U.S. Wheat and Barley Scab Initiative
Annual Progress Report
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Cover Page

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Year:	**FY2000**
Grant Number:	**59-0790-9-027**
Grant Title:	**Fusarium Head Blight Research**
Amount Granted:	**$57,450.00**

Project

Program Area	Objective/Project	Amount Requested
Chemical & Biological Control	Identify safe, effective fungicides for FHB through evaluation on wheat and/or barley varieties grown in relevant environments.	$5,000.00
Chemical & Biological Control	To identify other fungal antagonists, such as bacteria, that may be effective in controlling FHB when used as an anthesis time field spray, seed treatment, or residue treatment. Also, to examine other residue treatments that might interfere with perithecial development or spore release.	$40,000.00
Epidemiology & Disease Management	Identify the environmental conditions that trigger the initiation of ascospore release and affect the the duration of ascospore discharge.	$12,450.00
	Total Amount Requested	$57,450.00

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Principal Investigator Date

(Form – PR1)

Project 1: Identify safe, effective fungicides for FHB through evaluation on wheat and/or barley varieties grown in relevant environments.

S. O. Kawamoto, C.A. Stockwell, D. J. Otis, W. J. Cox, and G. C. Bergstrom

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

At present, there are no means to adequately control the infection of wheat and barley by *Gibberella zeae* that are both highly effective and widely regarded as safe. The objective was to evaluate a uniform set of fungicide treatments across a number of locations and crops, to achieve more information on potentially useful fungicides and bioprotectants to control FHB. Background and description of the Uniform Fungicide Trial is provided in the progress report by Marcia McMullen.

2. *Please provide a comparison of the actual accomplishments with the objectives established.*

We carried out all the procedures as outlined in the New York test location and produced a valuable data set on the effects of sprays on crop yield and quality (Table 1.1), in a season of high disease incidence but relatively modest impact of disease on yield, a circumstance frequently encountered by soft winter wheat producers. We invested over $5,000 in an irrigation system which provided mist during the critical period for infection. However, frequent precipitation during anthesis probably made the irrigation system unnecessary this year.

3. *What were the reasons established objectives were not met? If applicable.*

Nearly all objectives were met. We are awaiting vomitoxin results from Michigan State University. When those results are statistically analyzed, all objectives will have been met.

4. *What were the most significant accomplishments this past year?*

We were able to evaluate foliar fungicides and bacterial antagonists under a natural epidemic in which the major impact of the disease was on test weight and vomitoxin contamination rather than grain yield. These are conditions frequently encountered in the soft winter wheat production areas. Since there was no significant foliar disease during early grain development, it can be inferred that spray effects were directly attributable to Fusarium head blight suppression. Several treatments produced statistically significant reductions in disease incidence and Fusarium damaged kernels. Yet, despite excellent spray coverage and nearly perfect spray timing, based on previous research, no treatment reduced scab dramatically as hoped. No treatment resulted in a yield increase that was significantly better at the 95% confidence level than nontreated. Folicur 3.6F (at 4 fl oz, at 6 fl oz, and at 2 fl oz plus 6.2 fl oz BAS 500F) produced the largest (ca. 6 bu) increases in yield. Two treatments, Folicur 3.6F at 6 fl oz, and Folicur 3.6F at 4 fl oz plus the bioprotectant TrigoCor 1448, significantly increased test weight. The most important potential effect of scab fungicides from a New York producer viewpoint is vomitoxin reduction. We defer conclusions on this aspect of the study pending receipt of vomitoxin analyses from Michigan State University.

Project 2: To identify other fungal antagonists, such as bacteria, ...

