

**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:	59-0790-4-096
USDA-ARS Agreement Title:	Crop Residue Management and Screening Techniques for Improved Management of FHB.
FY08 USDA-ARS Award Amount:	\$ 88,620

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
MGMT	Factors Influencing the Accumulation of DON in Fusarium-Infected Wheat.	\$37,020
GDER	A Field Nursery for Testing Transgenic Spring Wheat and Barley from the USWBSI.	\$ 7,811
PBG	Sporulation on Residues: Environmental Influences on Fungal Development.	\$ 19,376
MGMT	The Targeting of Residues as a Management Strategy for FHB of Wheat and Barley.	\$ 24,413
	Total Award Amount	\$ 88,620

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: *Factors Influencing the Accumulation of DON in Fusarium-Infected Wheat.*

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

Few studies have closely examined the development of Fusarium head blight and deoxynivalenol (DON) accumulation in relation to the resistance of wheat cultivars, the relative aggressiveness of *F. graminearum* isolates, and the ability of *F. graminearum* isolates to produce DON, or the impact of environmental conditions, especially moisture, on the accumulation of DON in *Fusarium*-infested wheat. This project aimed to improve our knowledge of the development of Fusarium head blight and the accumulation of DON in wheat by examining; i) the effect of environmental conditions, principally moisture between anthesis and harvest, on the development of FHB and the accumulation of DON in wheat; ii) the impact of host genetic resistance on the development of FHB and the accumulation of DON in wheat; and iii) the effect of pathogen variability (aggressiveness and mycotoxin production capacity) on the development of FHB and the accumulation of DON in wheat.

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:

An inoculated, mist irrigated field experiment examining FHB development and DON accumulation was established in St. Paul in 2008, which was essentially a replication of the 2007 experiment. Entries were planted in a randomized split-split-plot design with five replicates. Main plots were irrigation regime (irrigation for 14, 21, 28 and 35 days after inoculation (dai)), subplots were host genetic background and sub-sub-plots were *F. graminearum* isolates. Plots were inoculated at anthesis and mist-irrigated following inoculation for 14 days to facilitate disease development and then the different irrigation treatments were imposed. Entries were assessed for FHB incidence and FHB severity and grain harvested and assessed for visually scabby kernels (VSK) and DON. Developing spikes were also harvested at regular intervals between inoculation and maturity and assessed for FHB symptoms and DON to develop accumulation profiles.

In addition to this field study a greenhouse experiment examining the impact of an extended period of head wetting to determine if leaching can result in reducing the DON in kernels heads. Our earlier findings suggested that periods of extended irrigation between disease assessment and harvest reduced DON so we want to test how readily DON could be leached from heads in situ.

Impact:

Our findings indicated that FHB incidence and severity, VSK and DON, across all isolates, were significantly higher in the susceptible wheat cultivar Wheaton. FHB severities were not significantly impacted by irrigation treatments, though this was to be expected as the first irrigation treatments was only imposed 14 days after inoculation and disease assessment was

at 21 dai. VSK values were significantly lower in the treatments receiving the least amount of mist-irrigation (14 DAI) suggesting that extended moisture promotes disease development after disease assessment in the field. DON was significantly lower in the longest (35 dai) misting treatment. DON in head samples was reduced with increased durations of irrigation, but was only significantly lower in grain from the 35 dai misting treatment. The reduction of DON was larger in Wheaton than other cultivars.

Our results suggest that DON may be reduced by late-season moisture despite increased grain colonization by the pathogen. We believe leaching may explain the reduction of DON observed with an increased misting duration, and that differences in tissue morphology and metabolism may determine the rate of leaching from specific tissues. Results from the leaching study indicate that reductions in DON in whole heads could be reduced by as much as 51% by placing plants under a continuous misting period of 6 hours. In addition testing of the water collected from the misting of the plants detected significant levels of DON (up to 7 ng/g wash water) in samples taken at both 3 and 6 hours following the commencement of the misting period. These results indicate that leaching can account at least for part of the reduction of DON in *Fusarium*-infested heads observed in the field trial. Thus it appears that environmental conditions, particularly irrigation or rainfall, up to harvest do impact the DON levels in harvested grain. This finding helps explain why predictions of DON in harvested grain, when based on disease severity reading taken on plants in the field prior to harvest, are frequently wrong.

Project 2: *A Field Nursery for Testing Transgenic Spring Wheat and Barley from the USWBSI.*

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

Developing effective FHB resistance through transgenics is one of the strategies that have been used by the USWBSI for reducing the impact of FHB in wheat and barley. Over the past decade the USWBSI has funded projects seeking to identify and utilize novel sources of resistance to Fusarium head blight. Since 1997, the University of Minnesota has established an annual nursery to provide field testing for transgenic spring wheat and barley lines developed by researchers in the USWBSI. In 2008 we established a single uniform nursery for the testing of transgenic materials from any/all the spring wheat and barley programs. The principle advantage for establishing this nursery was to make available independent testing for transgenic lines produced by researchers in the USWBSI and, perhaps more importantly, to provide comparative data across programs allowing us to more readily establish the merit of individual transgenes.

