

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY08 Final Performance Report (approx. May 08 – April 09)
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Cover Page

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Fiscal Year:	2008
USDA-ARS Agreement ID:	59-0790-6-068
USDA-ARS Agreement Title:	Starch Degradation by Gibberella zeae and its Role in Fueling Development.
FY08 USDA-ARS Award Amount:	\$ 71,690

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
MGMT	Analysis of DON Accumulation in Green and Senesced Tissues of Wheat.	\$38,751
PBG	Sporulation on Residues: Environmental Influences on Fungal Development.	\$ 32,939
	Total Award Amount	\$ 71,690

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
 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

(Form FPR08)

Project 1: *Analysis of DON Accumulation in Green and Senesced Tissues of Wheat.*

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

Previously, we had noticed from a limited number of wheat stem samples and early infected heads of barley that DON biosynthetic genes were much more highly expressed in green plant tissue than in senescent plant tissue. If this is true, it could impact management. Our specific objectives were: **Objective 1:** To determine if green plant tissue is associated with DON biosynthesis. We characterized expression of DON biosynthetic genes during various stages of head colonization through seed maturation using quantitative RT-PCR. We have completed this characterization in wheat heads, using kernels.

An infection course was performed in wheat cultivar "Wheaton", collecting kernels from infected heads at 6 days post-inoculation

(DPI) through 14 DPI. Visible symptoms (bleaching and curling of the awns; water-soaking of the wheat kernels) first appeared at 8 DPI; by 14 DPI, all or nearly all kernels were infected and the fungus was progressing down the stem. Quantitative reverse transcript PCR (qRT-PCR) detected *Fusarium graminearum* in the wheat kernels immediately above and below the inoculation point at 6 DPI, two days before any visible symptoms were observed on those kernels. Both the housekeeping gene encoding glyceraldehyde phosphate dehydrogenase (GAPDH) and one of the genes involved in deoxynivalenol biosynthesis (TRI5) were observed at 6 DPI. As the fungus progresses through the plant, GAPDH expression generally preceded TRI5 expression, albeit by no more than 24 hours [gray kernels in Fig. 1]. The highest concentration of TRI5 relative to GAPDH (Ct GAPDH/Ct TRI5; blue) was found at or close behind the infection front. Highest relative TRI5 expression continued to track the infection front, but TRI5 expression in kernels closer to the inoculation point did not cease during the time-course we studied, although expression decreased substantially.

In summary, the infection moves upward from the inoculation point as well as downward, albeit not as quickly. TRI5 and GAPDH are detected as high as 3 kernels above the inoculation point; GAPDH has been detected at 4 kernels above the inoculation point. By the later infection stages, kernels above the inoculation point are difficult to harvest; many do not develop fully. See Figure 1.

Objective 2: To characterize expression of DON biosynthetic genes in association with strobilurin treatment. Does the prolonged greening result in prolonged DON gene expression, and does DON gene expression shut off with eventual senescence?

We have examined 8, 11, 14, 17 and 21 DPI for treatment with the strobilurin fungicide Quadris. Wheat was treated at flag leaf and at stage 10-5. Similar to the inoculated, nontreated, DON expression was never entirely lost and expression appears to be similar to those of Obj. 1. However, we have noticed an increase in kernel size with Quadris treatment. This summary for Quadris was based on just 2 replicates. We are in the process of examining another replicate. We expect to have these done by mid-August.

For both of these Objectives, we are doing single kernel DON analysis to correlate gene expression with presence of the toxin. This part of the proposal will be completed this fall and is in collaboration with Gretchen Kuldau.

- 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:

We are confirming a picture of DON expression that suggests that the highest DON biosynthesis may be at freshly infected kernels. We still do not know how *Tri5* expression correlates with DON accumulation. Once the single DON kernel assays are completed, we will have a better picture.