C. A. Stockwell, S. O Kawamoto, W. C. da Luz, and G. C. Bergstrom

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

There is a need for safe, affordable and efficacious biological and bio-compatible protectants in the integrated management of FHB. This project, like the development of any effective biological control, follows a pattern of step-wise advancement from laboratory to greenhouse and finally to the field. Emphasis is being placed upon the selection of organisms which are likely to be robust under harsh field conditions. Candidate agents will be evaluated for use as a heading time field spray, seed treatment, or residue treatment. Test results from the FY2000 project will provide information to choose the most promising organisms and bio-compatible treatments to be included in the next level of evaluations thus advancing biological control of wheat and barley scab closer to the ultimate goal of commercial application.

2. Please provide a comparison of the actual accomplishments with the objectives established.

This project may be divided into the following 3 phases:

Phase 1. In this phase, a culture collection was assembled from locally-isolated organisms and elite Brazilian accessions provided by our collaborator, Dr. Wilmar Luz of the EMBRAPA-TRIGO program. This resulted in the isolation and preservation (stored in cryovials in 15 % glycerol at -80° C) of 120 candidate biocontrol organisms from 70 different sources. Concurrent with the creation of a culture collection, efforts were made to develop and evaluate bioassays for the characterization and screening of accessions.

This phase has been completed.

Phase 2. A substantial portion of the biocontrol culture collection has been screened using bioassays for antibiosis and tolerance to UV radiation. These bioassays were employed to identify organisms which are both tolerant to environmental stresses and are able to inhibit fungal growth *in vitro*. Several of the more promising isolates were tested in an evolving series of glasshouse experiments (Tables 2.4). Two of these experiments were designed to determine the optimal conditions for scab infection and efficacy of the elite biocontrol isolate, TrigoCor 1448. The goal of additional glasshouse experiments was to evaluate promising isolates for their ability to reduce scab under controlled environmental conditions.

Work on this phase is well underway.

Phase 3. Fall/Spring 2000 -

Debris treatment- Fourteen treatments (9 candidate biocontrol organisms, 3 bio-compatible treatments, and a control) were applied to artificially-infested maize stubble (nodes and internodes) and grain in December 1999 and then allowed to over-winter under ambient exterior environmental conditions. The purpose of this study (Table 2.1) was to evaluate the effect of various treatments on perithecial development and ascospore discharge with the goal of reducing the amount of initial inoculum of *Gibberella zeae* available for infection of the flowering wheat the following Spring.

Seed treatment- The bioprotectant TrigoCor 1448 was included at three concentrations as part of a larger a field trial on the effect of seed treatments on hard red winter wheat (cv. 'Crimson') with 25-32% incidence of seed infection by *Fusarium* sp. (assayed by freezer blotter test) (Table 2.2). The number of seedlings per meter was greater in the 10^9 cfu/cwt treatment than the untreated control but less than the Raxil-Thiram treatment. There was no significant difference between treatments in the seedling weight or grain yield. In an accompanying *in vitro* assay, the % emergence at the highest

concentration (10^9 cfu/cwt) was slightly higher (4.6%), although not significantly different, than the untreated control.

Anthesis-time spray- The most promising treatments were included in field evaluations on winter wheat. Three elite biocontrol bacteria and one bio-compatible fungicide (Armicarb SR) were added as treatments to the Uniform Fungicide Trial at the New York State location (Table 1.1). Two of these organisms (TrigoCor 1448 and TrigoCor 9790) were included based on their performance in Brazilian field evaluations. The third organism (TrigoCor 4712) had proved promising, along with TrigoCor 1448, in greenhouse evaluations. In this same trial, TrigoCor 1448 was combined with Folicur (4 oz) to determine if the combination would give enhanced scab control over either treatment alone. In addition, one or two isolates (TrigoCor 1448 and TrigoCor 9790) were sent to collaborators in 10 states for inclusion in the Uniform Fungicide trial or in a separate evaluation. Only a few of these sites had sufficient scab incidence for treatment evaluation.

Lastly, in order to evaluate additional promising biocontrol isolates under field conditions, a separate experiment was designed in which ten organisms and 3 binary combinations were applied to very small-sized replicated plots.