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 2008 field screening nursery, with 64 wheat and 208 barley plots was located at UMore Park, Rosemount MN. Trial entries were submitted by Rutgers University (Nilgun Tumer & Rong Di , 5 wheat entries), University of North Texas (Jyoti Shah, 2 wheat entries) and USDA-ARS (Lynn Dahleen, 48 barley entries). In addition to the submitted transgenic entries, untransformed controls were also submitted from each program. Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks used were the moderately resistant Alsen and the susceptible cultivars Wheaton, Norm and Roblin while the barley checks were the moderately resistant line M122 and the susceptible cultivars Robust and Stander. The experimental design was a randomized block with four replicates. Plots were 2.4 m long single rows. The trial was planted on May 8, 2008. All plots, except a non-inoculated Wheaton check, were inoculated twice. The first inoculation was applied at anthesis for wheat and at head emergence for barley. The second inoculation was applied three days after the initial inoculation (d.a.i.) for each plot. The inoculum was a composite of 41 *F. graminearum* isolates at a concentration of 100,000 macroconidia.ml⁻¹ with Tween 20 added at 2.5 ml.L⁻¹ as a wetting agent. The inoculum was applied using a CO₂-powered backpack sprayer fitted with a SS8003 TeeJet spray nozzle with an output of 10ml.sec⁻¹ at a working pressure of 275 kPa. Mist-irrigation was applied from June 26, two days prior to the first inoculation, till July 22 to facilitate FHB development (26 days total) . FHB incidence and severity were assessed visually 20-24 d.a.i. for wheat and 17-21 d.a.i. for barley on 20 arbitrarily selected spikes per plot. FHB incidence was determined by the percentage of spikes with visually symptomatic spikelets of the 20 spikes observed. FHB severity was determined as the percentage symptomatic spikelets of the total of all spikelets

observed in the 20 spikes examined. Plots were harvested at maturity, on August 11. The harvested seed from each plot was split using a Boerner Divider to obtain a 50 g sub-sample, which was then cleaned by hand. These sub-samples were used to estimate the percentage of visually scabby kernels (VSK) for wheat and then all samples (wheat and barley) were analyzed for deoxynivalenol (DON). The data indicated that resistance was expressed in some of the transformed lines.

Impact:

This trial increased the efficiency of individual programs to develop effective FHB resistance through transgenics. The data collected (FHB incidence, FHB severity, VSK and mycotoxin level) was forwarded, as soon as practical, to the researchers submitting entries in the nursery. This data helped them verify the efficacy of the new and novel sources of FHB/DON resistance in these transgenes and to make decisions on whether to discard or promote the further development of genes or lines. In association with expression data, the results from this nursery would also have been valuable in improving our understanding of the efficacy and mechanisms regulating the expression of R-genes.

Project 3: *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?

Colonization of wheat tissues by *Gibberella zeae* generally results in the formation of perithecium initials in the wheat stems close to harvest. These initials become dormant, presumably due to the drying of the tissues as the host plant reaches maturity, and enable the fungus to overwinter. Once they become dormant, the initials have to be stimulated to initiate the development of perithecia. The triggers for initiating perithecium production from dormant perithecium initials appear to be moisture and permissive temperatures, but are poorly understood. An understanding of the triggers for dormancy and for perithecium induction may be keys to understanding the development of this fungus in residues and thus should guide us in the development of new strategies to reduce the sporulation of the fungus on potential inoculum sources. This study was undertaken to determine the environmental parameters affecting the development of perithecial initials, the initiation and breaking of dormancy and subsequent formation of perithecia.

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:

To examine the effect of moisture levels on the dormancy of *Gibberella zeae* and the development of perithecia in the spring a field experiment was established in St Paul. Plots of the susceptible wheat cultivar Norm were grown, inoculated at anthesis with macroconidial inoculum and mist-irrigated to promote FHB infection. Plants were harvested and stem pieces, with colonized nodes, were transferred to mesh bags and placed in the field under two mist irrigation treatments. The two treatments included residues which had been irrigated for 20 days following inoculation and then maintained under ambient conditions and residues that were maintained under moist conditions (12 hours of mist irrigation/day) till the onset of freezing temperatures in the field. These residues were recovered in the spring and used to determine if the ability of the fungus to enter dormancy earlier in the fall had promoted the survival of the fungus over the winter and lead to greater inoculum potential in the spring. Preliminary results indicated that both treatments were able to produce perithecia. The inoculum potential, which will be determined by ascospore counts, is yet to be completed.

