USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY09 Final Performance Report
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Cover Page

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Fiscal Year:	2009
USDA-ARS Agreement ID:	NA
USDA-ARS Agreement Title:	Efficacy and Characterization of Fungicide and Cryptococcus flavescens Mixtures.
FY09- USDA-ARS Award Amount:	$ 20,000

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
MGMT	Efficacy and Characterization of Fungicide and Cryptococcus flavescens Mixtures.	$ 20,000
	Total Award Amount	**$ 20,000**

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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 Winter Wheat Region
SWW – Southern Sinter Wheat Region

Project 1: *Efficacy and Characterization of Fungicide and Cryptococcus flavescens Mixtures.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Employing integrated pest management (IPM) strategies to reduce Fusarium head blight (FHB) and DON contamination of wheat and barley is recognized as the best management practice for the disease. Management tools such as fungicides, crop rotation, predictive modeling of FHB risk, and resistant varieties have been used to reduce FHB disease and mycotoxin contamination of grain, yet consistently reducing disease and DON to acceptable levels remains an intractable problem. New pathogen infections and associated DON contamination of wheat heads after flowering can be especially vexing since fungicides are not labeled for application after flowering. The use of yeast biological control agent *Cyptococcus flavescens* OH 182.9 (NRRL Y-30216) as part of an IPM strategy against FHB is understudied yet has considerable potential for significantly contributing to the reduction of FHB and deoxynivalenol (DON). Because we have isolated a prothioconazole-tolerant (PTCT) variant of OH 182.9 (OH 182.9 C3) that frequently exhibits enhanced biocontrol activity over its wild type progenitor strain, the option now exists to apply strain OH 182.9 to wheat and barley after flowering when fungicides are not approved for use. Alternatively, a tank-mixed fungicide and OH 182.9 combination treatment applied at flowering would theoretically provide immediate protection from FHB and continuing protection due to OH 182.9 colonization of wheat head infection courts after the fungicide component was no longer effective. The OH 182.9 component of this tank mix could be especially useful in limiting the total DON content in harvested grain by combating new, DON producing infections by *F. graminearum* that can occur during early to late grain development. By quantifying the colonization dynamics of strain OH 182.9 under differing integrated application protocols and determining the level of FHB disease reduction associated with colonization levels observed on wheat head tissues, the direction of research on producing and formulating the biocontrol agent could be focused on improving the ability of cells to colonize pathogen infection courts and thereby improve biocontrol effectiveness. Additionally, studies were conducted to further our understanding of the chemical and physical properties of wheat head surfaces from the time of wheat head emergence from "boot" until the latter stages of kernel development. Such knowledge contributes to understanding how and when to apply fungicides and/or biocontrol agents to improve spray coverage and, concomitantly, treatment effectiveness. Formulations of fungicides and/or cells of biocontrol agents could also be better tailored to overcome surface characteristics that impede ideal spray coverage of infection courts on wheat heads.

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: *Documented successful colonization of wheat heads by yeast biological control agent Cryptococcus flavescens OH 182.9 when mixed with a fungicide that is active against Fusarium head blight (FHB) disease.*
Combining FHB biocontrol agent *C. flavescens* OH 182.9 with fungicides such as prothioconazole (PTC) could be especially useful in limiting FHB disease and pathogen

formation of the mycotoxin deoxynivalenol (DON) in grain since new pathogen infection can take place after fungicides can no longer be applied but when populations of the biocontrol agent could still be high. Experiments were conducted to quantify colonization of wheat head tissues by a PTC tolerant (PTCT) variant of OH 182.9 when the biocontrol agent was applied alone or in combination with PTC either at or seven days after wheat flowering. Populations of OH 182.9 3C were not effected by the presence of PTC and, after rain events, made up 40-95% of the total microbial population recovered from specific wheat head tissues from 8 to 12 days after flowering. FHB disease reduction associated with the various treatments supported the observation that the population of a PTCT variant of OH 182.9 on infection court tissues was not inhibited by the presence of PTC and that treatments that contained both the variant and PTC provided the greatest arithmetic reduction in FHB symptoms and DON.

