USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY12 Final Performance Report
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Cover Page

PI:	Ruth Dill-Macky
Institution:	University of Minnesota
Address:	Department of Plant Pathology 495 Borlaug Hall St. Paul, MN 55108
E-mail:	ruthdm@umn.edu
Phone:	612-625-2227
Fax:	612-625-9728
Fiscal Year:	FY12
USDA-ARS Agreement ID:	59-0206-9-069
USDA-ARS Agreement Title:	FHB Resistance and DON Accumulation in Wheat.
FY12 USDA-ARS Award Amount:	$ 51,042[*]

USWBSI Individual Project(s)

USWBSI Research Category[**]	**Project Title**	**ARS Award Amount**
GDER	A Field Nursery for Testing Transgenic Spring Wheat, Barley and Durum from the USWBSI.	$ 9,765
MGMT	Integrating Multiple Management Strategies to Minimize Losses Due to FHB and DON in Minnesota in Wheat and Barley.	$ 17,300
MGMT	Uniform Fungicide Test for the Control of FHB in Minnesota.	$ 7,544
MGMT	Influence of Variable Pre-anthesis Rainfall Patterns on FHB and DON in Wheat.	$ 16,433
	Total ARS Award Amount	**$ 51,042**

Ruth Dill-Macky 7/16/2013
Principal Investigator Date

[*] Partial funding for this research is under ARS agreement # 59-0206-9-070
[**] 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, Barley and Durum 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 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 2012 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):**

   <u>Accomplishment:</u>
   The 2012 field nursery consisted of 42 wheat and 24 barley entries evaluated in side-by-side experiments. Entries within each species experiment were arranged in a randomized complete block design with four replications in a field located at UMore Park, Rosemount MN. Trial entries and untransformed controls, were submitted by; Jyoti Shah (University of North Texas, 5+1 wheat), Nilgun Tumer (Rutgers University, 7+1 wheat), Gary Muehlbauer (University of Minnesota, 19+5 wheat), and Lynn Dahleen (USDA, 20+1 barley). Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks used were the moderately resistant Alsen, the susceptible cultivars Wheaton and Roblin, and a non-inoculated Wheaton check. The barley checks were the moderately resistant Quest and the susceptible cultivars, Robust and Stander. Individual plots were 2.4 m long single rows. The trial was planted on May 22, 2012. All plots, except a non-inoculated Wheaton check, were inoculated twice. The first inoculation, on 6 July 2012, was applied at anthesis for wheat and at head emergence for barley. The second inoculation was applied three days after the initial inoculation (dai) for each plot. The inoculum was a composite of 30 *F. graminearum* isolates at a concentration of 100,000 macroconidia.ml^{-1} with Tween 20 (polysorbate) added at 2.5 ml.L^{-1} as a wetting agent. The inoculum was applied using a CO_2-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 before the first inoculation on July 5 through July 25 to facilitate FHB development. FHB incidence and severity were assessed visually 19 d.a.i. for wheat and 14 d.a.i. for barley on 20 arbitrarily selected heads per plot. FHB incidence was determined by the percentage of spikes with visually symptomatic spikelets of the 20 heads observed. FHB severity was

determined as the percentage symptomatic spikelets of the total of all spikelets observed. Plots were harvested at maturity on August 13 (barley) and August 22 (wheat). Fifty heads (wheat and barley) were harvested from each plot, threshed and the seed cleaned manually. For wheat, sub-samples were used to determine the percentage of visually scabby kernels (VSK) and then all samples (wheat and barley) were ground and submitted for deoxynivalenol (DON) analysis.

Comparison of FHB severity data for the non-inoculated Wheaton entry and inoculated Wheaton entry indicated that inoculation was successful. The mean FHB severities for the untransformed Bobwhite and CB037 wheat checks were 39% and 13%, respectively. Mean DON content for the untransformed Bobwhite and CB037 checks were 12.7 ppm and 5.8 ppm, respectively. The data indicated that resistance was expressed in some of the transformed wheat lines compared to the untransformed checks submitted by each institution. For barley, the untransformed Conlon check had a mean FHB severity of 36% and the harvested grain a DON content of 12.2 ppm. The data also indicated that resistance was expressed in some of the transformed barley lines compared to the untransformed Conlon check, particularly in terms of DON content. None of the transformed barley lines demonstrated lower FHB severity than the resistant check Quest, however, some did have a lower DON content than Quest.