We also investigated the age and condition of host tissue for perithecium production and the effect of wet/dry cycles on inoculum levels in greenhouse studies. Tissue age and condition was examined by colonizing the node tissues of plants of a susceptible wheat cultivar soon after tillering. Colonized node tissues were excised from plants either; a) while the plants are still green but well colonized, or b) from plants at maturity. The sampled nodes (green and mature) were divided into treatments. The two treatments were; a) dried down, or b)

kept moist for 3 weeks. Following this incubation period all tissues were transferred to conditions that promote the production of perithecia. Our preliminary results indicate that the mature tissues support perithecium production while the tissues excised from the plants while green did not.

We also examined perithecium production in both standing residue and in residues in contact with the soil surface and thus subjected to longer wet periods following precipitation. Field plots were to be established to provide residues that have not been in direct contact with the soil (e.g. residues from no-till plots) and residues in contact with the soil (e.g. residues from chisel plowed plots), however maintaining field space with standing residues in St Paul was impractical so residues were cut and placed in bags either staked upright or placed horizontally on the soil surface. Residues from these treatments and are still to be examined for their level of perithecial production but early data suggest that residues in contact with the soil produce greater numbers of perithecia.

Impact:

The results of these experiments will provide valuable information that might be fed into models predicting the inoculum load based on previous crop, tillage and fall weather conditions. It appears that this study will confirm our previous findings, that while no-till plots maintain a greater volume of residue at or above the soil surface it appears likely that the production of inoculum, per unit residue by weight is lower than that from conventional tillage systems where the residue lies in contact with the soil and is thus exposed to more favorable conditions for perithecia production.

Project 4: *The Targeting of Residues as a Management Strategy for FHB of Wheat and Barley.*

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

Reduced tillage practices, which have been adopted worldwide in agriculture, have undoubtedly contributed to the global upsurge in FHB of wheat and barley. This study we investigated our ability to reduce the inoculum associated with *Fusarium*-infested residues. Targeted were treatments that either a) promoted the rate of decomposition of crop residues (particularly the residues of Bt-corn, which decomposes more slowly than those of regular corn residues), or b) reduced the survival of the *Fusarium* spp. which infest crop residues.

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:

Field experiments (examining crop residues from wheat, barley and corn, respectively) were established during the 2007 growing season in Rosemount, MN. The treatments were designed to examine the effect of a) biological control agents (*Trichoderma* spp. - fall application), b) fungicides (prothioconazole-tebuconazole mix - spring application) and c) soil amendments (bentonite clay, urea, and spent lime (spent lime is a byproduct of beet processing and has been shown to reduce *Aphanomyces* root rot in sugar beets) - fall application), on the rate of decomposition of *Fusarium*-infested crop residues or the production by *Fusarium* spp. of inoculum from these residues. Additionally fine mechanical chopping of residues was introduced as an additional treatment in the experiment examining corn residue. The residue cover of the soil surface of these trials was monitored in spring 2008 following the establishment of the residue treatments. The residue was sampled at a time coincident with the flowering of the subsequent wheat crop to determine its inoculum potential. The incidence of FHB within the wheat sown into each trial following the establishment of the crop residues was assessed at soft dough. Our results indicated that none of the treatments significantly impacted the soil cover provided by the residues before the establishment of the subsequent wheat crop. No treatment effects on inoculum potential were detected using either traditional mycological techniques to examining ascospore production or by PCR for the identification of the *Fusarium* species in the residues, none were treatment effects evident in the subsequent wheat crop. Dry environmental conditions likely diminished any impact of the treatments and may have contributed to this rather disappointing research outcome. However as part of this study suitable protocols for extracting DNA from the residues collected on the soil surface, and subsequently PCR testing, were developed and these tools are likely be of considerable valuable in future studies.

Impact:

The results from this two year study determined that the treatments examined did not reduce the residues on the soil surface in the spring - this is actually desirable as residue cover is required for soil conservation programs. However it was also evident that the treatments did not have the desired effect on residue decomposition or the inoculum load from these residues in combination with various soil amendments, biological control agents, or fungicides. The development of practical and repeatable protocols for DNA extraction and PCR testing from residues will aid subsequent studies of *Fusarium*-infested crop residues

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.

