USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY14 Final Performance Report
July 15, 2015

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:	FY14
USDA-ARS Agreement ID:	59-0206-4-016
USDA-ARS Agreement Title:	Management of Fusarium Head Blight in Small Grains.
FY14 USDA-ARS Award Amount:	$ 44,264

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	A Field Nursery for Testing Transgenic Spring Wheat and Barley from the USWBSI.	$ 10,596
MGMT	Developing More Robust Integrated Management Guidelines to Minimize Losses Due to FHB and DON in Wheat and Barley in MN.	$ 17,267
MGMT	Influence of Variable Pre-anthesis Rainfall Patterns on FHB and DON in Wheat.	$ 16,401
	FY14 Total ARS Award Amount	**$ 44,264**

7/15/2015

Principal Investigator Date

* MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
EC-HQ – Executive Committee-Headquarters
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
WES-CP – Western 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 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 being used by USWBSI researchers to reduce the impact of FHB in both wheat and barley. The USWBSI has multiple funded projects seeking to identify and utilize novel sources of resistance to Fusarium head blight. Since 1997, the University of Minnesota has established a summer nursery to field-test transgenic spring wheat and barley lines developed by researchers funded by 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 provide independent testing for transgenic lines produced by researchers in the USWBSI and, perhaps more importantly, to provide comparative data across programs allowing researchers 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 2014 field screening nursery consisted of 49 wheat and 11 barley entries evaluated in side by side experiments. Entries within each experiment (species) were arranged in a randomized complete block design with four replications. The trial was located at UMore Park, Rosemount MN. Trial entries and untransformed controls* were submitted by the University of Minnesota (UMN; 39 wheat lines + Bobwhite*, CB037* and Rollag*), Rutgers University (RU; 9 wheat lines + Bobwhite*) and the USDA (7 barley lines + Conlon*). Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks included were the moderately resistant cultivars Alsen, RB07 and Sumai 3 and the susceptible cultivar Wheaton. The barley checks were the moderately resistant cultivar Quest and line ND20448 and the susceptible cultivar Stander. Individual plots were 2.4 m long single rows. The trial was planted on June 6, 2014. All plots were inoculated twice. The first inoculation was applied at anthesis for wheat (July 16-July 29) and at head emergence for barley (July 21-July 25). The second inoculation was applied three days after the initial inoculation (d.a.i.) for each plot. The inoculum was a composite of 39 *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 at a rate of 33 ml.m^{-1} of row 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 14 to facilitate FHB development. FHB incidence and severity were assessed visually 22-27 d.a.i. for wheat and 20-23 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 September 9 (RU wheat & USDA barley) and September 16 (UMN wheat). Fifty heads where hand harvested from each plot and the seed threshed and 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.

Disease incidence, severity, VSK, and DON levels were high in the susceptible varieties indicating good disease pressure in the nursery. The disease severities in 2014 were generally higher than in the 2013 nursery. Mean FHB severities for the untransformed wheat checks, Bobwhite, CB037 and Rollag were 63%, 34% and 22%, respectively. Mean FHB severities for the other wheat checks, Alsen, RB07, Sumai 3 and Wheaton, were 26%, 25%, 18% and 83%, respectively. The mean DON concentrations for the untransformed checks Bobwhite, Rollag and CB037 were 12 ppm, 9 ppm and 22 ppm, respectively. The data indicated that resistance was expressed, in terms of FHB severity, in some of the transformed wheat lines in the Bobwhite background, when compared to the untransformed check. However, none of the transformations in the Bobwhite background performed significantly better than Bobwhite for either VSK or DON concentration. For wheat in the CB037 background, entries 1726 and 1383 had FHB severities, VSK and DON levels that were significantly improved compared to the untransformed check (CB037). For wheat in the Rollag background, nine entries had VSK levels that were statistically improved compared to the untransformed check (Rollag), however differences were not observed for either FHB severity or DON level in these lines. For barley, the untransformed barley check Conlon had a mean FHB severity of 22%. The barley check lines, Quest, Stander and ND20448 had mean FHB severities of 17%, 29% and 34%, respectively. The mean DON concentrations for barley ranged from 4 ppm to 10 ppm. Three of the barley transformations, 82ND2-2, 82Q3 and 321Q3, performed significantly better than the untransformed Conlon check in terms of FHB severity and two of these lines (82Q3, 321Q3) also performed better in terms of DON concentration.

Impact:

This trial increased the efficiency of individual programs to develop effective FHB resistance through transgenics. The data collected (FHB incidence, FHB severity, VSK - wheat only- and DON) was forwarded, as soon as practical, to the researchers submitting entries in the nursery. The data are used by researchers to verify the efficacy of the new and novel sources of FHB/DON resistance 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 have also been valuable in improving our understanding of the efficacy and mechanisms regulating the expression of R-genes to FHB.