These experiments have been completed for the 1999-2000 field year and the data is currently being compiled and analyzed. We are awaiting the results of the DON analysis of the field experiments. Experiments for the 2000-2001 field year are presently being designed based on the results from this year.

3. What were the reasons established objectives were not met? If applicable.

Established objectives are being met in a timely manner. Space and time constraints limit the number of treatments that can be evaluated in the greenhouse or in the field. Therefore only a small proportion of the isolates in the culture collection have been evaluated beyond *in vitro* bioassays.

4. What were the most significant accomplishments this past year?

Glasshouse evaluation of biocontrol isolates

a) Timing- Incubation of wheat plants for 48 hours in a mist chamber following inoculation with a spore suspension (10^5 cfu/ml) of *Fusarium graminearum* macroconidia resulted in both good incidence of infection (75% and 96%) and adequate seed set (Table 2.4). Treatment of the wheat with TrigoCor 1448 24 hours prior to inoculation with the spore suspension, reduced scab by 15 and 22% in the two experiments and increased 100-seed weight by 13.1 and 45%. TrigoCor 1448 was included as a benchmark in subsequent glasshouse evaluations of other candidate biological control organisms.

b) Isolate evaluation- Treatment with TrigoCor 1448 and TrigoCor 4712 consistently, with only one exception, resulted in decreased disease incidence and increased 100-seed weight when compared to the untreated control (Table 2.1). Furthermore, the biocontrol isolate TrigoCor 4712 had the highest 100 seed weight of any treatment in each of the four trials in which it was included. Several other isolates showed promising results as well. At this time, only one glasshouse experiment has been analyzed for the presence DON toxin. Treatment with TrigoCor 1448 and TrigoCor 4712 reduced the toxin content of the seed by 26.8 and 71.2 % respectively compared to that present in the nontreated conreol.

Debris treatment- Treatment of the stem pieces containing nodes with 5% acetic acid (sampled

early Spring: March 22, 2000) was the only treatment that resulted in the complete absence of perithecia and therefore spore discharge. All other treatments resulted in higher numbers of perithecia and ascospore discharge than the control (water). Internode stem pieces and kernels which were collected from the field site at later dates were in more advanced states of decomposition, but the 5% acetic acid treatment also did not produce any perithecia when incubated under favorable laboratory conditions.

Anthesis-time spray- Two of the three bacterial isolates (TrigoCor 1448 and TrigoCor 4712) tested in the Uniform Fungicide trial at the New York location gave slight reductions in the % incidence of scabby heads and % fusarium damaged kernels (%fdk), although test weight and yield were not significantly different. When Folicur (4 fl oz) was added to the TrigoCor 1448 treatment, % incidence of scabby heads and % fdk was the lowest and test weight the highest of any of the treatments included in the trial. Conversely, the bio-compatible fungicide Armicarb SR had a far greater scab incidence and a lower test weight than any other treatment.

Project 3: Identify the environmental conditions that trigger the initiation of ascospore release and affect the the duration of ascospore discharge.

S. L. Maldonado, D. M. Gadoury, and G. C. Bergstrom

1. *What major problem or issue is being resolved and how are you resolving it?*
   One critical yet unknown aspect of the aerobiology and epidemiology of *Gibberella zeae* is the effect of environmental parameters on the release of ascospores from mature perithecia. This knowledge is a necessary component of scab advisory and forecast systems. Our goal is to pinpoint environmental triggers that initiate ascospore release and affect the duration of release events. We are accomplishing this by two experimental approaches. Time course ascospore capture from ascocarp-bearing corn stalk tissues is being conducted with a volumetric spore trap in a natural environment where weather variables are monitored. Also we are utilizing a controlled environment laboratory wind tunnel to study ascospore release under variable conditions.