Impact: Basic information derived from this research provides further evidence for the feasibility of using strain OH 182.9 as part of an integrated disease management program against FHB on wheat and developing an OH 182.9-based commercial product for this use. Specifically, these studies showed that the yeast biocontrol agent could be tank mixed with a fungicide and still colonize wheat heads to high levels and contribute to overall FHB disease reduction.

Accomplishment: *Characterized the chemical and physical surface properties of lemma and glume tissues on wheat heads during the course of wheat head development using contact angle methods.*

The surfaces of lemma and glume tissues on wheat heads are covered by a waxy cuticle which mediates interactions between these tissues, and the biotic environment including microbial and pathogen colonists. The chemical and physical properties of the surfaces of these plant tissues contribute to the nature of these interactions. The contact angles of several solvents were measured for the glumes and lemmas of a moderately resistant and a susceptible soft red winter wheat cultivar. Experimental results showed that the surface chemistry and ultrastructure of glume and lemma tissues changed around the time of anthesis for both of the wheat cultivars tested, with the tissue surfaces reaching a minimum in hydrophobicity immediately after flowering, though the moderately resistant cultivar maintained a higher hydrophobicity reading during this time than the susceptible cultivar.

Impact: The discovery that the surface chemistry and ultrastructure of wheat head tissues changed during the course of wheat head development provides evidence that formulations of fungicides and/or biocontrol agents may need to be optimized for the specific stage of wheat head development in order to account for changing surface roughness and hydrophobicity levels on wheat heads. Improved delivery, coverage and retention of biocontrol agents and/or fungicides should ultimately result from spray formulations that are tailored to the stage of wheat head development.

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.

Schisler, D.A., Khan, N., and Boehm, M.J. 2010. Canadian Patent No. 2,322,370, Corresponds to USSN 09/414,097, "Bacillus Species for Reducing Fusarium Head Blight in Cereals" April 13, 2010.

Dunlap, C.A., and Schisler, D.A. 2009. Fluidized-Bed Drying and Storage Stability of *Cryptococcus flavescens* OH 182.9, a Biocontrol Agent of Fusarium Head Blight. Biocontrol Science and Technology 20:465-474.

Schisler, D.A., Khan, N., Boehm, M.J. and Slininger, P.J. U.S. Patent 7,601,346, "Choline-Utilizing Microbial Strains for Biologically Controlling Fusarium Head Blight" October 13, 2009.

Schisler, D.A., Boehm, M.J., Pierce, P., Dunlap, C.A. 2009. Colonization of Wheat Heads by Fusarium Head Blight Antagonist *Cryptococcus flavescens* OH 182.9 when Applied Alone or in Combination with Prothioconazole and the Treatment Effect on FHB Disease Development in Field-Grown Wheat. In: S. Canty, A. Clark, J. Mundell, E. Walton, D. Ellis and D. Van Sanford (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2009 Dec 7-9; Orlando, FL. Lexington, KY: University of Kentucky. pp. 80-84.

Dunlap, C.A., and Schisler, D.A. 2009. Characterization of the Surface Properties of Wheat Spikelet Components. In: S. Canty, A. Clark, J. Mundell, E. Walton, D. Ellis and D. Van Sanford (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2009 Dec 7-9; Orlando, FL. Lexington, KY: University of Kentucky. pp. 41-43.

Yuen, G.Y., Jochum C.C., Halley S.A., Misek K., Sweets L.E., Kirk, W. and Schisler, D.A. 2009. Results of 2009 Uniform Biological Control Trials. In: S. Canty, A. Clark, J. Mundell, E. Walton, D. Ellis and D. Van Sanford (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2009 Dec 7-9; Orlando, FL. Lexington, KY: University of Kentucky. pp. 101-105.

http://extension.osu.edu/news-releases/technology-offers-green-method-to-tackle-top-disease-of-cereal-crops

http://www.youtube.com/watch?v=3aD1J2lS62Q

http://www.allaboutfeed.net/news/sci-protek-protects-crops-from-mycotoxins-id4398.html

Schisler, D.A. contributed, compiled, edited, and delivered a “white paper” document entitled, “Summary of Research Progress on the Biological Control Component of the Integrated Management of Fusarium Head Blight” to the U.S. Wheat and Barley Scab Initiative, 27 pp. April, 2010.