USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY13 Final Performance Report
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Cover Page

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Fiscal Year:	FY13
USDA-ARS Agreement ID:	59-0206-9-069
USDA-ARS Agreement Title:	FHB Resistance and DON Accumulation in Wheat.
FY13 USDA-ARS Award Amount:	$ 50,992

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
MGMT	Uniform Fungicide Test for the Control of FHB in Minnesota.	$ 7,536
MGMT	Integrating Multiple Management Strategies to Minimize Losses Due to FHB and DON in Minnesota in Wheat and Barley.	$ 17,283
MGMT	Influence of Variable Pre-anthesis Rainfall Patterns on FHB and DON in Wheat.	$ 16,417
GDER	A Field Nursery for Testing Transgenic Spring Wheat, Barley and Durum from the USWBSI.	$ 9,756
	FY13 Total ARS Award Amount	**$ 50,992**

Ruth Dill-Macky 7/15/2014

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
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: *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 major disease, limiting the production capacity of wheat in Minnesota, especially 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 in control of FHB and the reduction of DON in harvested grain and have subsequently been widely adopted as a tool for the management of both FHB and the foliar diseases of wheat and barley. 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 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 accomplishments and their impact (i.e. how are they 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 but 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 the beginning of flowering (Feekes Growth Stage 10.5.1) or 3-7 days after flowering. At soft dough (Feekes 11.2), FHB incidence (FHBI) and severity (FHBS) were assessed for each plot by examining 20 heads per plot. Plots were harvested to determine yield, test weight and thousand kernel weight (TKW). 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 (Carl Bradley) for use in combined analyses and to support national recommendations for best management practices.

Analysis of the 2013 Minnesota trial data indicated:

- FHB Incidence and Severity (%): Fungicide treatments generally reduced FHB incidence (FHBI) and severity (FHBS) from that of the untreated control. The highest FHBS among the fungicide treatments was Thymol (an experimental chemistry from Repar Coro.) applied alone. The FHBS of the treatments that were applied late (3-7 days after Feekes 10.5.1) were similar and not significantly different to the control. The lowest FHB values (FHBI and FHBS) were recorded in the treatment where Caramba (metconazole) was applied at Feekes 10.5.1.
- VSK (%): Most treatments had VSK levels (range 11-34%) that did not differ significantly from the untreated check (34%). The only treatment that resulted in a VSK value significantly lower than that of the untreated control was Caramba, applied at Feekes 10.5.1.
- DON (ppm): The two treatments containing Thymol had the highest DON levels (22% and 23%), apart from the untreated check (29%). Caramba and Prosaro applied at Feekes 10.5.1 resulted in significantly lower DON levels, with average reductions of 21 ppm and 18 ppm, respectively.
- Yields ranged from 55 bu/A in the untreated control to 78 bu/A. Two treatments, Tebucon and Caramba applied at Feekes 10.5.1 and Caramba applied at Feekes 10.5.1 recorded the highest yeilds.

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 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 accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:

Accomplishment:
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 May 8, 2013 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 at heading (barley, June 1, 2013) and anthesis (wheat, June 3, 2013) 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 3 & 5, 2013 for barley and wheat respectively) 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). At each assessment, FHB was determined visually and incidence, head severity, and index calculated.

The presence and flag leaf severity (%) of any foliar diseases (in 2013 this was bacterial leaf streak in wheat) was also assessed. Plots were harvested (8/9/13 - barley; 8/16/13 - wheat) 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 0% to 40% in wheat and from 30% to 100% in barley. FHB severities (FHBS) ranged from <1% to 11% in the wheat and from <1% to 32% in the barley. FHB levels were significantly lower than in 2012 though the inoculated treatments were still generally higher than the non-inoculated treatments. The low disease level in wheat meant that differences in FHBI and FHBS among treatments were not significant. In the barley trial the fungicide treatments significantly reduced FHBI in the inoculated treatments. No significant differences in FHBS were recorded. The maximum DON content was 5 ppm in wheat and 1 ppm in barley, thus the impact of fungicides was not evident in the DON content of the harvested grain. We plan to repeat the trial in 2013.

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: *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 suggested 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 was used as in-field source of inoculum. FHB incidence and FHB severity, DON, VSK, spike spore density, and weather data was 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 accomplishments and their impact (i.e. how are they 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>

All pre-anthesis misting treatments resulted in higher FHB incidences (range 30-45%) and FHB severities (range 7-12%) than the non-misted controls (10% and 2% respectively). The highest FHB incidence (45%) and FHB severity (12%) was recorded in the treatment where mist-irrigation was supplied for all 8 days prior to anthesis. The second highest levels of FHB were observed in the treatment that provided the next longest continuous period of wetness (four consecutive days of mist-irrigation on days 3-6 prior to anthesis). Leaf diseases ranged from 7% in the control treatment (no-mist irrigation) to 12% in the treatment where mist irrigation was provided on days 1, 2, 7 and 8 prior to anthesis. The differences observed in the development of FHB among treatments were reflected the differences in yields, with all four pre-anthesis misting treatments resulting in yield losses of 2-6% in comparison to the non-mist irrigated control.

Impact:

Results from this study have provided 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 will 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.

