USDA-ARS / USWBSI
FY04 Final Performance Report
July 15, 2005

Cover Page

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Year:	**FY2004** (approx. May 04 – April 05)
FY04 ARS Agreement ID:	**59-0790-4-093**
FY04 ARS Agreement Title:	**Developing Technologies to Enhance Utility of B. subtilis Against Wheat Scab.**
FY04 ARS Award Amount:	**$ 47,276**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
CBC	Developing Technologies to Enhance the Utility of B. subtilis Against Wheat Scab.	$ 47,276
	Total ARS Award Amount	**$ 47,276**

_____Gary C. Bergstrom_____July 15, 2005_____
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

(Form – FPR04)

Project 1: ***Developing Technologies to Enhance the Utility of B. subtilis Against Wheat Scab.***

1. What major problem or issue is being resolved and how are you resolving it?
Effective and economic means of controlling Fusarium head blight (FHB) are needed, and new chemical or biological control strategies have yet to meet the immediate and long-term needs of growers. Within the arena of integrated pest management, microbial biocontrol agents to control FHB have had modest success. That includes TrigoCor 1448, a proprietary isolate of *Bacillus subtilis*, which in some cooperative field tests has reduced FHB and mycotoxin contamination comparable to synthetic fungicides. This success, however, is tempered by the fact that the biology of the microbial antagonist and its interaction with *Fusarium* within the plant environment is poorly understood, and biocontrol efficacy is not consistent across environments.

Our project focused on developing technologies to supplement and expand the biological control potential of TrigoCor 1448. A preliminary experiment under greenhouse conditions indicated that particle films, such as Surround (kaolin), might be useful in providing a matrix for TrigoCor 1448 on the grain surface. Through greenhouse experiments we evaluated the timing of applications of TrigoCor 1448, alone and in combination with Surround and other potential adjuvents.

We assessed the survival of populations of *Bacillus* on the sprayed wheat spikes. Our technique included a treatment in which the spikes were suspended in water and boiled such that only heat-resistant spores would survive for plating on agar medium.

To enhance the biological control ability of TrigoCor 1448 for field application we first need to develop microbial production protocols that maximize the active chemical components in the microbial product. Chemical profiling of TrigoCor 1448 broth cultures was used to identify and quantify active components as a function of days of bacterial growth in shake culture. Surface response modeling was used to predict the effect of temperature, aeration, and time of culture on component production, while principle component analysis was used to delineate which components identified via HPLC profile analysis correlated with activity.

2. What were the most significant accomplishments?
In a long series of greenhouse experiments, the application of *Bacillus subtilis* strain TrigoCor 1448 to wheat spikes, prior to inoculation with conidial suspensions of *Fusarium graminearum*, consistently reduced the incidence and severity of Fusarium head blight symptoms and produced dramatic (5- to 10-fold) reductions in contamination of grain by deoxynivalenol. This protection occurred as experimental protocols were varied from inoculation at peak anthesis through soft dough stages. Though preliminary experiments suggested that particle film technology, i.e., the application of Surround brand of kaolin clay, might enhance the biocontrol activity of TrigoCor, repeated experiments with Surround application at either 2% or 6% weight/volume failed to produce further reductions in disease or mycotoxin. Other adjuvents such as CS-7 and Tween also failed to enhance the biological control activity of TrigoCor on wheat spikes.

Large, viable populations of spore-forming bacteria were shown to persist on sprayed wheat spikes more than a week following the application of TrigoCor 1448, while no spore-forming

bacteria were found on the surface of wheat spikes sprayed with water. This is a striking demonstration of the potential for TrigoCor 1448 to persist during grain filling on spikes of wheat.

We found an optimal time window of 5-6 days for production of active chemistries in shake broth culture that correlated with greater biocontrol activity. The active antifungal components included iturins, fengycins, and surfactins: mass analysis was used to confirm active chemistries.

