USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY10 Final Performance Report
July 15, 2011

Cover Page

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Fiscal Year:	FY10
USDA-ARS Agreement ID:	59-0206-9-056
USDA-ARS Agreement Title:	FHB Management Research in New York.
FY10 USDA-ARS Award Amount:	$ 26,753

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
MGMT	Enhancement of Biocontrol of FHB/DON Through an Understanding of Microbial Ecology.	NCE**
MGMT	Integrated Management Strategies for FHB and DON in New York.	$ 14,634
MGMT	Within-Field Inoculum from Corn Debris and the Management of FHB/DON.	$ 12,119
	Total ARS Award Amount	**$ 26,753**

Gary C. Bergstrom July 15, 2011
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

** NCE – Carry-over from FY08 expired agreement was used to fund this project.

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 amyloliquefaciens* 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.

Previous work funded by the USWBSI in the Bergstrom lab has shown that population levels of *Bacillus* on wheat heads are one or more orders of magnitude lower in the field (10^6-10^7CFUs/ head) than they are in the greenhouse (10^8CFUs/head) for at least 14d post-*Bacillus* application. We conducted three greenhouse experiments to determine if this order of magnitude difference in *Bacillus* populations and their associated metabolites on wheat heads could be responsible for a loss in disease control efficacy. We consistently found that diluting a *Bacillus* inoculum 10-fold to produce population levels on wheat heads similar to those observed in the field provided significantly less disease and DON control than did a non-dilute inoculum. This finding indicates that the 10-fold reduction in *Bacillus* population levels on wheat heads in the field as compared to the greenhouse may contribute to the lack of disease control observed in the field.

To determine if raising the *Bacillus* population levels on wheat heads in the field would lead to better disease control, in the 2010 field season we increased the inoculum concentration and volume applied per head to wheat heads in two NY locations. *Bacillus* populations were monitored on wheat heads using dilution plating. At 0 and 1d post-*Bacillus* application, the level of *Bacillus* recovered from wheat heads at both locations was comparable to the level recovered from heads in the greenhouse (10^8CFUs/head), and at 3,7, and 14d post-application the level was lower (10^7CFUs/head) than populations in the greenhouse but still higher than in previous field seasons. At one field location there was a statistically significant decrease in FHB severity ($p=0.014$), but otherwise treatment with TrigoCor did not provide significant reductions in FHB or DON. The insufficient FHB control of TrigoCor in the 2010 field season, despite the *Bacillus* population numbers on wheat heads being initially similar to levels on greenhouse heads and overall fairly high, suggests that population levels alone do not explain the ability of *Bacillus* to control FHB.

In addition to bacterial population dynamics, we are assessing the persistence of antifungal metabolites relative to disease control in greenhouse 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 0d, 1d, 3d, and 7d post-*Bacillus* application in the greenhouse and in our field trials in 2010. In both environments, we found that metabolite levels decrease quickly by 3d post-*Bacillus* application. Despite this similar trend in metabolite loss, the level of metabolites was at least twice as high on heads in the greenhouse as compared to the field. Results from replicated well-diffusion assays on plates indicate that a two-fold reduction in iturin concentration has a significant effect on *Fusarium* growth inhibition, suggesting that the difference observed in metabolite levels between the greenhouse and the field may be relevant. We have conducted two greenhouse experiments to confirm that decreasing metabolite levels by a factor of two is significant for disease control, and DON data from those experiments are pending.

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:

We have demonstrated through repeated field and greenhouse experiments that there are major differences in the levels of *Bacillus* populations and their associated antifungal metabolites in the greenhouse and the field, such that population levels in the field are at least one order of magnitude lower than in the greenhouse, and metabolite levels are at least half. Most of our field experiments thus far have involved hand-sprayed plots, but our limited analysis of heads from commercially sprayed plots suggests that population and metabolite levels in commercially sprayed plots are even lower. These significant reductions in *Bacillus* populations and metabolites in the field are likely responsible for much of the insufficient FHB and DON control observed in the field.

Impact:

Based on our findings, the most viable approach to improving FHB control with *Bacillus* appears to be increasing inoculum concentrations of antifungal metabolites, particularly the iturins. A secondary focus should be on increasing inoculum population densities, although as seen in the 2010 field season, simply having a large initial population size on wheat heads does not necessarily equate to better disease control. Multiple applications of *Bacillus* may be helpful to maintain high metabolite levels over time, but this may not always be feasible in commercial settings. Cornell University has signed an agreement with Novozymes Inc., one of the world's foremost biological product companies, in which Novozymes will provide two years of assistantship support for a Cornell graduate research assistant (Julia Crane) in the Bergstrom Lab to conduct research on the mechanisms and enhancement of biological control of Fusarium head blight by *Bacillus.* This public/private research collaboration is the direct result of the promising data generated by this successful USWBSI-supported project.