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 researchers submitting entries in the nursery. This data helps those researchers verify the efficacy of the new and novel sources of FHB/DON resistance in these transgenic materials and to make decisions on whether to discard or promote the further development of genes and/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 to FHB.

Project 2: *Integrating Multiple Management Strategies to Minimize Losses Due to FHB and DON in Minnesota 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 USWBI has funded research on a number of different approaches to the control of Fusarium head blight (FHB). The research on fungicides, which has largely been conducted through the annual uniform collaborative fungicide trials (UFTs), has supported the finding that the triazoles are the most effective fungicides against FHB. The research has also provided us with a better understanding of application technologies for fungicides, including nozzle configurations appropriate for spraying fungicides onto heads and an appreciation for the importance of timing fungicide applications with respect to growth stage. Research has also resulted in the identification of host resistance and the development of moderately resistant cultivars of wheat and barley that are now available to growers. The ultimate goal of this project was to increase growers' adoption of an integrated management approach for FHB and DON. We recognize that a growers' willingness to adopt new technologies in agriculture is often driven by the effectiveness, convenience, practicality and economic benefit of using such technology. This project is, as a part of a large collaborative project, aimed to generate the data that will provide a sufficiently convincing body of evidence that will promote the adoption of best management practices for the control of FHB.

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>

Two field experiments, with hard red spring wheat and spring barley, respectively, were conducted in St. Paul MN to investigate the effects of variety resistance and fungicide application on FHB and DON accumulation. The experimental design used in each case was a split-split-plot, with variety as the whole-plot, inoculation as sub-plot and fungicide treatment as the sub-sub-plot. Each experiments had four replicates. The HRSW varieties included were Samson (S), Briggs (S), Steele-ND (MR) and Glenn (MR). The barley varieties included were Lacey (S), Robust (S), Tradition (S) and Quest (MR). The plots were planted on April 11, 2012 on land previously planted to soybeans. The trial was managed according to the standard agronomic practices for hard red spring wheat and barley. We followed the recommended design, so that in each whole plot, there were two sub-plots, one spray-inoculated and the other un-inoculated. Plots (wheat and barley) were inoculated on June 11, 2012 with a spore suspension (100,000 spores/ml) of macroconidia inoculum consisting of multiple *F. graminearum* isolates. Fungicide treated plots were sprayed at early anthesis (Feekes GS 10.5.1, June 12, 2012) with Prosaro (6.5 fl oz/A + 0.125% Induce).

FHB was assessed in each plot at the soft dough growth stage (Feekes 11.2; wheat on June 28 and barley on July 2). At each assessment, FHB was determined visually and incidence, diseased head severity, and index calculated. The presence and flag leaf severity (%) of any foliar diseases (wheat, tan spot and bacterial leaf streak; barley, powdery mildew and leaf rust) was also assessed. Plots were harvested (barley - August 1; wheat - August 7) with a plot combine and yield and test weight determined. The wheat samples were rated to determine the percentage of visually scabby kernels (VSK) and then all grain samples (wheat and barley) were sent to the USWBSI-funded mycotoxin laboratory in St. Paul for DON analysis. The will be used in combined analysis with data from other locations and years to support national recommendations for best management practices.

The FHB incidence (FHBI) ranged from < 2% to 86% in wheat and from 8% to 76% in barley. FHB severities (FHBS) ranged from <1% to 37% in the wheat and from <1% to 6% in the barley. FHB levels were significantly higher in all inoculated treatments. Barley Yellow Dwarf Virus was severe in all field trials in St. Paul in 2012. The cultivar "Tradition" was so severely impacted by BYDV that the line was excluded from the trial after planting. In the barley trial the fungicide treatments significantly reduced FHBI in the inoculated treatments with reductions of 27%, 44%, and 45% recorded in Robust, Lacey and Quest, respectively. No significant differences in FHBS were recorded, likely because the FHB severity was low. Despite the low FHBS, the fungicide treatments did result in lower DON levels in the grain; with reductions of 0.7 ppm, 0.7 ppm and 0.3 ppm recorded for Robust, Lacey and Quest, respectively. No impact of fungicide treatment on any yield parameter examined was observed for barley. In the wheat trial, fungicide treatments significantly reduced FHBI and FHBS and lowered DON in harvested grain. Differences among variety treatments were also evident and more pronounced than in the barley. The fungicide treatments significantly reduced FHBI in the inoculated treatments with reductions of 19%, 36%, 55% and 68% in Briggs, Glenn, Steele-ND and Samson, respectively. The fungicide treatments reduced FHBS in the inoculated treatments with reductions of 6%, 7%, 24% and 32% recorded in Briggs, Glenn, Steele-ND and Samson, respectively. In addition to reducing FHBI and FHBS, the impact of fungicides was also evident in the DON content of the grain harvested from inoculated treatments with reductions of 0.1 ppm, 0.5 ppm, 0.6 ppm and 1.6 ppm recorded in Briggs, Glenn, Steele-ND and Samson, respectively.

