at 0h, 24h, 72h, 7d, and 14d after *Bacillus* application. Population levels of *Bacillus* remained constant throughout the sampling period in four greenhouse experiments and in both field locations. The quantity of *Bacillus* on wheat heads was at least one order of magnitude higher in the greenhouse (10^8 CFUs/head) as compared to the field (10^6-10^7 CFUs per head) at all sampling times. We also recovered *Bacillus* from wheat heads in significant quantities (10^6 CFUs/head) at harvest. Treatment with TrigoCor did not provide significant reductions in FHB or DON in either field location. To follow-up on the results from 2009, in the 2010 field season we increased the *Bacillus* inoculum concentrations and sprayed a larger volume of inoculum onto heads, resulting in *Bacillus* population levels approaching those observed in the greenhouse (10^8 CFUs/head at 0h). Despite these higher population levels, we did not observe a significant reduction in FHB symptoms, although the DON results are pending.

In addition to viable bacterial populations, we are also assessing the production and persistence of antifungal metabolites relative to biological control in controlled and field environments. We have developed a protocol for detecting the iturin class of lipopeptides, which are the compounds largely responsible for the biocontrol activity of TrigoCor, from wheat heads and for analyzing them using HPLC. Using this protocol we measured the amount of iturins from wheat heads collected at 0h and 7d post-*Bacillus* application in the greenhouse, and at time points matching our population studies from two field locations in 2010. In the greenhouse, iturins are present on wheat heads at significant levels at both 0h and 7d post-*Bacillus* application. In the field, however, the level of iturins on heads treated with *Bacillus* is high at 0h, but then gradually decreases over 24h and 72h, and is barely detectible at 7d and undetectable at 14d post-application. These results suggest inadequate production or persistence of antifungal compounds on wheat heads in the field may contribute to limited disease control in this environment.

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):

<u>Accomplishment:</u>
We determined that the quantity of *Bacillus* cells on wheat heads in the field (applied with best currently available spray technology) is more than one order of magnitude lower than in

the greenhouse (applied with a hand-operated atomizer) throughout the 14-day post-application period. Additionally, we determined that antifungal iturin lipopeptides are present at significant levels on wheat heads for at least 7d post-application in the greenhouse, but that they decrease to barely detectable levels during the 7d post-application period in the field.

Impact:

We have identified two technical challenges to successful deployment of *Bacillus* as a biocontrol agent to wheat heads in the field: 1) Much higher cell/metabolite populations must be delivered to wheat heads than current technology allows, and 2) high iturin levels must be maintained on wheat heads for several days following application. Improvements in FHB biocontrol will most likely result from a combination of increasing the amount of *Bacillus* on wheat heads and facilitating better persistence/higher production of antifungal compounds. Higher *Bacillus* population levels can be achieved in part from raising inoculum cell concentrations, such as through optimization of fermentation conditions. However, increasing cell densities by one or more orders of magnitude may be extremely difficult and expensive, particularly on a commercial scale. Increasing the inoculum production of antifungal lipopeptides may be more practical and will likely have a greater impact on disease control, although it does not guarantee presence of these compounds during later infection periods. We plan to use the recently sequenced TrigoCor genome to investigate the production/regulation of key lipopeptides over critical infection periods by measuring expression of the genes controlling their synthesis. We also hope to determine if there are any triggers that can be used to enhance production of these compounds on wheat heads.

PI: Bergstrom, Gary
USDA-ARS Agreement #: 59-0206-9-056

Project 2: *Within-Field Inoculum from Corn Debris and the Management of FHB/DON.*