2. *Please provide a comparison of the actual accomplishments with the objectives established.*
   A protocol was developed for preparation of ascocarp-bearing corn stalk pieces for use in both field and wind tunnel experiments. Corn stalk pieces bearing mature perithecia were placed on a specially designed platform that was mounted at the orifice of the Burkard spore collector. Hourly ascospore counts and weather information were collected using one set of corn stalk pieces from 4 June to 2 July. Hourly captures of ascospores on Burkard tapes ranged from 0 to 3862. During the collection period, there were seven major spore release events (greater than 1000 spores) with durations of 1 to 6 hours. All but one major release occurred during daylight hours! Correlation of releases with weather variables is still being done. Similar data will be collected over other intervals. Experiments in the laboratory wind tunnel at the New York Agricultural Experiment Station in Geneva have just begun.

3. *What were the reasons established objectives were not met? If applicable.*
   Funded work on this objective was begun in May 2000 and is on schedule to be completed prior to May 2001.

4. *What were the most significant accomplishments this past year?*
   Protocols for conducting the ascospore release experiments have been refined. Preliminary evidence suggests that ascospore release occurs predominantly during daylight hours.

Included below is a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about the projects included in this grant:

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

- Small Grains Committee, New York Seed Improvement Cooperative. Waterloo, NY. (3/8/00)
- Small Grains Management Field Day, Aurora, NY (6/13/00)
- Cornell Seed Growers Field Day, Ithaca, NY. (7/6/00)
- Musgrave Research Farm Field Day, Aurora, NY. Fungicides and biocontrol bacteria for management of wheat scab and foliar diseases. (7/7/00)

Publication (non-peer reviewed):

Stockwell, C.A., G.C. Bergstrom, and W.C. da Luz. 1999. Selection of microbial antagonists for biological control of Fusarium head blight of wheat. Pages 82-84 in *Proc. 1999 National Fusarium Head Blight Forum*. USDA-ARS.

Table: 1.1 Effects of fungicides and bioprotectants applied at anthesis on Fusarium head blight incidence, *Fusarium* damaged kernels, yield, and test weight of Caledonia wheat at Aurora, NY in 2000.

Treatment		Scab Incidence (% spikes) 6/19/2000	6/26/2000	Test weight @ 13.5% moisture (lb/bu)	Yield @ 13.5 % moisture &13.5% tw (bu/A)
Nontreated	.....	3.5	14.1	56.1	74.0
AMS 21616	.....	na	na	56.7	77.4
Armicarb SR (5 lb/A)	.....	7.5	23.5	54.5	76.7
BAS 500F (12.3 fl oz/A)					
+ Agridex COC (1% v/v)	.....	3.0	11.7	56.7	73.5
BAS 500F (6.2 fl oz/A)					
+ Agridex COC (1% v/v)					
+ Folicur 3.6F (2 fl oz/A)	.....	1.9	14.0	56.4	80.1
Folicur 3.6F (4 fl oz/A)					
+ Induce (0.125% v/v)	.....	3.3	11.1	57.0	82.4
Folicur 3.6F (6 fl oz)					
+ Induce (0.125% v/v)	.....	na	na	58.0	80.4
Quadris 2.08SC (9.2 fl oz/A)					
+ Benlate 50WP (0.25 lb/A)	.....	2.3	16.2	56.0	76.2
Stratego E (14 fl oz/A)					
+ Induce (0.125% v/v)	.....	2.1	11.6	56.6	74.1
Tilt (4 fl oz/A)	.....	2.6	10.5	56.8	74.1
TrigoCor 1448	.....	2.2	11.7	56.6	74.2
TrigoCor 1448					
+ Folicur 3.6F (4 fl oz/A)					
+ Induce (0.125% v/v)	.....	1.4	8.7	58.3	80.1
TrigoCor 4712	.....	3.3	11.9	56.6	77.3
TrigoCor 9790	.....	3.3	17.0	56.5	77.2
LSD (*p*=0.05)		0.4	0.6	1.1	NS
cv (%)		25.4	14.0	0.1	12.1

Table: 2.1 Summary of evaluations of candidate biocontrol organisms in glasshouse/mist chamber to control *Fusarium graminearum* applied as a spore suspension (1.0×10^5 cfu/ml) on spring wheat (cv. Norm).