Impact:

No single management strategy is fully effective in controlling FHB or the contamination of *Fusarium*-infected grains with mycotoxins. The current recommendations for FHB management include the use of fungicides, genetic resistance, and cultural practices targeting residue management, including crop rotation or tillage. This cooperative research effort has generated the data that supports our understanding that integrating the use of cultivar resistance and fungicide applications provides greater control than either strategy used in isolation. The ultimate goal of this collaborative project is to increase growers' adoption of an integrated management approach for the control of FHB. We recognize that a growers' willingness to adopt new technologies is driven by the effectiveness, convenience, practicality and the economic benefit of a given technology. The additional data from these trials adds to the body of knowledge that we can use to support our efforts to promote an integrated approach to the management of FHB.

Project 3: *Uniform Fungicide Test for the Control of FHB in Minnesota.*

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

   Fusarium head blight (FHB), caused predominantly by *Fusarium graminearum*, remains a disease of significance, limiting the production capacity of wheat in Minnesota in years when environmental conditions favor initial infection and disease development. Since the resurgence of FHB in the Upper Midwest in the early 1990's, fungicides have proven to be effective and have subsequently been widely adopted as a tool for the management of both FHB and the foliar diseases of wheat. This project represents Minnesota's contribution to the multi-state cooperative uniform fungicide trial. This cooperative effort helps determine the efficacy of registered, unregistered and experimental fungicides on multiple classes of wheat and in barley across diverse environments. The data are used to identify compounds, mixtures of compounds, and to determine the most appropriate rates and timing of application of these fungicides to best manage FHB development and reduce DON accumulation in grain. The data generated by this project may be used to support the registration of new chemistries and to provide recommendations at the regional and national level for best management practices.

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 effect of fungicides on Fusarium head blight (FHB) and deoxynivalenol (DON) was examined in the cultivar 'Samson', a regionally-adapted FHB susceptible hard red spring wheat in an inoculated and mist-irrigated trial. The trial was located in Crookston MN. To help ensure development of FHB the trial was inoculated with *Fusarium graminearum*-colonized corn kernels, which were spread throughout the trial shortly before heading. Irrigation during head development through soft dough (Feekes 11.2) was used to supplement natural rainfall to provide a favorable environment for *F. graminearum* infection and disease development.

   The experimental design was a randomized complete block with 4 replications, with plot being 5 ft wide x 20 feet long. The fungicide treatments, established cooperatively by the project participants, were applied at tillering, flag leaves fully emerged and at the beginning of flowering (Feekes Growth Stages 5, 9 and 10.51, respectively). At soft dough (Feekes 11.2), FHB incidence and severity (FHBS) were assessed for each plot by examining 20 heads per plot. Additionally, the incidence and severity of foliar diseases (predominately tan spot and bacterial leaf streak) were assessed on the flag leaves. Plots were harvested to determine yield, test weight, thousand kernel weight, and grain protein. Grain samples from each plot were used to evaluate the percentage of Fusarium-damaged kernels (VSK) and were then submitted to the mycotoxin laboratory at the University of Minnesota for deoxynivalenol (DON) analysis. The data were submitted to the project coordinator for use in combined analyses and to support national recommendations for best management practices.

Analysis of the 2012 Minnesota trial data indicated:

- FHB Severity (%): Fungicide treatments generally reduced FHBS significantly from that of the untreated control. The highest FHBS among the fungicide treatments was Headline applied alone, at Feekes 9 and in this case the difference from the control was not significant. The lowest FHBS values were recorded where a triazole fungicide (prothiconazole, tebuconazole, or metconazole) was applied at Feekes 10.5.1.
- VSK (%): Three treatments (Headline applied at Feekes 9, Folicur applied at Feekes 10.5.1 and Folicur and Caramba applied at Feekes 10.5.1) had VSK levels (33%, 34%, and 33%, respectively) that did not differ significantly from the untreated check (34%). The triazole fungicides applied at Feekes 10.5.1 did however generally result in lower VSK levels with reductions ranging from 0% (Folicur applied at Feekes 10.5.1) to 18.8 % (Caramba applied 5 days after Feekes 10.5.1).
- DON (ppm): Headline applied at Feekes 9 had the highest DON level (31%), apart from the untreated check (31%). Triazole fungicides applied at Feekes 10.5.1 generally resulted in lower DON levels with reductions ranging from 4 ppm (Folicur applied at Feekes 10.5.1) to 16.7 ppm (Caramba applied 5 days after Feekes 10.5.1).
- Yields ranged from 30 to 60 bu - the average yield responses to fungicides ranged from 5 to 23%.

Impact:

The results of this experiment contribute to our ability to determine the efficacy of fungicides in the management of FHB and determine if they are able to reduce the level of DON in harvested grain. This information had already been used regionally as the results have been made available to growers, extension agents and others in the wheat industry through the University of Minnesota Extension Service. The results have also been used to support the chemical control component of SCABSMART and thus are part of our national effort to provide information on the best management guidelines for the control of FHB and DON.

Project 4: *Influence of Variable Pre-anthesis Rainfall Patterns on FHB and DON in Wheat.*

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

The effects of pre-anthesis rainfall patterns (frequency and duration) on the development of Fusarium head blight (FHB) and the accumulation of deoxynivalenol (DON) in harvested grain are not fully understood. This constitutes a major knowledge gap in the epidemiology of FHB and has led to uncertainty in the assessment of the risk of FHB/DON and interpretation of results from the FHB forecasting systems. Preliminary data suggest that certain intervals of dryness during the pre-anthesis period may be sufficient to reduce the forecasted FHB risk level without actually reducing the real risk of kernel damage and DON accumulation, especially where substantial infected residues are present. This study was designed to investigate the specific effects of intermittent moisture during the 7-day pre-anthesis window on FHB and DON. The pre-anthesis moisture periods will be achieved through the use of mist-irrigation systems programmed to run on different schedules. Similar experiments were planned for three locations - Minnesota, North Carolina and Ohio. The Minnesota site represents the hard red spring wheat-producing region while the other two sites represent the soft red winter wheat (SRWW) regions with a distinctly different weather pattern.

At each location and beginning 7 days prior to anthesis, four mist-irrigation regimens will be used to enhance inoculum production, infection, and FHB development. The four treatments are as follows:

- i) mist every day
- ii) two intermittent misting periods (days 1, 2, 6 and 7 only)
- iii) one intermittent misting period (days 3, 4 and 5 only)
- iv) mist every other day (days 1, 3, 5, and 7)

An FHB susceptible cultivar will be used in the trial. Grain spawn or naturally-infected host crop residue will be used as in-field sources of inoculum. Different planting dates and cultivar maturities will be used to increase the flowering window, and consequently, the chance of some plots receiving each of the mist treatments at the designated time relative to anthesis. FHB incidence and FHB severity, DON, VSK, spike spore density, and weather data will be collected for all plots. Linear mixed model analyses will be performed to quantify the effects of mist regimen, planting date/cultivar and their interaction on all measured responses and to develop equations to estimate the probability of infection (or the probability of disease being above a threshold, *e.g.* 10%) and the probability of DON exceeding critical threshold (*e.g.* 2 ppm), given weather conditions and intermittency of pre-anthesis misting.

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:

We were inly notified that funding for this project was approved in September 2012 - which was after the 2012 cropping season. Although the experiment proposed in this project was not planted, we did establish a small unfunded experiment in the 2012 cropping season in Minnesota that has helped us in the planning for the 2013 field season - when the experiment outlined above will actually be carried out.

The results of the pilot study have allowed us to more precisely time the application of grain spawn inoculum to the field so that maximal spore release coincides with heading and to establish when to initiate the pre-anthesis misting treatments to coincide with heading.

At the time of writing (July 2013) the experiment was planted, inoculated and we will be assessing disease development before the end of the month. A report of the findings of this experiment will be submitted along with our FY13 final report.

Impact:

Results from this study are providing further insight into the role of moisture in the development of FHB and contamination of grain with DON, and will contribute to ongoing disease/toxin risk assessment efforts. Data from this trial should help identify predictor variables for future FHB and DON modeling. The results will aid users of the current FHB risk tools to better interpret the model output, especially for cases of intermittent rainfall that may be on the cusp of low-moderate risk.

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.

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