Project 2: *Developing More Robust Integrated Management Guidelines to Minimize Losses Due to FHB and DON in Wheat and Barley in MN.*

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 the control of 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 help develop more robust integrated management guidelines to minimize losses due to FHB and DON in spring wheat and spring barley.

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>

   Two field experiments, with hard red spring wheat (HRSW) and spring barley, respectively, were conducted in St. Paul, MN to investigate the effects of variety resistance and timings of fungicide application on FHB and DON accumulation. The experiments each examined three cultivars with different levels of resistance to FHB and four fungicide treatments, with the emphasis on different post-anthesis application timings and new genotypes. Additional goals were to provide data to support an economic analysis of the integrated effects of fungicide and resistance on FHB/DON and to develop more robust "*best-management practices*" for the control of FHB and DON.

   The experimental design used in each case was a split-plot, with variety as the whole-plot, and fungicide treatment as the sub-sub-plot. Each of the experiments had four replicates. The HRSW varieties included were Samson (FHB-8), Linkert (FHB-5) and SY-Soren (FHB-4). The barley varieties included were Lacey (FHB-8), Conlon (FHB-6) and Quest (FHB-5). The plots were planted on May 6, 2014 on land previously planted to soybeans. The trial was managed according to the standard agronomic practices for hard red spring wheat and barley. In each whole plot (cultivar), there were six sub-plots, five spray-inoculated and one non-inoculated. Plots (wheat and barley) were inoculated at heading (barley, June 26, 2014) and anthesis (wheat, June 30, 2014) with a spore suspension (100,000 spores/ml) of macroconidia inoculum consisting of multiple *F. graminearum* isolates. Fungicide treated plots were sprayed at mid-anthesis (Feekes GS 10.5.1), 2 days after anthesis (daa), 4 daa or 6 daa with Prosaro® (6.5 fl oz/A + 0.125% Induce). Both inoculated and non-inoculated treatments, that were not treated with a fungicide, were included as controls.

FHB was assessed in each plot at the soft dough growth stage (Feekes 11.2). At each assessment, FHB was determined visually and FHB incidence, FHB severity determined. The presence and flag leaf severity (%) of any foliar diseases was also assessed. In 2014 the predominant disease was bacterial leaf streak, especially in the wheat. Plots were harvested (August 4, 2014) 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 plan was to use the data in a combined analysis with data from other locations and years to support national recommendations for best management practices, however both the FHB incidences and FHB severities were low. The low disease level meant that differences in disease levels among treatments were not significant. We plan to repeat the trial in 2015, without additional funding, using the same design with the intent of obtaining useful data and these trials have already been planted

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 better define the application window where a fungicide is effective and therefore to increase growers' adoption of an integrated management approach for the control of FHB even when optimal timing may ot be possible. 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. 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.

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 and the 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 (HRSW) producing region while the other two sites represent the soft red winter wheat (SRWW) regions with a distinctly different weather pattern.

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>

The experimental design used was a completely randomized design, with four replicates. The FHB susceptible cultivar Samson (FHB-8) was used for the trial. The plots were planted on May 14, 2014 on land previously planted to soybeans. *Fusarium graminarum*-colonized corn grain (grain-spawn inoculum) was applied at tillering and served as an in-field source of inoculum. Beginning 8 days prior to anthesis (June 27, 2014), four mist-irrigation regimens were used with the intent of enhancing inoculum production, infection, and FHB development. The four mist-irrigation treatments were as follows: i) mist-irrigation every day, ii) two intermittent misting periods (days 8, 7, 2 and 1 only), iii) one intermittent misting period (days 6, 5, 4 and 3 only), iv) mist every other day (days 7, 5, 3, and 1). A non-irrigated treatment was also included as a control. FHB incidence and FHB severity, yield, thousand kernel weight, percent visually scabby kernels (VSK) and DON was collected for all plots. Spike ascospore density at anthesis as well as 7 and 14 days after anthesis, were estimated by collecting 20 spikes per plot (5 spikes from each of 4 locations in the plot) and washing in sterile water (50 ml water/5 spikes). Spore suspensions were plated on Komada's media, semi-selective for *Fusarium* species. Colony-forming units (CFUs) were counted five days after plating. A weather station within 500 m of the trial site was used to collect weather parameters from stem elongation till harvest.

In 2014 the quantity of wetness did not consistently correspond with greater FHB development. Furthermore, data patterns by treatment did not follow those observed in the 2013 trial although precipitation and temperature patterns appear to have been similar in 2013 and 2014. The spike washing method for estimating spore density on wheat spikes was reliable, straightforward, and demonstrated an increase in disease potential over the three sampling times in 2014. We plan to repeat the trial in 2015, with no additional funding, with the aim of obtaining a minimum of two years of useful data that would be suitable for publication.

