

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY05 Final Performance Report (May 05 – May 07)
July 16, 2007**

Cover Page

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Fiscal Year:	2005
FY05 ARS Agreement ID:	59-0790-4-093
FY05 Agreement Title:	Developing Technologies to Enhance Utility for B. Subtilis Against Wheat Scab.
FY05 ARS Award Amount:	\$ 73,707

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
CBC	Defining Quality Parameters of B. subtilis for Optimized Performance in the Field.	\$ 44,439
EDM	Airborne spores of Gibberella zeae: Release, Viability, and Deposition Over Kilometer Distances.	\$ 29,268
	Total Award Amount	\$ 73,707

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 – FPR05)

Project 1: *Defining Quality Parameters of B. subtilis for Optimized Performance in the Field.*

1. What major problem or issue is being resolved and how are you resolving it?

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 focuses on developing technologies to supplement and expand the biological control potential of TrigoCor 1448. 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 and in solid substrate fermentation. Surface response modeling was used to predict the effect of growth medium 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. List the most important accomplishment and its impact (how is it being used?).

Complete all three sections (repeat sections for each major accomplishment):

Accomplishment:

We have optimized the small-batch laboratory fermentation of *Bacillus subtilis* strain TrigoCor 1448 on tryptic soy broth plus yeast extract for 7 days at room temperature. This produces stable liquid suspension products that are stored for several months at -80C before distribution to USWBSI collaborators for field testing. Optimal levels of antifungal compounds including iturins, fengycins, and surfactins were present in these preparations and have a shelf life of many months at refrigerated or freezing temperatures. When applied to wheat spikes in the glasshouse, 7 days or 1 day prior to inoculation with conidial suspensions of *Fusarium graminearum*, these preparations consistently reduced the incidence and severity of Fusarium head blight symptoms and produced reductions in contamination of grain by deoxynivalenol. When preparations were separated into cells and supernatant, both fractions produced detectable biological control against FHB. The greatest biocontrol was produced by the complete mixture, followed closely by supernatant alone, and finally by washed cells alone. This suggests that the preformed water-soluble, lipopeptides play a major role in the biocontrol of FHB by the bacterium.

Impact:

Correlation of biological control activity with peaks in antifungal lipopeptides can be exploited to produce an enhanced, commercial biocontrol product useful in the integrated management of FHB.

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?

Knowledge of cultural conditions that increase the biocontrol activity of TrigoCor 1448 makes the production of a commercial biocontrol product more feasible. Two companies are now examining the biocontrol agent for plant disease control and considering the licensing of this technology.

Project 2: Airborne spores of *Gibberella zeae*: Release, Viability, and Deposition Over Kilometer Distances.

3. What major problem or issue is being resolved and how are you resolving it?

The prevailing thought within the FHB research and grower community still maintains that aerial spore inoculum for annual epidemics of FHB comes predominantly from debris within or very near to the cereal crop to be infected, though such distance has never been clearly defined. We have observed severe epidemics of FHB in rotational wheat fields in the Northeast and have produced several lines of evidence suggesting that the inoculum arrives in the air from sources at considerable distance from where those spores are liberated. We hypothesized that the majority of spores deposited in rotational wheat fields comes from well-mixed, regional atmospheric sources, and therefore that the number of spores deposited over a square kilometer area in a given sample period will be of the same general magnitude. Our objective was to assess regional patterns of viable spore deposition of *Gibberella zeae* over landscape environments on a kilometer scale. We monitored the daily deposition of viable airborne spores of *G. zeae* over two winter wheat fields, two soybean fields, one alfalfa hay field and one fallow corn field, all within 1 square km, in central New York during the period of local wheat flowering in 2005.

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

Complete all three sections (repeat sections for each major accomplishment):

Accomplishment:

We demonstrated that, for any particular day or night collection period, the atmospheric deposition of viable spores of *G. zeae* was of a very similar magnitude in seven different agricultural fields (within 1 square kilometer) that varied by growing crop species and the presence or absence of corn residues on the soil surface. Thus flowering wheat and barley crops within 1 km of infected cereal debris (and probably greater distances) are exposed to similar spore loads as those cereal crops that are in direct proximity to infected cereal debris. This work is published in *Can. J. Plant Pathol.* 28:100-108 (2006).

Impact:

Though the presence of infested debris within a wheat or barley canopy is a documented risk factor for FHB, this research documents that the risk of exposure to airborne, viable spores extends to at least a kilometer from inoculum sources, and probably much greater. Airborne spores in a local region are highly mixed and the magnitude of their deposition by gravitational settling or rain-washout is very similar over a fairly wide area.

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?

The community should now have a more realistic concept that spore inoculum for annual epidemics of FHB comes from over a considerable distance from the source of residue on the ground, not just in close proximity to infested debris. Localized control of debris-borne inoculum is not likely to provide highly effective management of FHB unless practiced over wide production areas.