	Weight (g) / 100 seeds					
Treatment[1]	expt 2[2]	expt 3	expt 6	expt 7	expt 9	expt 10
H2O/F.g.	3.40	1.46	0.87	1.82	1.57	2.37
TrigoCor 1448/F.g.	3.10	nd	1.64	2.29	1.84	2.60
TrigoCor 4712/F.g.	3.90	2.85	1.67	nd	nd	2.77
TrigoCor 4821/F.g.	nd	nd	nd	2.20	nd	nd
TrigoCor 5152/F.g.	nd	nd	nd	nd	2.08	2.29
TrigoCor 5231.1/F.g.	nd	nd	nd	nd	2.02	2.56
TrigoCor 5394/F.g.	3.00	2.30	0.84	nd	nd	nd
TrigoCor 6224.1.2/F.g.	nd	nd	nd	nd	1.86	2.67
TrigoCor 6388/F.g.	3.40	1.49	nd	nd	nd	nd
TrigoCor 9725.1/F.g.	nd	nd	1.58	2.61	nd	nd
TrigoCor 9770/F.g.	nd	nd	1.47	nd	nd	nd
TrigoCor 9786/F.g.	3.20	nd	0.94	2.36	nd	nd
TrigoCor 9788.1.1.1/F.g.	nd	nd	nd	nd	1.64	nd
TrigoCor 9788/F.g.	nd	nd	nd	1.95	nd	nd
TrigoCor 9790.2/F.g.	nd	nd	nd	nd	1.60	nd
TrigoCor 9790.4/F.g.	nd	nd	nd	nd	nd	2.22
TrigoCor 9828/F.g.	nd	nd	nd	1.99	nd	nd

nd = not done

[1]Wheat heads sprayed with water or suspension of biocontrol isolate and allowed to air dry for 24 hours before inoculation with conidial suspension (10^5 cfu/ml) of *Fusarium graminearum*; 48 hours post-inoculation incubation in mist chamber, plants grown in glasshouse until maturity.
[2]All treatments replicated 5 times except expt. 6 (replicated 4 times)

Table 2.2 1999-2000 Debris Treatment Trial (partial data set). Artificially infested pieces of maize stem pieces and kernels were over-wintered in the field at the Musgrave Research Farm, Aurora, NY.

	Stem tissue		Seeds	
Treatment	**perithecia[1]**	**% discharge[2]**	**perithecia[3]**	**% discharge**
Check (H20)	9	30	1.5	0
Biocompatible fungicides				
acetic acidd4 (5%)	0	0	0	0
NH4-bicarb (.59% w/w)	11	80	0	0
Urea-bicarb (.59% w/w)	17	60	4	0
Biological control isolates[5]				
TrigoCor 1448	27	80	0.5	0
TrigoCor 4712	26	50	3	0
TrigoCor 5394	10	50	0.5	0
TrigoCor 6941	42	60	1.5	0
TrigoCor 9537	30	80	8.5	0
TrigoCor 9708	25	60	2	0
TrigoCor 9725.2	48	70	7.5	0
TrigoCor 9770	20	100	2	0
TrigoCor 9786	50	80	1	0

[1] Removed from field on March 22, 2000; average of 5 replications number of perithecia on stem surface counted using a standardized grid (total area= 94.2 mm^2).
[2] Removed from field on March 22, 2000; average of 4 replications % of stem pieces from which ascospores were discharged onto inverted plates of Komada's medium.
[3] Removed from the field on June 7, 2000; number of pieces of pericarp on which perithecia developed
[4] food grade white vinegar
[5] biocontrol isolates were grown in flasks of 200 ml nutrient broth yeast extract agitated continuously at 100 rpm for up to 48 hours (turbid) on a shaker table. Population counts for TrigoCor 1448 and TrigoCor 4712 were 10^9 and 10^8 cfu/ml.

