USDA-ARS / USWBSI
FY04 Final Performance Report
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Cover Page

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Year:	**FY2004**
FY04 ARS Agreement ID:	**NA**
FY04 ARS Agreement Title:	**Pilot-scale Production, Stabilization and Field Testing of a Biocontrol Product.**
FY04 ARS Award Amount:	**$ 19,512**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
CBC	Pilot-scale Production, Stabilization and Field Testing of a Biocontrol Product.	$ 19,512
	Total ARS Award Amount	**$ 19,512**

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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

(Form – FPR04)

Project 1: *Pilot-scale Production, Stabilization and Field Testing of a Biocontrol Product.*

1. What major problem or issue is being resolved and how are you resolving it?
The development of a dried biocontrol product active against Fusarium head blight would have potential advantages of ease of handling, convenience in transportation, favorable economics and acceptance by consumers and commercial developers. In the development process, dehydration of antagonist biomass can adversely affect antagonist viability and efficacy as can cryoprotectants if they can be metabolized by the pathogen target. In this research, we developed a liquid culture production technique to produce drying-tolerant biomass of our effective FHB biocontrol agent *Cryptococcus nodaensis* OH 182.9 and tested various inert materials to act as economically feasible carriers for maintaining cell viability and efficacy when air-drying biomass of OH 182.9.

2 What were the most significant accomplishments?

Accomplishments: *Discovery that cold shocking Fusarium head blight biocontrol agent Cryptococcus nodaensis* OH 182.9 *during inoculum production improves cell survival of air drying stress and that a diverse array of diatomaceous earth products can act as carriers that maintain product viability and efficacy after air drying.*
A key impediment to the commercialization of biocontrol agents is the lack of knowledge of cultivation and formulation technologies needed to maintain agent efficacy via maximizing the tolerance of stresses encountered during cultivation, separation, processing and storage. In the past year, in our research conducted at the National Center for Agricultural Utilization Research in Peoria, IL in collaboration with The Ohio State University, the effects of temperature shocking during liquid cultivation of patented biocontrol agent *Cryptococcus nodaensis* OH 182.9 at two different growth stages were tested for their impact on air-drying tolerance of biomass products. We discovered that OH 182.9 cells tolerated air-drying better up to 36 weeks after stored at 4ºC when produced in liquid cultures that were moderately cold shocked (10 and 15ºC) during the late exponential stage of growth and maintained at cold temperatures until harvest than did cultures that were cold shocked during the early stationary stage of cell growth. Exposing cell cultures to short duration cold incubation or heat shock (31ºC) did not influence the survival of air-dried cells. Additionally, we discovered several grades of near neutral pH diatomaceous earth (DE) carriers that, when combined with biomass of *C. nodaensis*, can be air-dried to a friable product that maintains cell viability at near initial concentrations for at least 52 weeks when stored at 4 C. A DE product containing dried *C. nodaensis* biomass was successful in reducing FHB in some 2004 field tests, reducing FHB severity by as much as 42%.

Impact: These discoveries make it possible to develop a more effective, air-drying-tolerant product thereby enhancing the possibility of producing a commercially available FHB biocontrol product for agricultural customers and, concomitantly, reduce the agricultural chemical input already burdening our environment. Air-drying of biomass is an economically feasible dewatering method. DE is relatively inexpensive and wettable powder formulations of pest control products generally receive broad end-user acceptance. Additionally, the discovery of the benefit of cold-shocking in producing a more stress tolerant biocontrol inoculum should encourage other biocontrol researchers to pursue this technique to help solve otherwise intractable problems with maintaining biocontrol agent efficacy after drying.

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 your grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Peer Reviewed Journal Publications:

Zhang, S., Schisler, D. A., Boehm, M. J. and Slininger, P.J. 2005. Carbon-to-nitrogen ratio and carbon loading of production media influence freeze drying survival and biocontrol efficacy of *Cryptococcus nodaensis* OH 182.9. Phytopathology 95:626-631.

Symposium Publications:

Schisler, D.A., Zhang, S., Boehm, M.J. and Lipps, P.E. 2004. USDA-ARS, Ohio State University cooperative research on biological control of Fusarium head blight 1: use of diatomaceous earth as a carrier for formulations of the antagonist *Cryptococcus nodaensis* OH 182.9, In: Canty, S. M., Boring, T., Versdahl, K., Wardwell, J. and Ward, R. W. (Eds.), Proceedings of the 2nd International Symposium of Fusarium Head Blight; incorporating the 8th European Fusarium Seminar; 2004, 11-15 December; Orlando, FL, USA. Michigan State University, East Lansing, MI. pp. 369-373.

Zhang,S., Schisler, D.A., Jackson, M.A., Boehm, M.J., and Slininger, P.J. 2004. USDA-ARS, Ohio State University cooperative research on biological control of Fusarium head blight 2: cold temperature shock during production of *Cryptococcus nodaensis* OH 182.9 enhances cell survival after air-drying, In: Canty, S. M., Boring, T., Versdahl, K., Wardwell, J. and Ward, R. W. (Eds.), Proceedings of the 2nd International Symposium of Fusarium Head Blight; incorporating the 8th European Fusarium Seminar; 2004, 11-15 December; Orlando, FL, USA. Michigan State University, East Lansing, MI. pp. 383-387.

Meeting abstracts:

Schisler, D.A., Behle, R.W., Slininger, P.J., and Jackson, M.A. 2003. Production and formulation of microbial products active against plant pests. Proceedings of the II Moscow International Congress of Biotechnology:State of the art and prospects of development, P&I JSC "Maxima", Moscow, Russia. p. 263.

Schisler, D.A., Khan, N.I., Boehm, M.J., Zhang, S. and Slininger, P.J. 2004. Selection and field evaluation of choline-utilizing microbial strains as potential biocontrol agents of Fusarium head blight. Phytopathology 94:S93.

VanCauwenberge, Schisler, D.A., and Slininger, P.J. 2004. Utilization of the osmolyte melezitose and its effect on the growth and freeze-drying tolerance of Cryptococcus nodaensis OH 182.9. Phytopathology 94:S106.

Zhang, S., Schisler, D.A., Jackson, M.A., Boehm, M.J., and Slininger, P.J. 2004. Cold shock increases air-drying survival of Cryptococcus nodaensis OH 182.9. Phytopathology 94:S115.