Project 2: *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 2010, 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 and foliar fungicides (Caramba, Prosaro, and Folicur on wheat yield and the integrated management of Fusarium head blight (FHB) and deoxynivalenol (DON) under two natural environments in New York. All experiments were performed at the Musgrave Research Farm in Aurora, NY following cultural practices recommended for soft winter wheat in the region. The four cultivars included in the experiment were 'Pioneer 25R47' (red, susceptible to FHB), 'Truman' (red, moderately resistant to FHB), 'Jensen' (white, moderately resistant to FHB), and 'Richland' (white, susceptible to FHB). The two experimental wheat environments were characterized by the planting of winter wheat 1) no-till into soybean residue on 10/12/09 and 2) no-till into a fallow field on 10/20/09. 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 a 10 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) Caramba 13.5 fl oz/A & Induce 0.125%; 3) Folicur 4.0 fl oz/A & Induce 0.125%; and 4) Prosaro 6.5 fl oz/A & Induce 0.125%. The experiment was inoculated with conidial suspension of Fusarium graminearum (100,000 conidia/ml) on the same day as treatments were applied after the fungicides had dried. Fungicide and Fusarium application was made with paired Twinjet nozzles mounted at an angle (30^o from horizontal) forward and backward and calibrated to deliver at 35 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 an USWBSI-supported mycotoxin laboratory.

   Although planting dates varied by a week, any developmental head start in the earlier planted environment was inconsequential by spring. Flowering occurred simultaneously in both environments during a dry and hot period unfavorable to FHB development. The two weeks following flowering were considered medium risk of FHB due to increased rainfall and more moderate temperatures. The overall average of FHB incidence observed for both environments was 20%. Inoculation with the conidial suspension proved fruitful as only 2% FHB incidence was observed in adjacent non-inoculated experiments. There is little evidence to suggest inocula from within-plot crop residues impacted FHB development. Only two cultivars, Jensen and Richland, had significantly higher FHB indexes in the soybean residue plot (environment 1). There were no significant differences observed in the DON levels between the two environments for all cultivars. Due to the similar levels of FHB development in both environments, FHB is unlikely an explanation to the greater yields observed in environment 1. One potential explanation of yield differences is that more foliar disease, especially Stagonospora leaf blotch, was observed in the previously fallow plot (environment 2). In both environments, foliar diseases were reduced significantly by application of any of the fungicides.

All fungicide treatments impacted at least some aspect of FHB development. All fungicide treatments resulted in FHB indices lower than nontreated. In two plots, Jensen and Richland in environment 2, there was no significant difference in FHB index between any of the treatments. When significant reductions of FHB index due to fungicide application were observed, there was no difference between fungicides except for Pioneer 25R47 in environment 1 where Folicur did not differ from nontreated. Pooling cultivar data to determine treatment averages minimized the observed decrease in FHB index due to fungicide application.

Contamination of grain by DON was decreased significantly by all fungicide applications in all cultivars and in both environments, but not always below the 2.0 ppm threshold for sale at flour mills. In both environments, nontreated grain had DON levels greater than the threshold with the exception of the Truman plots. For the Jensen plots in both environments, no fungicide treatment reduced DON levels below 2.0 ppm. In Pioneer 25R47 and Richland plots in both environments, Prosaro and Caramba reduced DON below the threshold. Reduction of DON below 2.0 ppm was observed only once with the Folicur treatment (in environment 2 on Pioneer 25R47).

Fungicide application increased yield for all cultivars in both environments. Prosaro was the only fungicide that resulted in significantly higher yields than the nontreated in all cultivars in both environments. The other two fungicides had significantly higher yields in all plots except for the Folicur treatments on Truman in environment 1 and on Pioneer 25R47 in environment 2, and the Caramba treatment on Richland in environment 2. Of the three fungicide treatments, none consistently increased yields significantly in comparison with the others. However, in situations where FHB is severe, treatment with Caramba or Prosaro would more likely reduce DON below 2.0 ppm than would treatment with Folicur.

The four cultivars demonstrated differences in both yield capability and disease response. Observations did not necessarily conform with expectations based on defined FHB response. The white wheat varieties, Jensen (previously categorized as moderately resistant) and Richland (susceptible), had similarly high FHB indexes and DON levels. Richland had significantly higher yields than Jensen in environment 2. The red wheat varieties, Pioneer 25R47 (susceptible) and Truman (moderately resistant), had similarly low FHB indexes and DON levels. Pioneer 25R47 had significantly higher yields than Truman in both environments. Only Truman demonstrated cultivar FHB resistance with DON levels below 2.0 ppm in the nontreated plots. Under the moderate disease levels of this experiment, fungicide application resulted in marketable grain even in the highest yielding, albeit more susceptible, cultivar Pioneer 25R47.