- Shin, S., Mackintosh, C.A., Lewis, J., Heinen, S.J., Radmer, L., Dill-Macky, R., Baldrige, G.D., Zeyen, R.J. and Muehlbauer, G.J. (2008). Transgenic wheat expressing a barley class II chitinase gene has enhances resistance against *Fusarium graminearum*. *J. Exp. Bot.*, **59**:2371-2387.
- Pereyra, S.A. and Dill-Macky, R. (2008). Colonization of the residues of diverse plant species by *Giberella zeae* and their contribution to *Fusarium* head blight inoculum. *Plant Dis.*, **92**:800-807.
- Pérez, C., Dill-Macky, R. and Kinkel, L.L. (2008). Management of soil microbial communities to enhance populations of *Fusarium graminearum*-antagonists in soil. *Plant Soil.*, **302**:53-69.
- Gautam, P. and Dill-Macky, R. (2008). Post inoculation moisture and deoxynivalenol production by *Fusarium graminearum* in wheat. *Phytopathology*, **98**:S58.
- Gautam, P. and Dill-Macky, R. (2008). Post inoculation moisture impacts deoxynivalenol accumulation in *Fusarium graminearum*-infected wheat. Proceedings of the X International *Fusarium* and *Fusarium* Genomics Workshop, Alghero, Sardinia (ITALY), August 30 - September 2, 2008, *Journal of Plant Pathology*, **90** (3, Supplement):81.
- Gautam, P. and Dill-Macky, R. (2008). Post inoculation moisture impacts deoxynivalenol accumulation in *Fusarium graminearum*-infected wheat. Proceedings of the 3rd International Symposium on *Fusarium* Head Blight Symposium and 10th European *Fusarium* Seminar, Szeged, HUNGARY, September 1-5, 2008, *Cereal Research Communications*, **36** (Supplementum B):493.
- Dill-Macky R. (2008). Cultural control practices for *Fusarium* Head Blight: Problems and solutions. Proceedings of the 3rd International Symposium on *Fusarium* Head Blight Symposium and 10th European *Fusarium* Seminar, Szeged, HUNGARY, September 1-5, 2008, *Cereal Research Communications*, **36**(Supplementum B):653-657.
- Pérez, C., Dill-Macky, R. and Kinkel, L.L. (2008). Enhanced populations of indigenous soil microorganisms antagonistic to *Fusarium graminearum* obtained by the incorporation of green manures. Proceedings of the Second Uruguayan Workshop on Microbial Agents for Biological Control (Segundo Taller Uruguayo de Argentes Microbianos de Control Biológico), Colonia, URUGUAY, September 4-5, 2008.

- Dill-Macky, R. (2008). Cultural control practices in the management of Fusarium head blight. In: *Proceedings of the 2008 National Fusarium Head Blight Forum*, Indianapolis, Indiana, USA, December 2-4, 2008, p. 19.
- Gautam, P. and Dill-Macky, R. (2008). Impact of extended periods of mist-irrigation on deoxynivalenol accumulation in *Fusarium*-infected wheat. In: *Proceedings of the 2008 National Fusarium Head Blight Forum*, Indianapolis, Indiana, USA, December 2-4, 2008, p. 21.
- Gale, L.R., Dill-Macky, R., Anderson, J.A., Smith, K.P., Lyøse, E. and Kistler, H.C. (2008). Links between population affiliation and toxigenic potential in *Fusarium graminearum*. In: *Proceedings of the 2008 National Fusarium Head Blight Forum*, Indianapolis, Indiana, USA, December 2-4, 2008, p. 82.
- Dahleen, L.S., Dill-Macky, R., and Neate, S.N. (2008). 2008 Fusarium head blight analysis of transgenic barley lines. In: *Proceedings of the 2008 National Fusarium Head Blight Forum*, Indianapolis, Indiana, USA, December 2-4, 2008, p. 106.
- Dill-Macky, R., Elakkad, A.M., Wennberg, K.J., Tumer, N.E., Di, R., Shah, J. and Dahleen, L.S. (2008). Testing transgenic spring wheat and barley lines for reaction to Fusarium head blight: 2008 field nursery report. In: *Proceedings of the 2008 National Fusarium Head Blight Forum*, Indianapolis, Indiana, USA, December 2-4, 2008, p. 107.
- Beaubien, K.A., Dill-Macky, R., Dong, Y., Steffenson, B.J. and Smith, K.P. (2008). Characterizing barley near-isogenic lines for a DON QTL on chromosome 3H. In: *Proceedings of the 2008 National Fusarium Head Blight Forum*, Indianapolis, Indiana, USA, December 2-4, 2008, pp. 134-136.
- Beaubien, K.A., Dill-Macky, R., Dong, Y., Roy, J.K., Steffenson, B.J. and Smith, K.P. (2008). Investigating host variation for DON accumulation in wild barley. In: *Proceedings of the 2008 National Fusarium Head Blight Forum*, Indianapolis, Indiana, USA, December 2-4, 2008, pp. 137-141.
- Massman, J., Horsley, R.D., Cooper, B. Neate, S.N., Dill-Macky, R., Chao, S., and Smith, K.P. (2008). Investigating host variation for DON accumulation in wild barley. In: *Proceedings of the 2008 National Fusarium Head Blight Forum*, Indianapolis, Indiana, USA, December 2-4, 2008, p. 184.

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