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):

- Pryor, S.W., D.M. Gibson, G.C. Bergstrom, and L.P. Walker. 2007. Minimization of between-well sample variance of antifungal activity using a high-throughput screening microplate bioassay. *Biotechniques* 42:168-172.
- Schmale, D.G. III and G.C. Bergstrom. 2007. The aerobiology and population structure of *Gibberella zeae*. *Plant Health Progress: in press*.
- Schmale, D.G. III, J.F. Leslie, K.A. Zeller, A.A. Saleh, E.J. Shields, and G.C. Bergstrom. 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. *Phytopathology* 96: 1021-1026.
- Schmale, D.G. III, E.J. Shields, and G.C. Bergstrom. 2006. Night-time spore deposition of the fusarium head blight pathogen, *Gibberella zeae*, in rotational wheat fields. *Can. J. Plant Pathol.* 28:1-9.
- 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* 132: 20-27.
- 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: 376-382.
- 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.
- Schmale, D.G. III, D. A. Shah, and G.C. Bergstrom. 2005. Spatial patterns of viable spore deposition of the ear rot pathogen, *Gibberella zeae*, in first-year corn fields. *Can. J. Plant Pathol.* 27: 225-233.

Publications (not peer-reviewed):

- Schmale, D.G. III, A.K. Wood-Jones, G.C. Bergstrom, and C. Cowger. 2006. Spatial patterns of trichothecene genotypes of *Gibberella zeae* in wheat fields. Page 140 in Proc. 2006 National Fusarium Head Blight Forum, Sheraton Imperial Hotel, Research Triangle Park, North Carolina, December 10-12, 2006.
- Schmale, D.G. III, A.K. Wood-Jones, and G.C. Bergstrom. 2006. Trichothecene genotypes in atmospheric populations of *Gibberella zeae*. Page 141 in Proc. 2006 National Fusarium Head Blight Forum, Sheraton Imperial Hotel, Research Triangle Park, North Carolina, December 10-12, 2006.
- Bergstrom, G.C. and D.G. Schmale III. 2005. Aerobiology and regional epidemiology of *Gibberella zeae*. Page 76 in Proc. 4th Canadian Workshop on Fusarium Head Blight. Ottawa Congress Centre. Ottawa, Canada, November 1-3, 2005.

- Schmale, D.G. III, J.F. Leslie, R.L. Bowden, K.A. Zeller, A.A. Saleh, E.J. Shields, and G.C. Bergstrom. 2005. Genetic structure of atmospheric populations of *Gibberella zeae*. Page 149 in Proc. 2005 National Fusarium Head Blight Forum, Hilton Milwaukee City Center, Milwaukee, WI, December 11-13, 2005.
- Schmale, D.G. III, J.F. Leslie, A.A. Saleh, E.J. Shields, and G.C. Bergstrom. 2005. Temporal scales of genetic diversity within New York atmospheric populations of *Gibberella zeae*. Page 149 in Proc. 2005 National Fusarium Head Blight Forum, Hilton Milwaukee City Center, Milwaukee, WI, December 11-13, 2005.

Ph.D. Theses:

- Pryor, Scott W. 2005. Optimization of the solid state fermentation of *Bacillus subtilis*: Production of a antifungal lipopeptides and use a a biological control agent. Cornell University, Ithaca, NY 203pp.
- Schmale, David G. III. 2006. The aerobiology and population genetic structure of *Gibberella zeae*. Cornell University, Ithaca, NY 214 pp.

United States Patent:

United States Patent No. 6,896,883 B2 issued on May 24, 2005. Title: Biocontrol for plants with *Bacillus subtilis*, *Pseudomonas putida*, and *Sporobolomyces roseus*. Inventors: Gary C. Bergstrom and Wilmar Corio da Luz. Assignees: Cornell Research Foundation and Embrapa Trigo (Brazil)

Presentations:

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

- Small Grains Management Field Day, Aurora, NY (6/2/05).
Seed Growers Field Day, Ithaca, NY (7/7/05).
Agriculture and Food Systems In-Service, Ithaca, NY (11/4/06).
New York State Agribusiness Association, Auburn, NY (1/10/06).
Vermont Pesticide Applicators, Middlebury, VT (1/19/06).
Northeast Organic Farm Association of New York, Syracuse, NY (1/29/06).
Small Grains Seed Committee, Waterloo, NY (3/8/06).
New York Certified Organic Crops Group (NYCO). Geneva, NY. (03/15/06).
Franklin County Crops Day, Malone, NY (3/28/06).
Small Grains Management Field Day. Aurora, NY. (6/8/06).
Seed Growers Field Day, Ithaca, NY. (7/8/06).
Western New York Soybean and Small Grains Congress, Batavia, NY. (2/7/07).
Finger Lakes Soybean and Small Grains Congress, Waterloo, NY. (2/8/07).
Steuben County Crop Symposium, Bath, NY. (2/27/07.)
Vermont Crops n' Critters Conference, Bridport, VT. (3/20/07).