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:

Contamination of grain by DON was decreased significantly by all fungicide applications in all cultivars, but not always below the 2.0 ppm threshold for sale at flour mills. Varietal responses did not conform to expectations based on previously described FHB response. The white wheat varieties, Jensen (previously categorized as moderately resistant) and Richland (susceptible), had similarly high FHB indexes and DON levels. The red wheat varieties, Pioneer 25R47 (susceptible) and Truman (moderately resistant), had similarly low FHB indexes and DON levels. Only Truman demonstrated cultivar FHB resistance with DON levels below 2.0 ppm in the nontreated plots. For the Jensen plots in both environments, no fungicide treatment reduced DON levels below 2.0 ppm. In Pioneer 25R47 and Richland plots, Prosaro and Caramba reduced DON below the threshold. Under the moderate disease levels of this experiment, fungicide application resulted in marketable grain even in the highest yielding, albeit more susceptible, cultivar Pioneer 25R47.

Impact:

Integrated management is the most promising strategy for reducing DON, but we need varieties with DON levels at or below that of Truman, but with higher yield potential than Truman. In situations where FHB is severe, treatment with Caramba or Prosaro would more likely reduce DON below 2.0 ppm than would treatment with Folicur.

Project 3: *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?**

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 eleven variable wheat environments in 2010, 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 commercial-scale wheat fields in Illinois, Missouri, Nebraska, New York, and Virginia, each following a non-susceptible crop. Replicated (six) microplots containing corn debris from a nearby field or no added debris were set out in each field and were separated by a minimum of 100 ft in each dimension. Wheat spikes above each microplot were rated at soft dough stage for FHB incidence, severity, and index. At grain maturity, at least 100 spikes from each microplot were harvested, dried and shipped to Cornell where grain was threshed from a subsample of spikes and sent to the assigned USWBSI Testing Lab for DON analysis. Mature spikes from each microplot were also suface-disinfested and plated on Fusarium selective media to determine the incidence of spikes infected by *G. zeae*.

Characterization of epidemics over the 11 environments differed through the lenses of visual symptom development, incidence of mature spike infection, and toxin contamination. At every location except Chatham, VA, more than 20% of mature spikes were infected by *G. zeae*, regardless of the degree of symptom development at soft dough stage or the level of DON observed. This suggests that post-anthesis infection was quite common across environments in 2010. Based strictly on FHB index at soft dough, we observed five moderate epidemics (in Illinois, Missouri, and Nebraska) and six mild epidemics (in Nebraska, New York, and Virginia). On the other hand, three of the moderate epidemics, based on symptoms, were associated with toxin levels above 2 ppm. Mean DON levels in the no-debris microplots were 2.9 ppm in Urbana, IL, 4.4 ppm in Columbia, MO, and 12.2 ppm in Novelty, MO, and there was detectable DON at every site except Chatham, VA. Across the 11 environments, there was significantly (P=0.05) higher DON in grain from corn debris microplots (1.8 ppm) than from no-debris microplots (0.2 ppm) only in Bath, NY. It is especially noteworthy that DON levels were not significantly higher in corn debris microplots than no-debris microplots in any of the high DON locations, suggesting the predominance of regional atmospheric inoculum in those locations. FHB incidence, severity, or index was not significantly (P=0.05) higher in corn debris-containing than no-debris microplots in any of the 11 fields at soft dough stage. And only at Wilbur, NE did mature wheat spikes from microplots containing locally overwintered corn debris show a statistically significant increase in infection incidence by *G. zeae* over those from microplots with no corn debris.

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 levels did not differ significantly between corn debris and no debris microplots in 20 of the 21 winter wheat environments studied over two years. The single exception was in Bath, New York in 2010, an isolated valley environment with less surrounding grain corn acreage than other locations. It is especially noteworthy that DON levels were not significantly higher in corn debris microplots than no-debris microplots in any of the high DON locations, suggesting the predominance of regional atmospheric inoculum over within-field inoculum in severe epidemic circumstances.

Impact:

By inference of our results over two years and 21 winter wheat environments, it appears that elimination of corn debris from single wheat fields in major corn-producing regions may have rather limited benefits in terms of reducing FHB and especially of reducing DON contamination of grain. One caveat regarding this interim conclusion is that the microplot experimental design (small area sources of corn debris) we used may have resulted in an underestimation of the contribution of large area sources of corn debris to wheat infection and DON contamination. Much larger replicated plots will be necessary to definitively assess the quantitative contribution of corn debris to local wheat infection and DON accumulation on an agricultural field scale. This is the approach being taken in the FY11 project by Bergstrom et al and being conducted in wheat fields in seven states.