1. **What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?**
Knowledge of the relative contribution of within-field inoculum sources of *Gibberella zeae* to infection of local wheat and barley is important for developing and/or excluding strategies for managing FHB. Our experimental objective was to quantify the relative contribution of within-field corn debris as an inoculum source of *Gibberella zeae* for Fusarium head blight and DON contamination in ten variable wheat environments in 2009, all in regions where corn is the predominant crop in the agricultural landscape and corn debris is left on the land surface over large areas. Our research is based on the hypothesis that spores of *Gibberella zeae* that are deposited on wheat spikes and that result in Fusarium head blight come primarily from well-mixed, atmospheric populations in an area. The research was conducted in two commercial-scale wheat fields in Illinois, Missouri, Nebraska, New York, and Virginia, each following a non-susceptible crop. Over these environments we encountered six severe epidemics (in Illinois, Missouri, and Virginia), two moderate epidemics (in New York), and two mild epidemics (in Nebraska). Locally overwintered, natural corn stalks were collected in spring from two different sources in each locale by placing a 33 inch diameter plastic 'Hoola Hoop' onto four arbitrarily selected areas in a corn stubble field, and then removing all of the stubble within the hoop and placing it in a paper bag. Replicated (four) microplots containing corn debris and without debris were set out in each field and were separated by a minimum of 100 ft in each dimension. Debris was secured within the source circles by using cages fashioned of 2 ft high hardware cloth and shaped with the same 33 inch diameter plastic 'Hoola Hoop', fastened with plastic zip-ties, and secured to the soil with metal ground staples. Wheat heads above each microplot were rated at soft dough stage for FHB incidence, severity, and index. At grain maturity, at least 100 heads from each microplot were harvested, dried and shipped to Cornell where grain was threshed from a subsample of heads and sent to Virginia Tech for DON analysis.

   Despite a 'low' predicted risk of FHB (Penn State model) in each location, significant FHB symptoms (>5% with no debris) developed in four fields, and DON was detected in every field. FHB symptoms at soft dough were a poor predictor of DON ppm, e.g., in Columbia, MO and Riner, VA. *Gibberella zeae* was recovered from a large percentage of mature spikes at every location except Lincoln and Mead, NE, suggesting late infections in some fields with no or few symptoms at soft dough. Local corn debris resulted in significant (P=0.05) increases over no debris in FHB only in one field in Riner, VA, and did not result in a significant increase in DON in any location. Released clonal inoculum resulted in a significant (P=0.05) increase (over no debris) in FHB only in Urbana, IL, and resulted in significant increases in DON only at Dixon Springs and Urbana, IL.

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:
The astounding result is that DON level did not differ significantly between corn debris and no debris microplots in any of the ten wheat environments.

Impact:
By inference of our results, it appears that elimination of corn debris from single wheat fields in a major corn- producing region may have rather limited benefits in terms of reducing FHB and especially of reducing DON contamination of grain. The experiments will be repeated in ten additional environments in 2010.

Project 3: *Integrated Management Strategies for FHB and DON in New York.*

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

In response to the USWBSI goal to validate integrated management strategies for FHB and DON, the Disease Management RAC of USWBSI initiated a multi-state, multi-year, coordinated field study. In New York during 2009, we conducted two separate experiments each with unique environmental conditions during flowering and early grain development. Our objective was to evaluate the individual and interactive effects of moderately resistant cultivars, foliar fungicide (Prosaro), and a biological control agent (*Bacillus subtilis*) on wheat yield and the integrated management of Fusarium head blight (FHB) and deoxynivalenol (DON) under two natural environments in New York. The two experimental wheat environments were characterized by the planting of winter wheat 1) no-till into soybean residue in late September 2008 and 2) no-till into corn residue in late October 2008. Each experimental design was a split plot with four wheat cultivars as whole plots and four spray treatments as subplots, and four replicate blocks. Main plots were planted with a10 ft wide commercial grain drill. Sprayed areas in each subplot were 8 ft wide by 20 ft long. Spray treatments applied at Feekes GS10.5.1 were 1) non-sprayed; 2) Prosaro 6.5 fl oz/A & Induce 0.125%; 3) *Bacillus subtilis* TrigoCor ca. 1.5×10^{14} cfu/A & Induce 0.125%; and 4) TrigoCor & Prosaro & Induce. Application was made with paired Twinjet nozzles mounted at an angle (30^{o} from horizontal) forward and backward and calibrated to deliver at 20 gallons per A. FHB and foliar diseases were assessed at soft dough stages. Grain was harvested from a 4 ft wide x 20 ft long area in each subplot using a Hege plot combine. Grain moistures, plot yields, and test weights were recorded and the latter two were adjusted for moisture. Means were calculated and subjected to Analysis of Variance. Fisher's protected LSD was calculated at P=0.05. Analysis of DON content in grain was conducted in the USWBSI-supported mycotoxin laboratory of Dr. Schmale.

