USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY07 Final Performance Report (approx. May 07 – April 08)
July 15, 2008

Cover Page

PI:	Gretchen Kuldau
Institution:	Pennsylvania State University
Address:	Dept. of Plant Pathology 321 Buckhout Laboratory University Park, PA 16802
E-mail:	kuldau@psu.edu
Phone:	814-863-7232
Fax:	814-863-7217
Fiscal Year:	2007
USDA-ARS Agreement ID:	58-0790-5-079
USDA-ARS Agreement Title:	Factors and Mechanisms Favoring Deoxynivalenol Presence in Asymptomatic Wheat.
FY07 ARS Award Amount:	$ 43,902

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
EEDF	Factors and Mechanisms Favoring Deoxynivalenol Presence in Asymptomatic Wheat.	$43,902
	Total Award Amount	**$ 43,902**

_____

Principal Investigator Date

* CBCC – Chemical, Biological & Cultural Control
EEDF – Etiology, Epidemiology & Disease Forecasting
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GET – Genetic Engineering & Transformation
HGR – Host Genetics Resources
HGG – Host Genetics & Genomics
IIR – Integrated/Interdisciplinary Research
PGG – Pathogen Genetics & Genomics
VDUN – Variety Development & Uniform Nurseries

Project 1: *Factors and Mechanisms Favoring Deoxynivalenol Presence in Asymptomatic Wheat.*

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

We are investigating the conditions that lead to asymptomatic grain containing >2 ppm deoxynivalenol (DON). Understanding this phenomenon is important for grain buyers and millers to protect them from inadvertently buying contaminated grain and for breeders to understand how this phenomenon interacts with disease resistance. We are studying the environmental conditions leading to asymptomatic, DON contaminated grain and we are conducting experiments to understand the *in planta* mechanisms.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishment:
A field study to evaluate the impact of moisture timing on disease severity, Fusarium Damaged Kernels (FDK), and DON was conducted using the susceptible variety Hopewell, and the moderately resistant varieties Truman and Valor. Our results demonstrate that moisture during the grain fill period can promote infection leading to disease, FDK and DON accumulation. We observed however that disease severity was lower than when moisture was available at anthesis. For the susceptible cultivar Hopewell, there was no significant difference between the ambient conditions and those with moisture during grain fill for disease severity or FDK but there was a significant difference for DON with the moisture at grain fill treatment having significantly more.

Impact:
These results confirm and expand on the research of others which point to the importance of moisture and infection during grain filling in addition to anthesis. A manuscript describing these results and those from a previous field season is complete and will be submitted for publication. This knowledge will help those preparing disease prediction models to refine their predictions and potentially incorporate information from weather events at more times during the season.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:
Data is now available using cultivars of wheat being developed now that indicates the importance of moisture during grain filling for disease development and DON accumulation. Additionally, our data provides the first evidence that moisture during grain filling can result in sound appearing grain that is contaminated with DON.

Accomplishment:
To understand the in planta mechanisms responsible for asymptomatic, contaminated wheat kernels we conducted several growth chamber experiments to assess the impact of temperature and to evaluate the movement of toxin and fungus in the wheat head. Wheat heads were point inoculated at a central floret and individual florets were harvested at various times after inoculation. For each spikelet, one floret was frozen for later chemical

analysis and one was plated to check for fungal colonization. Three separate runs of this experiment were run using the spring wheat varieties Wheaton (susceptible) and Alsen (resistant). Wheat heads of both varieties were fully colonized by eight days after inoculation at 22 C. At 15 C Alsen heads were fully colonized at eight days while Wheaton heads were not fully colonized until 14 days. Colonization of Alsen occurred before Wheaton at 15 C despite being moderately resistant. This suggests that current methods used in resistance trials may be insufficient. Trials that assess multiple types of resistance might be more effective.

Impact:
This new knowledge may influence breeders and their decisions regarding resistance screening.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:
The chemical analysis of the florets is not complete so the full story is not yet known.

Accomplishment:
Single wheat florets from growth chamber experiments described above will be analyzed for DON and the fungus specific sterol ergosterol. The ergosterol measurement will give an indication of fungal biomass in the floret. A gas chromatography method was developed for this analysis. The method uses a standard extraction for DON followed by saponification of the wheat floret for extraction of ergosterol. The samples are derivitzed with HFBA and detected by electron capture. Mirex is used as an internal standard. DON elutes at 20.4 minutes and has a limit of detection of 0.005 ng/ul. Ergosterol elutes at 47.7 minutes and has a limit of detection of 0.05 ng/ul. We have successfully detected and quantitated DON and ergosterol in single wheat florets. Preliminary data from a small number of wheat heads indicates that in susceptible varieties ergosterol and DON both accumulate at the point of inoculation and beyond. In the resistant variety however levels of DON were very high compared to ergosterol and ergosterol was restricted the point of inoculation while DON was seen above and below the point of inoculation.

Impact:
Ours is the first method to utilize gas chromatography with electron capture detection for quantitation and detection of DON and ergosterol in single wheat florets.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:
A method now exists for the study of the movement of the fungus and the toxin in wheat heads. This will permit dissection of differences amongst varieties in terms of build up toxin and fungus and this data can be paired with information on disease symptoms.

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.

Willyerd, K. T., De Wolf, E. D., Nita, M. and **Kuldau, G. A.** 2007. Timing of Infection: The effects on Fusarium Head Blight severity and toxin accumulation in wheat kernels. *Phytopathology* 97:S123. (Oral presentation.)

Willyerd, K. T., Boroczky, K., **Kuldau, G. A.** 2007. A method for quantifying trichothecenes and ergosterol in single wheat florets using gas chromatography with electron capture detection. In: Canty, S. M., Clark, A., Ellis, D., and Van Sanford, D. Proceedings of the National Fusarium Head Blight Forum; 2007 Dec. 2-4; Kansas City, MO. Lexington, KY: University of Kentucky. pp 142. (poster)

Willyerd, K. T., Nita, M., DeWolf, E. D., **Kuldau, G. A**. 2007. Influence of infection timing on Fusarium head blight severity, wheat kernel damage and deoxynivalenol accumulation during a 2007 field study. In: Canty, S. M., Clark, A., Ellis, D., and Van Sanford, D. Proceedings of the National Fusarium Head Blight Forum; 2007 Dec. 2-4; Kansas City, MO. Lexington, KY: University of Kentucky. pp 143. (poster)