USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY10 Final Performance Report
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Cover Page

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Fiscal Year:	FY10
USDA-ARS Agreement ID:	59-0206-9-069
USDA-ARS Agreement Title:	FHB Resistance and DON Accumulation in Wheat.
FY10 USDA-ARS Award Amount:	NCE*

USWBSI Individual Project(s)

USWBSI Research Category**	Project Title	ARS Award Amount
GDER	A Field Nursery for Testing Transgenic Spring Wheat, Durum and Barley.	NCE
MGMT	Epidemiology of Late FHB Infections in Wheat and Barley.	NCE
	Total ARS Award Amount	**NCE**

Principal Investigator Date

* NCE – Carry-over from FY08 expired agreement was used to fund this grant and projects.
** 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
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Soft Winter Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: *A Field Nursery for Testing Transgenic Spring Wheat, Durum and Barley.*

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 being used by USWBSI researchers to reduce 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 2010 we established a single uniform nursery for the testing of transgenic materials from any/all the spring wheat, durum 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):**

   <u>Accomplishment:</u>

   The 2010 field screening nursery, with 88 barley plots was located at UMore Park, Rosemount MN. No durum or spring wheat lines were entered by USWBSI researchers in 2010. The barley entries (n=18 transgenic) and an untransformed 2-row control Conlon (susceptible) were all submitted by USDA-ARS, RRVARC Fargo. Barley lines with known reactions to Fusarium head blight (FHB) were also included as checks. The checks included the moderately resistant cultivar Quest (previously breeding 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 4, 2010. All plots were inoculated twice, with the first inoculation applied at head emergence. The second inoculation was applied three days after the initial inoculation (dai) for each plot. The inoculum was a composite of 51 *F. graminearum* isolates at a concentration of 200,000 macroconidia ml^{-1} with Tween 20 (polysorbate, 2.5 ml L^{-1}) added as a wetting agent. Inoculum was applied at a rate of ca. 30 ml per meter of plot row using a CO_2-powered backpack sprayer fitted with a SS8003 TeeJet spray nozzle. Mist-irrigation was applied from the first inoculation on June 28 till July 15 to facilitate FHB development. FHB incidence (FHBI) and severity (FHBS) were assessed visually 14 dai on 20 arbitrarily selected spikes per plot. FHBI was determined by the percentage of spikes with visually symptomatic spikelets of the 20 spikes observed. FHBS was determined as the percentage symptomatic spikelets of the total of all spikelets observed on the 20 spikes. Plots were harvested at maturity on August 5. The harvested seed from each plot was split to obtain a 25 g sub-sample, which was then cleaned by hand. The samples were ground and submitted for deoxynivalenol (DON) analysis. FHBI for all treatments ranged from 86 to 99%. FHBS ranged from 13 to 36% for the 18 entries examined. The FHBS for the untransformed

control Conlon was 23%. The FHBS for the moderately resistant check Quest was 15% while FHBS for the susceptible checks Robust and Stander were 15% and 22%, respectively. The level of disease was similar to the 2009 nursery. The DON data provide additional information on the response of these entries to FHB. The level of DON in the grain of the untransformed control Conlon was 9 ppm, while the moderately resistant check Quest was 8 ppm and the susceptible checks Robust and Stander were 18 ppm and 22 ppm, respectively. The best transgenic barley lines had DON levels below that of Quest and 11 of the 18 lines examined had average DON levels less than 7 ppm.

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 DON) was forwarded, as soon as practical, to the researcher 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 2: *Epidemiology of Late FHB Infections in Wheat and Barley.*

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

The timing of infection is known to be important to the development of FHB. This project aimed to better define the window of susceptibility, understand the factors that change the timing of infection, and determine how different infection timings may result in different degrees of kernel damage and mycotoxin accumulation at harvest. It is generally accepted that infections of wheat at anthesis frequently result in a total failure in kernel development, while later infections, which may occur either as direct infections or from the spread of FHB throughout the head, lead to shriveled, but DON-contaminated, kernels. It has been postulated that very late infections may result in healthy-appearing kernels that contain significant levels of DON. Our investigations focused on defining the window of maximum host susceptibility starting at anthesis, and examining if this period of susceptibility is cultivar-dependent and/or influenced by environmental conditions. In field and greenhouse studies we examined, with the goal to more precisely define, how long wheat and barley remain susceptible after anthesis, and how quickly that susceptibility declines and the influence of moisture on FHBdevelopment and DON accumulation. We also tracked the progress of Fusarium infection and DON accumulation during the period from flowering to harvest in a time course study to precisely relate how each of the variables related to growth stage, the time of initial infection and current environmental conditions.