Impact:
The fact that bacterial endospores of this biocontrol agent can survive long periods on dry wheat spikes in the greenhouse suggests that the bacterium has the capacity to persist in field environments as well. The knowledge that the anti-*Fusarium* activity of TrigoCor 1448 is maximal in broth shake cultures harvested after 5-6 days, when most bacterial cells have been transformed into endospores, and that this biological activity is correlated with peaks in certain antifungal lipopeptides, can be exploited to produce an enhanced, commercial biocontrol product.

As a result of the accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?
Knowledge of cultural conditions that increase the biocontrol activity of TrigoCor 1448 makes the production of a commercial biocontrol product more feasible. BioWorks, Inc. of Rochester, NY is currently assessing TrigoCor 1448 as a potential biocontrol product for broad-spectrum control of fungal diseases in plants.

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 you 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):

Maldonado-Ramirez, S.L, D.G. Schmale III, E.J. Shields, and G.C. Bergstrom. 2005. The relative abundance of viable spores of *Gibberella zeae* in the planetary boundary layer suggests the role of long-distance transport in regional epidemics of Fusarium head blight. *Agricultural and Forest Meteorology* : in press.

Schmale, D.G. III, Q.A. Arntsen, and G.C. Bergstrom. 2005. The forcible discharge distance of ascospores of *Gibberella zeae*. *Can. J. Plant Pathol*. 27: in press.

Schmale, D.G. III, D. A. Shah, and G.C. Bergstrom. 2005. Spatial patterns of viable spore deposition of *Gibberella zeae* in rotational wheat fields. *Phytopathology* 95*:* 472-479.

Del Ponte, E.M., J.M.C. Fernandes, C.R. Pierobom., and G.C. Bergstrom. 2004. Giberela do trigo – aspectos epidemiologicos e modelos de previsao. Fitopatologia Brasiliera 29:587-605.

Publications (not peer-reviewed):

Bergstrom, G.C. 2004. The promise and challenge of employing biological control in the integrated management of Fusarium head blight. Page 291 in Proc Second International Symposium on Fusarium Head Blight. Wyndham Orlando Resort, Orlando, FL, December 11-15, 2004.

Schmale, D.G. III, Q.A. Arnsten, and G.C. Bergstrom. 2004. The forcible discharge distance of ascospores of *Gibberella zeae*. Page 515 in Proc Second International Symposium on Fusarium Head Blight. Wyndham Orlando Resort, Orlando, FL, December 11-15, 2004.

Schmale, D.G. III, D.A. Shah, and G.C. Bergstrom. 2004. Spatial patterns of viable spore deposition of *Gibberella zeae* in wheat and corn fields. Page 514 in Proc Second International Symposium on Fusarium Head Blight. Wyndham Orlando Resort, Orlando, FL, December 11-15, 2004.

Presentations:

Presentations made by Gary C. Bergstrom on Fusarium head blight research and management:

Small Grains Management Field Day, Aurora, NY. (6/3/04).
Seed Growers Field Day, Ithaca, NY. (7/8/04).
Field Crop Dealer Meeting. Clifton Park, NY. (10/26/04)
Field Crop Dealer Meeting. New Hartford, NY. (10/27/04)
Field Crop Dealer Meeting. Batavia, NY. (10/28/04)
Field Crop Dealer Meeting. Auburn, NY. (10/29/04)
Agriculture and Food Systems In-Service, Ithaca, NY. (11/18/04).
Small Grains Seed Committee, Waterloo, NY. (3/24/05).
Western New York Soybean/Small Grains Congress. Waterloo, NY. (2/9/05)
Finger Lakes Soybean/Small Grains Congress, Batavia, NY. (2/10/05)
Cayuga County IPM Meeting at Monroe Tractor. Auburn, NY. (3/24/05)

Patent:
Support of this biocontrol research project in past years was instrumental in the development of **U.S. Patent No. 6,896,883 B2 (Biocontrol for plants with *Bacillus subtilis* …)**, issued May 24, 2005, to Cornell Research Foundation and Embrapa Trigo in Brasil. Inventors: Gary C. Bergstrom and Wilmar C. da Luz