USDA-ARS / USWBSI
FY04 Final Performance Report
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Cover Page

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Year:	**FY2004** (approx. May 04 – April 05)
FY04 ARS Agreement ID:	**59-0790-1-079**
FY04 ARS Agreement Title:	**Development and Evaluation of Biological Control Agents for Fusarium Head Blight Control.**
FY04 ARS Award Amount:	**$ 18,341**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
CBC	Enhancement of Biological Control by Lysobacter enzymogenes C3.	$ 11,512
CBC	Standardized Evaluation of Biological Control Agents.	$ 8,829
	Total ARS Award Amount	**$ 18,341**

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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

Project 1: ***Enhancement of Biological Control by Lysobacter enzymogenes C3.***

1. What major problem or issue is being resolved and how are you resolving it?
C3, a strain of the bacterial species *Lysobacter enzymogenes*, was consistently and highly efficacious in reducing the severity of Fusarium head blight (FHB) in over two-years of greenhouse experimentation, but field efficacy has been difficult to achieve using this agent. The goal of this project is to identify strategies that will improve field efficacy using C3. The objectives were to: 1) identify application strategies to enhance its efficacy, 2) assess the potential of combining C3 with other bacterial biocontrol agents, 3) identify cultural methods that will enhance antagonism by C3. In two field experiments, a formulation was tested in which C3 cells were collected from broth cultures by centrifugation and resuspended at a concentration 10-fold higher than in original broth cultures. Other strategies tested for improving C3 efficacy included applying C3 at head emergence, applying C3 twice (at head emergence and at anthesis), and applying C3 in combination with tebuconazole. C3 also was applied in combination with the *Bacillus* strains TrigoCor 1448 and 1BC. Laboratory experiments were conducted to determine the effects of mixing cultures of C3 and *Bacillus* strains on the survival of the agents. Strains of bacteria that induced systemic resistance to other pathogens were obtained from other researchers and were investigated in the greenhouse for potential control of FHB and possible combination with C3. C3 was cultured in various broth media and then cells and liquid from these cultures were compared for FHB suppression in the greenhouse.

2. What were the most significant accomplishments?
In field tests, application of C3 in high cell concentration provided little improvement over the standard C3 treatment (full-strength chitin broth culture); this may have been due to C3 cells not surviving well in concentrated form and to induced plant resistance elicited by C3 not requiring high cell numbers, as discovered in separate laboratory experiments. Single (at heading or at anthesis) and double applications of C3 provided similar reduction of scab severity as tebuconazole alone; the best treatment in the trial was a tank mix of C3 and tebuconazole. In laboratory experiments to combine C3 with *Bacillus* biocontrol agent strains, C3 metabolites inhibited the growth of the other bacteria. This supports results from field tests in which C3 alone, *Bacillus* strains alone, and C3-*Bacillus* combinations yielded similar levels of control. Two strains of bacteria reported to induce systemic host resistance in dicot plants were found in a greenhouse test to suppress FHB. In experiments comparing culture media, chitin broth was the best medium in terms of producing biocontrol-effective cells, whereas cells grown on other common media (TSB and LB) were less effective in biocontrol. Some media (chitin broth) induced higher levels of lytic enzymes, but lower concentrations of antibiotics than other media (e.g. TSB). Nutrients in common media used to culture C3 and other bacteria were found to be utilized by *Fusarium graminearum*. Various carbon sources have been identified that can be utilized by C3 in exclusion of *F. graminearum*, but are too costly to utilize in preparing media for biocontrol agent propagation.
Impact: The results from this project show no immediate benefit from manipulating C3 culture media or from developing formulations with high C3 cell numbers. Finding that combining C3 with *Bacillus* strains does not lead to improved scab control indicates a need to for new groups of biocontrol agents that may be synergistic with C3 or *Bacillus* strains. Promising results with the combination of C3 with a fungicide point to this strategy being a potential avenue for improving control efficacy using C3 and other biological agents.

Project 2: ***Standardized Evaluation of Biological Control Agents.***

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

Biological control methods continue to be a difficult strategy to develop for management of Fusarium head blight (FHB). None of the biological control agents being investigated as part of the USWBSI have been developed to the same level as commercial fungicides as to scale of production and formulation. Therefore, a special program is needed to evaluate biological control agents over a wide geographic range and across different genotypes of wheat and barley while maintaining standard experimental procedures. The goal of this project was to provide a basis for such standardized testing. My specific objectives were to: 1) coordinate efforts for a standardized evaluation of biological control agents across multiple states, and 2) provide a field test site in Nebraska as part of this effort.

Five trials were conducted on wheat across four states (Arkansas, Missouri, Nebraska, and South Dakota) on a range of market classes; a sixth trial was conducted in South Dakota on barley. In each trial, four biological agents (Gram-positive bacterium AS 54.6 *Bacillus subtilis* TrigoCor 1448 *Bacillus* sp.1BC *Lysobacter enzymogenes* C3R5) were tested. A culture of each organism was provided to the researcher in each location and inoculum for treatment was propagated by the researcher following instructions provided by the organism's supplier. The pre-application population of each agent in the inoculum was determined by the local researcher using dilution plating. In addition to the biological agents, there was a non-treated control and a treatment with the fungicide tebuconazole, as Folicur 432SC. Applications were standardized across site as to timing of applications (Feekes 10.51), spray methods, and data collection. Results from all trials were analyzed together.

What were the most significant accomplishments?

None of the treatments with a biological agent or with Folicur 432SC had a significant effect on disease incidence, severity, or deoxynivalenol level compared to the control across the trials. The agents also were ineffective in all of the individual trials. Folicur 432SC also had no effect in individual trials except to provide a significant reduction in deoxynivalenol in one site. Biocontrol agent numbers in the inoculum cultures varied considerably among agents and among locations. In many instances, cell concentrations determined at the time of application were several orders of magnitude lower than expected. The low population numbers applied could have been a contributing factor to lack of efficacy in the biological treatments.

Impact: This experience points to the need for better control over microorganism numbers when testing biological agents. The fact that Folicur also was ineffective across these trials is an indication that suppression of FHB under field conditions remains a difficult objective to achieve using biological or chemical treatments.

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

Yuen, G.Y., B.H. Bleakley, M.A. Draper, C.C. Jochum, E.A. Milus, K.R. Ruden, and L.E. Sweets. 2004. Results From the 2004 Standardized Evaluation of Biological Agents for the Control of Fusarium Head Blight. Proceedings of the 2nd International Symposium on Fusarium Head Blight Forum, pages 380-382.

Yuen, G.Y. and C.C. Jochum. 2004. Factors That Can Affect Field Efficacy of Biological Control Against Fusarium Head Blight (Abstract). Proceedings of the 2nd International Symposium on Fusarium Head Blight Forum, pages 379.

Presentation

Gary Y. Yuen and C. Christine Jochum. Factors That Can Affect Field Efficacy of Biological Control Against Fusarium Head Blight. Poster presented at the 2nd International Symposium on Fusarium Head Blight Forum, 2004.