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 with wheat and barley were conducted to examine the effect of late infection of *Fusarium graminearum* and post-inoculation moisture on FHB development. The 2010 experiments were each a randomized split-split-plot design with four replications. The main-plot treatments were duration of mist-irrigation (14, 21, 28, or 35 d after inoculation). Sub-plots were host genetic background (3 cultivars of each wheat and barley). The sub-sub-plots were timing of inoculation (0, 7, or 14 days after anthesis (daa)) of *F. graminearum*. The three wheat cultivars examined included Tom (moderately resistant), 2375 (moderately resistant-moderately susceptible), and Wheaton (susceptible). The three barley cultivars were Quest (moderately resistant), Robust (moderately resistant-moderately susceptible), and Stander (susceptible). Individual plots consisted of three rows, 1.8 m in length, at 30 cm spacing. All plots were inoculated twice, with the second inoculation applied 3 d after the initial inoculation. The first inoculation of the 0 daa treatment was applied at anthesis for wheat and head emergence for barley. Inoculum consisted of macroconidia (1×10^5 spores ml^{-1}) and Tween 20 (2 ml L^{-1}) of a mixture of ca. 50 *F. graminearum* isolates. The inoculum was applied at a rate of 30 ml per meter of plot row. The inoculum was applied using a CO_2-powered backpack sprayer fitted with a SS8003 TeeJet spray nozzle with an output of 10 ml sec^{-1} at a working pressure of 275 kPa. Visual assessment of FHB was determined on whole heads (10 per plot) that were arbitrarily sampled 0, 5, 10, 15, 20, 25, and 30 d after inoculation (DAI). The sampled heads were stored at -20°C for later processing. In a preliminary study, conducted in 2009, the window of infection examined was 0 till 7 days after anthesis (daa) for both wheat and barley. In 2010, an additional inoculation treatment (14 daa) was examined. Our data indicated that initial infections are still effective in establishing disease up to 14 daa. Our results also suggest that the rate of disease development increases as the plants near physiological maturity. The rate of disease development was not surprisingly greater in the susceptible cultivars, Wheaton (wheat) and Stander (barley). Our results support our preliminary findings indicating that wheat and barley may be susceptible to infection by *F. graminearum* for a prolonged period after anthesis. As FHB appears to develop more rapidly in plant tissues nearing natural senescence, late infections appear to contribute proportionally more to disease symptoms than would be expected in comparison to infections that occur closer to anthesis. The analysis of the toxin time course data from whole heads sampled throughout the season provided additional information on the accumulation of DON and associated Fusarium-associated mycotoxins. The rate of DON accumulation was significantly lower in the treatments inoculated at 7 and 14 days after anthesis compared to the inoculation treatment at anthesis in all cultivars of wheat and barley. DON levels were also observed to decline with increased durations of mist-irrigation. DON accumulation was, however, observed to increase after the cessation of any mist-irrigation period. The observed increase in DON following the cessation of mist irrigation treatments was most pronounced in treatments that received shorter mist-irrigation periods. The largest reduction in DON levels from irrigation was observed was in Wheaton, a susceptible wheat, under the longest mist-

irrigation treatment. These results suggest that DON levels may be significantly influenced, by either mist-irrigation or precipitation following infection.

Impact:

The study yielded useful data that, along with the results of other work, are helping to improve our understanding of the interaction of factors including; host genetics, pathogen aggressiveness and toxin production capacity, the timing of infection and the influence of environmental conditions, particularly moisture, on the development of FHB and the accumulation of mycotoxins in FHB-infested wheat and barley. The time course data has proven particularly useful in furthering our understanding of the influence of environmental conditions, particularly moisture, on mycotoxin accumulation between the time of initial infection and harvest.

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.

Di, R., Blechl, A., Dill-Macky, R., Tortora, A., and Tumer, N. (2010). Expression of a truncated form of yeast ribosomal protein L3 in transgenic wheat improves resistance to Fusarium Head Blight. *Plant Science*, **178**:374-380.

Gautam, P. and Dill-Macky, R. (2010). Influence of moisture on the development of Fusarium head blight in wheat and accumulation of mycotoxins. In: *Book of Abstracts of the 11th European Fusarium Seminar: Fusarium – Mycotoxins, Taxonomy, Pathogenicity and Host Resistance*, Radzików, POLAND, September 20-23, 2010, p.165-166.

Dill-Macky, R. (2010). Strategies used for breeding for improved resistance to Fusarium head blight in the United States. *Bioforsk Fokus,* **5**:37.

Dill-Macky, R., Elakkad, A.M., Dahleen, L.S., Skadsen, R.W. and Abebe, T. (2010). Testing transgenic spring barley lines for reaction to Fusarium head blight: 2010 field nursery report. In: *Proceedings of the 2010 National Fusarium Head Blight Forum*, Milwaukee, Wisconsin, USA, December 7-9, 2010, p. 16.

Ng, E., Abebe, T., Jurgenson, J.E., Dill-Macky, R., Dahleen, L.S. and Skadsen, R.W. (2010). Greenhouse evaluation of transgenic barley expressing *gastrodianin* for resistance to Fusarium head blight. In: *Proceedings of the 2010 National Fusarium Head Blight Forum*, Milwaukee, Wisconsin, USA, December 7-9, 2010, p. 28-32.

Scanlan, T.C. and Dill-Macky, R. (2010). Effect of late infection and post-inoculation moisture on Fusarium head blight development in wheat and barley. In: *Proceedings of the 2010 National Fusarium Head Blight Forum*, Milwaukee, Wisconsin, USA, December 7-9, 2010, p. 97.