Due to moist weather through grain maturation, FHB occurred in both experimental environments. A moderate FHB epidemic was observed in the timely-planted plot following no-till soy, and a severe FHB epidemic was observed in the late-planted plot following no-till corn. Difference in epidemic severity for the two experiments is best explained by differences in flowering dates and moisture conditions through flowering. Wheat cultivars reached Feekes GS10.5.1 on June 5 and June 12 for the timely and late-planted experiments, respectively, while rain occurred frequently from June 9 through early July. The impact of crop residue type on FHB development was apparently less important than weather conditions as an adjacent experiment on plowed ground with no corn residue planted to Jensen wheat on the same date had a similar incidence of FHB (40%) as compared to nontreated Jensen (49%) in the late-planted management experiment into no-till corn.

Foliar diseases, including leaf rust and leaf spots, were observed in both experimental environments and were reduced significantly by application of Prosaro. In the timely-planted experiment, no foliar spray treatment (fungicide, biological control, or combination) had a significant effect on the yield of any cultivar. In the late-planted experiment, significantly greater yields due to fungicide treatment were observed in the two white cultivars, Jensen and Richland. Under the lower disease pressure of the timely-planted experiment, the fungicide application decreased DON levels to below 2.0 ppm for the two red cultivars, Pioneer 25R57 and Truman. Under the higher disease pressure of the late-planted experiment, Prosaro did not consistently reduce DON contamination and, when reductions were observed, remaining DON levels still greatly exceeded the 2.0 ppm threshold for sale at flower mills. Therefore, under severe epidemic conditions, the combination of the best available cultivar and fungicide did not reduce DON to satisfactory levels. While TrigoCor alone was not able to inhibit FHB, it neither impaired disease control of the fungicide when applied as a mixture nor reduced yield. Moderate FHB resistance was observed with the cultivar Truman, averaging the lowest FHB incidence and DON levels of all of the cultivars in both experimental environments. While Truman is a lower yielding cultivar, the benefit of resistance under high disease pressure was shown by the yield results of the no-till corn plot. Designation of moderate resistance status of cultivars including Jensen is based primarily on observations of FHB symptoms at soft dough stage. The finding of very high DON levels in a cultivar designated as moderately resistant suggests that DON production should be given greater weight in future designation of cultivar reaction.

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:
Lowest levels of DON were achieved when Prosaro fungicide was applied at Feekes 10.5.1 to varieties with moderate resistance to FHB. However, the challenge ahead for DON reduction was illustrated under severe epidemic conditions in New York where the combination of the best available cultivar (Truman) and fungicide (Prosaro) was still insufficient to reduce DON to below 2 ppm.

Impact:
Integrated management is the most promising strategy for reducing DON, but we need varieties with still lower DON levels than those currently available and/or improvements in fungicide efficacy consistently to achieve acceptable DON levels in grain.

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.

Publications (non-peer reviewed):

Bergstrom, G.C., and K.D. Waxman. 2009. Integrated management of Fusarium head blight in wheat: Introduction of *Scab Smart*. *What's Cropping Up?* Volume 19, No. 5: 7-9.
http://css.cals.cornell.edu/cals/css/extension/cropping-up-archive/wcu_vol19no5_20094scab.pdf

Bergstrom, G.C., K.D. Waxman, D.G. Schmale III, C.A. Bradley, L.E. Sweets, S.N. Wegulo, and M.D. Keller. 2009. Effects of within-field corn debris in microplots on FHB and DON in ten U.S. wheat environments in 2009. Page 22 in *Proc. 2009 National Fusarium Head Blight Forum*, Wyndham Orlando Resort, Orlando, FL, Dec7-9, 2009.

Crane, J.M., D.M. Gibson, and G.C. Bergstrom. 2009. Ecology of *Bacillus subtilis* on wheat florets in relation to biological control of FHB/DON. Page 39 in *Proc. 2009 National Fusarium Head Blight Forum*, Wyndham Orlando Resort, Orlando, FL, Dec7-9, 2009.