Project 4: *A Field Nursery for Testing Transgenic Spring Wheat, Barley and Durum from the USWBSI.*

2. 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 2013 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 accomplishments and their impact (i.e. how are they 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 2013 nursery consisted of 22 wheat and 15 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 the University of Minnesota (16 wheat lines + Bobwhite* and CB037*), and the USDA (10 barley lines + Conlon* and Golden Promise*). Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks included were the moderately resistant cultivars Alsen, Rollag and Sumai 3 and the susceptible cultivar Wheaton. The barley checks were the moderately resistant cultivar Quest and the susceptible cultivars, Robust and Stander. Individual plots were 2.43 m long single rows. The trial was planted on June 3, 2013. All plots were inoculated twice. The first inoculation was applied at anthesis for wheat (July 16-Aug 2) and at head emergence (July 19-Aug 2) 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 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 10 ml.sec^{-1} at a working pressure of 275 kPa. Mist-irrigation was applied from the first inoculation on July 16 through August 12 to facilitate FHB development. FHB incidence and severity were assessed visually 21 d.a.i. for wheat and around 18 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 hand harvested at maturity on August 27 (barley) and August 21 (wheat). Fifty heads where harvested from each plot, threshed and the seed cleaned manually. The wheat grain was 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. In 2013 the disease severities were generally a little higher than in the 2012 nursery. Mean FHB severities for the untransformed wheat checks, Bobwhite and CB037 were 27 and 17 %, respectively. Mean FHB severities for the other standard wheat checks, Alsen, Wheaton, Rollag and Sumai 3, were 11, 33, 7 and 3%, respectively. For barley, the untransformed check variety Conlon had a mean FHB severity of 15%. The untransformed check, Golden Promise, and one transformation in the Golden Promise background, were very late heading and did not produce seed. The barley standard checks, Quest, Robust and Stander had mean FHB severities of 2, 16 and 24%, respectively. For the wheat entries in the Bobwhite background, the FHB severity data indicated that resistance was significantly expressed ($P<0.05$) in all transformed lines compared to the untransformed Bobwhite check, with all transformed lines similar to Sumai 3 in response. For the entries with a CB037 background, resistance (FHB severity) was significantly ($P<0.05$) expressed in one transformed line compared to the untransformed background. The FHB severities of all the barley entries in the Conlon background were statistically similar to the untransformed Conlon check, which ranked between Quest and Stander.

Disease incidence, severity, VSK, and DON levels were high in susceptible varieties indicating good disease pressure in the nursery. The mean FHB severities for the untransformed Bobwhite and CB037 wheat checks were 27% and 17%, respectively. Mean DON content for the untransformed Bobwhite and CB037 checks were 9.1 ppm and 16.8 ppm, respectively. The data indicated that resistance was expressed in all of the transformed wheat lines in the Bobwhite background compared to the untransformed check in terms of FHB severity. All transformations in the Bobwhite background performed similarly to Sumai 3 in terms of FHB severity, VSK and DON however most had no significant improvement over the untransformed Bobwhite check in terms of VSK and DON concentration. For wheat in the CB037 background, entry 1726 had FHB severity, VSK, and DON levels that were all statistically similar to the resistant Sumai 3 check and significantly improved compared to the untransformed CB037 check. For barley, the untransformed Conlon check had a mean FHB severity of 15% and the harvested grain had a DON content of 6.7 ppm. Six of the barley transformations performed similarly to Quest in terms of FHB severity and all untransformed and transformed barley entries performed similarly to Quest in terms of DON concentration. Three of the barley entries exhibited significantly lower FHB severities than the susceptible check Stander and all but one entry had significantly less DON than the susceptible check Stander.

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.

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 FY13 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Smith, K.P., Budde, A.D., Dill-Macky, R., Rasmussen, D.C., Schiefelbein, E.L., Steffenson, B.J., Wiersma, J.J., Wiersma, J.V., and Zhang, B. (2013). Registration of 'Quest' spring malting barley with improved resistance to Fusarium head blight. *Journal of Plant Registrations*, 7**:**125-129.

Anderson, J.A., Wiersma, J.J., Linkert, G.L., Kolmer, J.A., Jin, Y., Dill-Macky, R, Wiersma, J.V., Hareland, G.A. and Busch R.H. (2012). Registration of 'Tom' wheat. *Journal of Plant Registrations*, 6**:**180-185.

Anderson, J.A., Wiersma, J.J., Linkert, G.L., Kolmer, J.A., Jin, Y., Dill-Macky, R, Wiersma, J.V., and Hareland, G.A. (2012). Registration of 'Sabin' wheat. *Journal of Plant Registrations*, 6**:**174-179.

Koeritz, E.J., Elakkad, A.M., Muehlbauer, G.J., Li, X., Dahleen, L.S., Abebe, T., Skadsen, R.W., and Dill-Macky, R. (2013) Testing transgenic spring wheat and barley lines for reaction to Fusarium head blight: 2013 field nursery report. In: *Proceedings of the 2013 National Fusarium Head Blight Forum*, Milwaukee, Wisconsin, USA, December 3-5, 2013, pp. 70-71.

Li, X., Shin, S., Heinen, S.J., Dill-Macky, R., Berthiller, F., Clemente, T.E., McCormick, S.P. and Muehlbauer G.J. (2013). Transgenic wheat carrying a barley UDP-glucosyltransferase exhibits high levels of Fusarium head blight resistance by detoxifying trichothecenes. In: *Proceedings of the 2013 National Fusarium Head Blight Forum*, Milwaukee, Wisconsin, USA, December 3-5, 2013, p. 73.