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 (peer-reviewed journals):

Schmale, D.G. III, A.K. Wood-Jones, C. Cowger, G.C. Bergstrom, and C. Arellano. 2011. Trichothechene genotypes of *Gibberella zeae* from winter wheat fields in the Eastern United States. *Plant Pathology*: (4 March 2011) DOI: 10.1111/j.1365-3059.2011.02443.x.

Keller, M.D., K.D. Waxman, G.C. Bergstrom, and D.G. Schmale III. 2010. Local distance of wheat spike infection by released clone of *Gibberella zeae* disseminated from infested corn residue. *Plant Dis.* 94:1151-1155.

Publications (non-peer reviewed):

Bergstrom, G.C. and K.D. Waxman. 2010. Where do the fungal spores come from that cause Fusarium head blight of wheat? *What's Cropping Up?* Volume 20, No. 4:13-15.

Bergstrom, G.C., K.D. Waxman, D.G. Schmale III, C.A. Bradley, L.E. Sweets, S.N. Wegulo, and M.D. Keller. 2010. Effects of within-field corn debris in microplots on FHB and DON in eleven U.S. wheat environments in 2010. Pages 69-70 in *Proc. 2010 National Fusarium Head Blight Forum*, Hyatt Regency Milwaukee, Milwaukee, WI, Dec 7-9, 2010.

Crane, J.M., D.M. Gibson, and G.C. Bergstrom. 2010. Ecology of *Bacillus amyloliquifaciens* on wheat florets in relation to biological control of FHB/DON. Pages 77-78 in *Proc. 2010 National Fusarium Head Blight Forum*, Hyatt Regency Milwaukee, Milwaukee, WI, Dec 7-9, 2010.

Keller, M.D., D.G. Schmale III, K.D. Waxman, and G.C. Bergstrom. 2010. Tracking released clones of *Gibberella zeae* within wheat and barley fields. Page 51 in *Proc. 2010 National Fusarium Head Blight Forum*, Hyatt Regency Milwaukee, Milwaukee, WI, Dec 7-9, 2010.

Waxman, K.D. and G.C. Bergstrom. 2010. Evaluation of integrated methods for managing FHB and DON in winter wheat in New York. Pages 104-107 in *Proc. 2010 National Fusarium Head Blight Forum*, Hyatt Regency Milwaukee, Milwaukee, WI, Dec 7-9, 2010.

Willyerd, K., L. Madden, M. McMullen, S. Wegulo, B. Bockus., L. Sweets, C. Bradley, K. Wise, D. Hershman, G. Bergstrom, A. Grybauskas, L. Osborne, P. Esker, and P. Paul. 2010. Pages 109-110 in *Proc. 2010 National Fusarium Head Blight Forum*, Hyatt Regency Milwaukee, Milwaukee, WI, Dec 7-9, 2010.

Waxman, K.D., G.C. Bergstrom, R.J. Richtmyer III, and R.R. Hahn. 2010. Evaluation of integrated methods for management of Fusarium head blight and foliar diseases of winter wheat in New York, 2009. *Plant Disease Management Reports* 4:CF016.

Waxman, K.D., G.C. Bergstrom, R.J. Richtmyer III, and R.R. Hahn. 2010. Evaluation of foliar fungicides for control of foliar diseases and Fusarium head blight of winter wheat in New York, 2009. *Plant Disease Management Reports* 4:CF017.

Bergstrom, G.C. 2010. Fungicide options for wheat. Page 11 in: *Ag Focus*, June 2010, www.nwnyteam.org. NWNY Extension Dairy, Livestock & Field Crops Team, Batavia, NY.

Extension presentations by Gary C. Bergstrom in 2010-11 that included updates on Fusarium head blight research:

Cattaraugus County Crop Meeting, Randolph, NY (4/8/11)

Small Grains Seed Committee, Waterloo, NY (3/25/11)

Cayuga County Grain Day, Auburn, NY. (3/17/11)

Oneida County Crop Congress, Clinton, NY. (3/16/11)

Western New York Crop Management Association Annual Meeting, Varysburg, NY. (2/24/11)

Wheat Management Workshop, Batavia, NY. (2/16/11)

Finger Lakes Soybean and Small Grains Congress, Waterloo, NY. (2/10/11)

Western New York Soybean and Small Grains Congress, Batavia, NY. (2/9/11)

Madison County Crop Congress, Cazenovia, NY (2/1/11)

Northeast Certified Crop Advisors Conference, Waterloo, NY. Advanced Training Course: How to grow a healthy wheat crop in the Northeast. (11/30/10)

2010 Agriculture and Food Systems Cornell Cooperative Extension In-Service, Ithaca, NY. (11/17/10)

Seed Growers Field Day, Ithaca, NY. (7/8/10)

Small Grains Management Field Day, Aurora, NY. Small grains pathology update. (6/3/10)