Waxman, K.D. and G.C. Bergstrom. 2009. Evaluation of integrated FHB management methods under moderate and severe epidemics in New York. Pages 90-92 in *Proc. 2009 National Fusarium Head Blight Forum*, Wyndham Orlando Resort, Orlando, FL, Dec7-9, 2009.

Waxman, K.D., G.C. Bergstrom, P.M. Atkins, P.J. Stachowski, and W.J. Cox. 2009. Evaluation of cultural, chemical, and biological control methods of winter wheat in New York, 2007-2008. *Plant Disease Management Reports* 3:CF026.

Willyerd, K., L. Madden, G. Bergstrom, C. Bradley, A. Grybauskas, D. Hershman, M. McMullen, K. Ruden, L. Sweets, S. Wegulo, K. Wise, and P. Paul. 2009. Integrated management of FHB and DON in small grains: 2009 coordinated trials. Pages 95-99 in *Proc. 2009 National Fusarium Head Blight Forum*, Wyndham Orlando Resort, Orlando, FL, Dec7-9, 2009.

Extension presentations by Gary C. Bergstrom in 2009-10 that included updates on Fusarium head blight research:

Cayuga County Pesticide Spray Clinic at Monroe Tractor, Auburn, NY. Overcoming disease barriers to field crop yield and quality: Update on corn, soybeans, wheat, alfalfa and switchgrass. (ca. 150 persons) (3/18/10)

Great Lakes Wheat Consortium Meeting, Ithaca, NY. FHB epidemiology research. (ca. 25 persons) (3/15-16/10)

Going the Whole Grain Conference, University of Vermont, Burlington, VT. Management of diseases in organic wheat production. (ca. 50 persons) (3/9/10)

Alleghany/Cattaraugus Winter Crop Update, Randolph, NY. Field crop diseases affecting corn, soybeans, and small grains. (ca. 30 persons) (3/4/10)

Southern Tier Field Crops Workshop, Horseheads, NY. Field crop diseases affecting corn, soybeans, and small grains. (ca. 40 persons) (3/2/10)

Organic Grain Meeting, MOFGA Education Center, Unity, ME. Disease management for organic small grains. (ca. 35 persons) (2/26/10)

Quebec Conference on Phytoprotection of Field Crops, St. Remi, Quebec, Canada. Strategies for management of diseases in field crops. (ca. 100 persons) (2/17/10)

Finger Lakes Soybean and Small Grains Congress, Waterloo, NY. Advances in the management of Fusarium head blight and other wheat diseases. (ca. 125 persons) (2/4/10)

Western New York Soybean and Small Grains Congress, Batavia, NY. Advances in the management of Fusarium head blight and other wheat diseases. (ca. 200 persons) (2/3/10)

Cornell Cooperative Extension, Agriculture and Food Systems In-Service, Ithaca, NY. 1. Mycotoxin Update (joint Dairy/Field Crops Session) 2. Field Crop Pathology Update (ca. 30 persons) (11/10-12/09)

Field Crop Dealer Meeting, Auburn, NY. Overcoming disease barriers to field crop yield and quality. (ca. 50 persons) (10/30/09)

Field Crop Dealer Meeting, Stafford, NY. Overcoming disease barriers to field crop yield and quality. (ca. 70 persons) (10/29/09)

Field Crop Dealer Meeting, New Hartford, NY. Overcoming disease barriers to field crop yield and quality. (ca. 45 persons) (10/28/09)

Field Crop Dealer Meeting, Latham, NY. Overcoming disease barriers to field crop yield and quality. (ca. 50 persons) (10/27/09)

Northeast Wheat Management Symposium, Branton Farms, Stafford, NY. Overcoming disease barriers to wheat yield and quality in New York. (ca. 80 persons) (8/21/09)

Third Annual New York Crops Tour, Avon, NY. Ask the educators booth – Q&A. (ca. 250 persons) (8/18/09)

Seed Growers Field Day, Ithaca, NY. Update on diseases of cereals, forages, and biofuel feedstock crops (ca. 70 persons) (7/7/09)

Grain Storage IPM Workshop, Ithaca, NY. Molds and mycotoxins affecting grain. (ca 20 persons) (6/10/09)

Small Grains Management Field Day, Aurora, NY. Integrated management of FHB. (ca. 80 persons) (6/4/09)