USDA-ARS / USWBSI
FY03 Final Performance Report (approx. May 03 – April 04)
July 15, 2004

Cover Page

PI:	**Martin Carson/William Bushnell**
Institution:	**USDA-ARS**
Address:	**Cereal Disease Laboratory** **1551 Lindig St.** **University of Minnesota** **St. Paul, MN 55108**
E-mail:	**mcarson@umn.edu**
Phone:	**612-625-3156**
Fax:	**612-649-5054**
Year:	**FY2003 (approx. May 03 – April 04)**
FY03 ARS Agreement ID:	**NA**
FY03 ARS Agreement Title:	**Cytological and physiological role of deoxynivalenol in Fusarium head blight.**
FY03 ARS Award Amount:	**$ 59,512**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
EDM	Cytological and physiological role of deoxynivalenol in Fusarium head blight.	$ 59,512
	Total Amount Recommended	**$ 59,512**

_____
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: ***Cytological and physiological role of deoxynivalenol in Fusarium head blight.***

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

Fusarium head blight produced catastrophic damage to wheat and barley crops in the US in the 1990s and continues to cause significant loss, especially in wet years. The disease is caused by the fungus pathogen, *Fusarium graminearum*, which infects grain heads directly from spores produced in crop residues. The fungus causes yield loss but also produces deoxynivalenol (DON) and other trichothecene toxins that reduce suitability of grain for animal feed or human consumption. Some protection against head blight is provided by genes for disease resistance but available genes give only partial resistance. Efforts to improve resistance are hampered by lack of knowledge of how the fungus enters, spreads, and causes damage in the grain head. DON produced by the pathogen in diseased heads is suspected to promote disease development by injuring plant cells or reducing their natural, active defense responses. DON is known to inhibit protein synthesis and also to induce programmed cell death (PCD) in animal cells. PCD is characterized by orderly self-destruction of organelles and membranes leading to cell death in a few hours or days. To learn if PCD is a response to DON, we investigated the physiological and cytological effects of the toxin on plant leaf cells.

2. What were the most significant accomplishments?

Earlier results were confirmed and extended, showing that DON causes loss of chloroplast pigments (chlorophyll and carotenoids) in treated leaf cells. Calcium ions (Ca^{2+}), known to enhance programmed cell death (PCD) in plant tissues, were found to greatly enhance the effect of DON. When applied with 10 mM Ca^{2+}, DON at a concentration of 10 mM consistently caused tissues to lose virtually all pigment after two days of treatment. Without Ca^{2+},much higher DON concentrations were required (30-90 ppm) and pigment loss was slow and erratic. The enhanced effect of DON by Ca^{2+} not only provided indirect evidence that PCD is involved in pigment loss, but also improved our ability to investigate effects of DON experimentally.

Using transmission electron microscopy to monitor changes in DON-treated tissues (in presence of Ca^{2+}), we found an orderly sequence of degenerative changes in cellular membranes and organelles, consistent with PCD. By 12 hr after treatment, the tonoplast degenerated. This allowed cytoplasm to be disbursed into the central vacuole of the cell, an irreversible first step toward the ultimate death of cells. By 18 hr, chloroplasts changed shape and lost integrity of internal structures. In addition, the plasmalemma (the principal membrane enclosing the cell protoplast) was no longer visible nor functional. By 24 hr, mitochondria lost internal cristae, essential for respiratory activity cells. By 48-72 hr, tissues were devoid of recognizable organelles or membranes. Fragmented remnants of nuclei were present in some cells, a characteristic of PCD in plant and animal cells. In summary, the dissolution of the tonoplast as the initial response to toxin, the sequential degradation of cellular membranes and organelles, and the fragmented nuclei all provided evidence that cells were undergoing PCD. This finding will lead to experiments to determine if PCD occurs in head tissues infected with the head blight fungus.

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.

Bushnell, W.R., T.M. Seeland, P. Perkins-Veasie, D.E. Krueger, J. Collins, and V.M. Russo. 2003. Calcium ions increase toxicity of deoxynivalenol to barley leaf tissues. Page 123 in: 2003 National Fusarium Head Blight Forum Proceedings, U.S. Wheat and Barley Scab Initiative, Bloomington, MN, December 13-15.

Bushnell, W.R., T.M. Seeland, P. Perkins-Veasie, D.E. Krueger, J. Collins, and V.M. Russo. 2004. The effects of deoxynivalenol on detached barley leaf segments. In: Genomics and Genetic Analysis of Parasitism and Defense. S.Tsuyumu et al., eds., APS Press, St. Paul, MN (In press).