Impact:

Understanding where and when DON is produced in greatest quantities is vital to our ability to control and reduce contamination. Understanding where and when *Tri5* is expressed is also vital, as we may be able to target and eliminate this expression. It is important to know that much of the expression occurs in green tissue. This indicates that genetic engineering of wheat to express genes that quell *Tri5*, such as RNAi techniques, would be possible, as the host cells will still be vital.

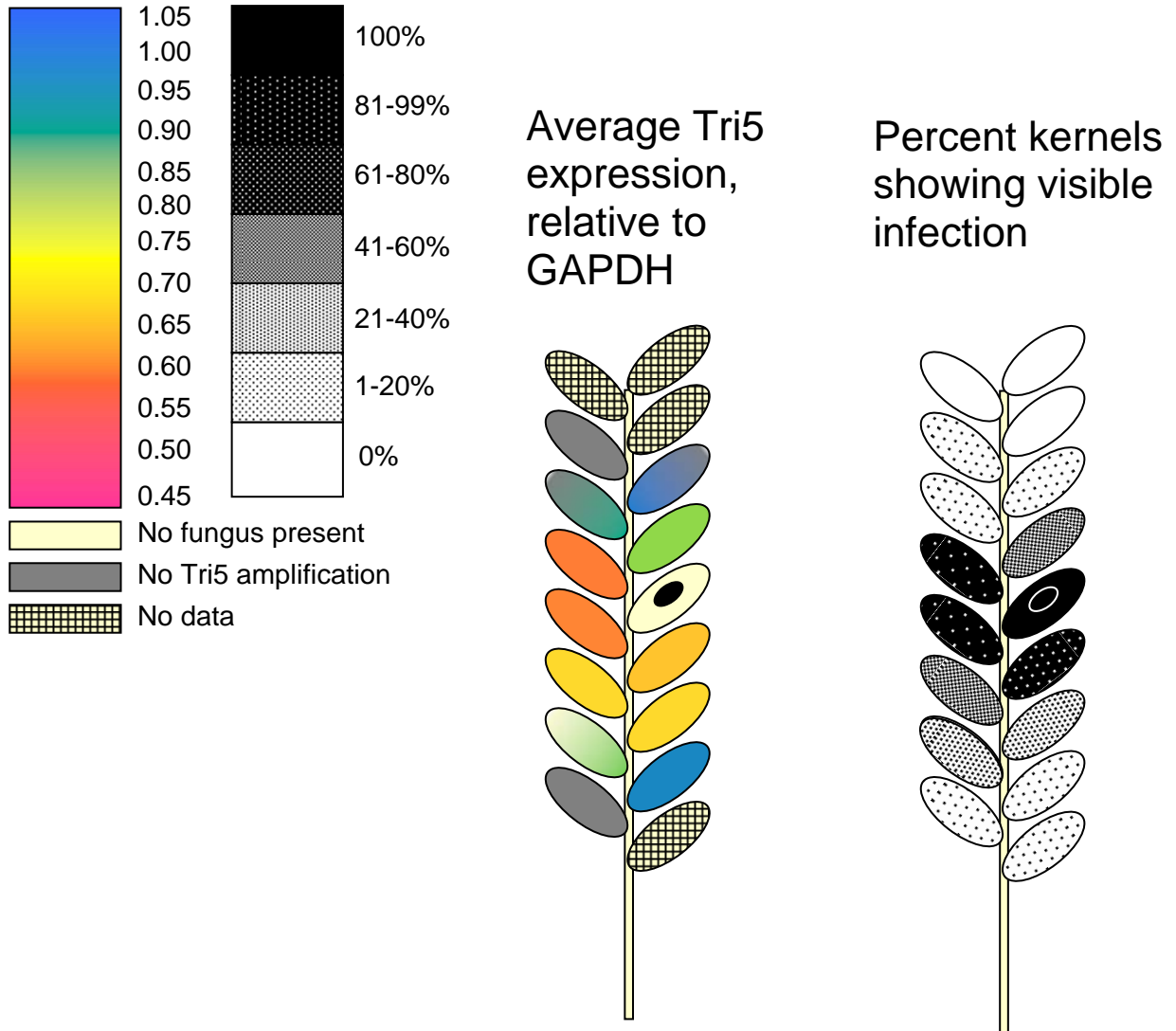


Figure 1. *Tri5* expression in kernels (left) in coordination with symptoms (right). Note that highest expression is in the blue kernels, right behind the infection front (grey kernels, no *Tri5* expression). Inoculated kernel has a black oval on it and was not assessed. The scale for relative expression indicates a very broad range of *Tri5* expression from lowest (older infection) to highest (just behind the front).