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.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY14 award period. The term "support" below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student's stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY14 award period?** No

   If yes, how many? N/A

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY14 award period?** No

   If yes, how many? N/A

3. **Have any post docs who worked for you during the FY14 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?** None

   If yes, how many? N/A

4. **Have any post docs who worked for you during the FY14 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?** None

   If yes, how many? N/A

Include below a list of all germplasm or cultivars released with full or partial support of the USWBSI during the FY14 award period. List the release notice or publication. Briefly describe the level of FHB resistance. *If not applicable because your grant did NOT include any VDHR-related projects, enter N/A below.*

N/A

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 FY14 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Dill-Macky, R., Dong, Y., Van Sanford, D.A., Knott, C.A., and De Wolf. E. (2014). Examination of commercial grain samples to ascertain how deoxynivalenol contamination exceeded anticipated levels in some 2014 wheat crops from western Kentucky. In: *Proceedings of the 2014 National Fusarium Head Blight Forum*, St. Louis, Missouri, USA, December 7-9, 2014, pp. 13-14.

Friskop, A.J., Brueggeman, R.S., McMullen, M.P., Gross, P.L., Ransom, J.K., Halley, S.A., Gautam, P., Dill-Macky, R., Osborne, L.E., Ruden, K.R., and Paul, P.A. (2014). The Effectiveness of an integrated strategy to manage Fusarium head blight in barley production using a meta-analysis approach. In: *Proceedings of the 2014 National Fusarium Head Blight Forum*, St. Louis, Missouri, USA, December 7-9, 2014, p. 15.

Salgado, J.D., Ames, K.A., Bergstrom, G.C., Bradley, C.A., Byamukama, E., Cummings, J.A., Dill-Macky, R., Friskop, A.J., Gautam, P., Kleczewski, N.M., Madden, L.V., Milus E.A., Nagelkirk, M., Ransom, J.K., Ruden, K.R., Wegulo, S.N., Wise, K.A., and Paul, P.A. (2014). Best FHB management practices: a 2014 multi-state project update. In: *Proceedings of the 2014 National Fusarium Head Blight Forum*, St. Louis, Missouri, USA, December 7-9, 2014, pp. 31-36.

Dill-Macky, R., Elakkad, A.M., Muehlbauer, G.J., Li, X., Dahleen, L.S., Skadsen, R.W., Bregitzer, P.P., McLaughlin, J.E., and Tumer, N.E. (2014). Testing transgenic spring wheat and barley lines for reaction to Fusarium head blight: 2014 field nursery report. In: *Proceedings of the 2014 National Fusarium Head Blight Forum*, St. Louis, Missouri, USA, December 7-9, 2014, pp. 52-53.

Li, X., Shin, S., Heinen, S.J., Dill-Macky, R., Berthiller, F., Clemente, T.E., McCormick, S.P., Chao, S., and Muehlbauer, G.J. (2014). Transgenic wheat and barley carrying a barley UDP-glucosyltransferase exhibit high levels of *Fusarium* head blight resistance. In: *Proceedings of the 2014 National Fusarium Head Blight Forum*, St. Louis, Missouri, USA, December 7-9, 2014, p. 57.

Muehlbauer, G.J., Li, X., Shin, S., Huang, Y., Boddu, J., Schweiger, W., McCormick, S.P., Dill-Macky, R., Clemente, T.E., Berthiller, F., and Adam, G. (2014). Developing transgenic wheat and barley that exhibit resistance to *Fusarium graminearum* via glucoside conjugation of trichothecene mycotoxins. In: *Proceedings of the 2014 National Fusarium Head Blight Forum*, St. Louis, Missouri, USA, December 7-9, 2014, p. 59.

Dill-Macky, R., Van Sanford, D.A., and Knott, C.A. (2014). Examination of commercial grain samples to ascertain how deoxynivalenol contamination exceeded anticipated levels in some 2014 wheat crops from western Kentucky. NJF Seminar 478, Nordic-Baltic Fusarium Seminar, Helsinki FINLAND, November 18-19, 2014. Nordic Association of Agricultural Scientists, *NJF Report*, **9**:30.

Adhikari, A., Dill-Macky, R., Wiersma, J.J., and Smith M.J. (2014). Fungicides as a first line of defense for effective control of stem rust in hard red spring wheat. *Phytopathology*, **104** (Suppl. 3):S3.8.

Stanton, J.L., Curland, R.D., Ishimaru, C.A., Smith, M.J., and Dill-Macky, R. (2014). Developing inoculation methods for screening wheat for reaction to Xanthomonas translucens pv. undulosa (Bacterial leaf streak). *Phytopathology*, **104** (Suppl. 3):S3.113.