Each treatment was applied by immersing the plant tissue into a liter flask containing 50 ml of the cell suspension, biocompatible solution or sterile distilled water. The plant material was agitated with the fluid for exactly 3 minutes before the fluid was drained and the plant tissue was spread out and allowed to air dry.

Treatments were randomly arranged on the soil surface in nylon mesh pouches in a field planted to wheat. Ten stem pieces or 50 seeds of maize were placed in each pouch.

The pouches were secured to the ground with metal pins.

Table 2.3 Effect of treatment of Fusarium-infested[1] hard red winter wheat (cv. Crimson) seed with the bacterial bioprotectant TrigoCor 1448 on emergence, seedling weight and grain yield. Musgrave Research Farm Aurora, NY Fall 1999. Data was extracted from a larger experiment.

Treatment	Seedling Wt.[2] (g)	#seedlings/m	grain yield (bu/A) [3]
Nontreated	0.4	58	38.6
TrigoCor 1448			
(1.1 x 10^7cfu/100 lbs seed)	0.4	60.3	39.4
(1.1 x 10^8cfu/100 lbs seed)	0.4	54.3	43.5
(1.1 x 10^9cfu/100 lbs seed)	0.3	68.8	38.2
Raxil-Thiram (3.5 fl oz)	0.3	79.3	39.7
LSD (p=0.05)	NS	16.7	NS
cv (%)	10.9	17.1	12.4

[1] 25-32% of the seed infested with *Fusarium* sp.; assayed by freezer blotter method.
[2] Seedling weight taken at 2 leaf stage (Feekes growth stage 1)
[3] yield calculated for 13.5% moisture

Table 2.4 Glasshouse/mist-chamber experiment to determine optimal conditions for scab incidence and efficacy of the biocontrol organism TrigoCor 1448.

	Timing Experiment 1[1]		
	visual incidence	Wt. (g) / 100 seed	freezer blotter
Treatment	mean (%)	mean	mean (%)
NoBC/No Fg	2.2	4.16	2.6
No BC/+Fg 24h	11.8	3.42	14.8
No BC/+Fg 48h	95.6	1.49	29.6
No BC/+Fg 72h	100	0.46	12.8
BC 0/+Fg 24	6.2	3.27	18.8
BC 0/+Fg 48	73.6	2.16	47.2
BC 0/+Fg 72	100	0.59	17.5

	Timing Experiment 2 [2]		
	visual incidence	wt/100 seed (g)	freezer blotter
Treatment	mean (%)	mean	mean (%)
NoBC/NoFg 48hr [3]	3	3.91	0.6
NoBC/+Fg 24hr	9	3.77	3.7
No BC/+Fg 48hr	75	3.13	7.2
BC 0/+Fg 24hr	8	3.89	2.1
BC 0/+Fg 48hr	15	3.54	6.6
BC24/+Fg 24hr	3	3.97	4.0
BC24/+Fg 48hr	60	3.00	6.8

[1] Average of 5 replicated treatments
[2] Average of 3 replicated treatments
[3] Explanation of treatments:
NoBC = Biocontrol organism **not** applied to wheat head
BC 0 = TrigoCor 1448 applied 24 hours before inoculation with pathogen;
0 hrs incubation in mist chamber before subsequent inoculation with pathogen
BC24 =TrigoCor 1448 applied 24 hours before inoculation with pathogen;
24 hrs incubation in mist chamber before subsequent inoculation with pathogen
/NoFg 48hr= wheat **not** inoculated with pathogen; 48 hrs in mist chamber
/+Fg 24hr= plants inoculated with pathogen; 24 hrs in mist chamber
/+Fg 48hr= plants inoculated with pathogen; 48 hrs in mist chamber
/+Fg 72hr= plants inoculated with pathogen; 72 hrs in mist chamber