Project 2: *Sporulation on Residues: Environmental Influences on Fungal Development.*

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

Define environmental parameters affecting perithecium formation.

A. We investigated the following parameters: (A) temperatures for perithecium induction and ascospore discharge; (B) the critical moisture for drying down and critical moisture for inducing perithecium production.

B. To identify physiological processes important to the commitment of *Fusarium graminearum* to over-wintering in wheat stems and to sexual development.

We found that the perithecia develop best above 9 C, although they will develop at 6 C. We also ran tests of active ascospore discharge at these temperatures. Interestingly, under equivalent light intensity, at 8 C, ascospore discharge is reduced by 35%, but at 10 C or above, there is no change. We are still running trials at lower temperatures.

Analysis of gene expression of colonized stems showed that lipid oxidation pathways and lipid biosynthetic pathways fluctuate with colonization and development of wheat plants. They appear to be extremely important in the survival of the fungus in residues and in perithecium formation. We are putting together a federal competitive proposal to explore the role of lipids in survival and perithecium production.

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:

The most important outcome of this work was to define the temperature parameters for perithecium development and ascospore discharge.

We are taking apart the processes that allow the fungus to survive in crop residues and disperse to the next year's crop. Although the impact in the field will be longer term, this work is nonetheless important. We seek other federal funding to continue this line of inquiry.

Impact: It is important to add as many environmental parameters for inoculum production to the prediction model as possible. This work, when completed, will provide new data for the model.

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.

Publications in Refereed Journals arising from this and previously funded projects:

Triacylglyceride metabolism by *Fusarium graminearum* during colonization and sexual development on wheat. Guenther, Hallen-Adams, Bücking, Shachar-Hill¹, and Trail. MPMI, minor revisions in progress.

Trail, 2009. For Blighted Waves of Grain: *Fusarium graminearum* in the post-genomics era. Plant Physiology 149: 103-110.

Hallen, H., and F. Trail. 2008. The L-type calcium ion channel, Cch1, affects ascospore discharge and mycelial growth in the filamentous fungus *Gibberella zeae* (anamorph *Fusarium graminearum*). Eukaryotic Cell. 7:415-424.

Presentations:

Spore cannons: Ascospore discharge and the life cycle of *Fusarium graminearum*. Seminar presented at the University of Arizona, Tucson. February 2009..

Deoxynivalenol gene expression during wheat head infection by *Fusarium graminearum*. Hallen and Trail. Presented at the Fungal Genetics Conference at Asilomar, CA, 2009.

A role for *MID1* and *CCH1* in ascospore discharge in *Gibberella zeae*. Trail, Cavinder and Hallen. Presented at the Fungal Genetics Conference at Asilomar, CA, 2009.

Understanding the life cycle of *Fusarium graminearum* and its impact on disease. Trail, Cavinder, Hallen and Guenther. Presented at the Fusarium Forum, Indianapolis, IN, Dec. 2008.

With work previously funded by the USWBSI, we were recently awarded a \$600,000 NSF grant to explore gene expression of perithecium development in *Fusarium graminearum* and related fungi. We are very cognizant of the role that USWBSI played in the success of the grant and are optimistic that information gleaned from the funded study will impact our approach to scab control.

If your FY08 USDA-ARS Grant contained a VDHR-related project, include below a list all germplasm or cultivars released with full or partial support of the USWBSI. List the release notice or publication. Briefly describe the level of FHB resistance. If this is not applicable (i.e. no VDHR-related project) to your FY08 grant, please insert 'Not Applicable' below.

Not Applicable.