

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY13 Final Performance Report
July 15, 2014**

Cover Page

PI:	Robert Brueggeman
Institution:	North Dakota State University
Address:	Department of Plant Pathology NDSU Dept. # 7520 PO Box 6050 Fargo, ND 58108-6050
E-mail:	Robert.Brueggeman@ndsu.edu
Phone:	701-231-7078
Fax:	701-231-7851
Fiscal Year:	FY13
USDA-ARS Agreement ID:	59-0200-3-002
USDA-ARS Agreement Title:	Management and Resistance Sources for Control of FHB in Barley.
FY13 USDA-ARS Award Amount:	\$ 24,434

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Coordination of NABSEN and Utilizing Historic Data Set for Association Mapping.	\$ 20,845
BAR-CP	Field Tests of Transgenic Barley Lines.	\$ 3,589
	FY13 Total ARS Award Amount	\$ 24,434

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: *Coordination of NABSEN and Utilizing Historic Data Set for Association Mapping.*

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

Barley production in the Midwestern United States has declined over the past twenty years and the most influential factor resulting in producer's decisions to grow other crops has been Scab. This project is aimed at helping to resolve these issues by assisting the Upper Midwestern barley breeding programs development of genetically resistant barley varieties. Adequate genetic resistance in combination with fungicide use and cultural practices can mitigate the effects of the disease. The main objective of the project was to coordinate the North American Scab Evaluation Nursery (NABSEN) screening of elite barley germplasm in uniform FHB nurseries in the Upper Midwestern United States and Canada. The second objective was to utilize the historic NABSEN data for association mapping to possibly identify novel resistance quantitative trait loci (QTL) present in the elite materials that could be utilized for marker assisted selection (MAS) and/or genome wide selection (GWS) in elite-by-elite crosses.

Mist-irrigated and naturally irrigated nurseries were established at six sites over the past two years to evaluate advanced barley lines from the North Dakota State University, University of Minnesota, Busch Ag, and Agriculture and Agri-Food Canada breeding programs. We coordinated the NABSEN by received and redistributed all the seed, establishing two of the mist irrigated nurseries at Fargo and Langdon, ND, produced the corn spawn inoculum to supplement with *Fusarium graminearum* spores for three nurseries and received collated and prepared the final NABSEN reports. The FHB nurseries have been continually established and evaluated for more than 13 years with all the NABSEN nurseries containing the same set of elite breeding germplasm with putative FHB resistance. It was proposed to utilize this robust set of historic NABSEN data for association mapping analysis, however during the life of the project it was determined that a different analysis strategy must be employed and this process has been developed in the past year. First, the phenotyping data set was analyzed using best linear unbiased prediction (BLUP) using a mixed model with multiple error terms. The BLUP analysis should allow for a more robust QTL analysis utilizing the unbalanced data sets over multiple years and locations allowing for the better identification of marker trait associations. Second we retooled our genotyping platform such that we could utilize our recently acquired Ion Torrent next generation sequencer for genotype-by-sequencing all of the historic NABSEN lines because a majority of them had not been genotyped and the ones that were genotyped as part of the barley T-CAP project had already been analyzed for FHB marker trait associations.

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:

In 2013 the NABSEN contained 48 elite breeding lines, 3 resistant and 3 susceptible controls. Short rows with three replications were planted at each location. Heading dates

were recorded at Fargo, Langdon, Crookston, St. Paul and Brandon and FHB incidence were recorded at Fargo, Langdon, Casselton, and Brandon. FHB severity was recorded at Fargo, Langdon, Casselton, St. Paul, Crookston and Brandon; all of these locations were under mist irrigation except Casselton. DON levels were recorded from all seven locations in U.S. and Manitoba, Canada. Disease levels in 2013 were very low at the Crookston and Casselton dryland nursery locations, thus no FHB incidence or severity data were taken from these nurseries. FHB disease levels were highest at Fargo and St. Paul misted locations; while Langdon, Crookston and Brandon locations had moderate FHB severity. DON levels were highest at Brandon and the other locations had moderate levels. Temperatures were close to the 30-year average, except for St. Paul, which had temperature, 2.7 degrees below normal in May. Precipitation was above the 30-year average in May and June for all locations except Langdon and Crookston, which were below average in June. Precipitation was below the 30-year average in July and August for all locations.

Plants were harvested and samples analyzed for DON in Paul Schwarz's lab at North Dakota State University or measured by ELISA technique at ECORC, Ottawa. DON levels varied much between locations with the dryland locations having the lowest levels with a range from 0.03-1.97 pap and misted nurseries ranged from 7.57 to 44.36 pap. The Agriculture and Agri-Food Canada two-rowed line TR13609 had the lowest mist irrigated DON level (7.57 pap), which was lower than Conlon the two-rowed standard with a mean DON level of 14.35. The University of Minnesota six-rowed line M159 had the lowest DON accumulation levels of all the six-rowed lines grown under irrigation (12.86 pap), which was lower than the six-row resistant standard ND20493, which had a mean DON level of 17.27. Seed was also redistributed and sent to Nanjing, China for testing. Thus, overall the NABSEN nurseries in 2013 were a success and robust evaluation of the elite material was accomplished.

Accomplishment: A second important accomplishment was the development and optimization of the PCR based genotype-by-sequencing platform utilizing Ion Torrent next generation sequence technology. In the last funding period we had developed the PCR GBS protocol, which was adequate for genotyping homozygous lines, but due inefficient ligation step in the procedure and the low efficiency of barcoding as a result of degenerate nucleotide primers in the barcoding adaptor step we were losing up to 2/3 of our sequences. We modified the protocol to contain 20 nucleotide terminal specific adaptors, designed by fluidigm, which allows us to add the Ion Torrent adaptors by PCR and avoid the ligation step. This optimization is relatively more expensive up front in the barcode and adaptor primers synthesis (~ \$4,000), however, the primers are good for thousands of genotyping runs, thus the upfront cost is balanced out overtime by less steps in the GBS process and cutting out the ligation enzyme. Thus, we still can maintain the cost of genotyping barley lines with 384 SNP markers for approximately \$4.00/ line. The 384 marker platform was designed based on SNPs from the barley 9k bead express Illumina platform, which will allow the integration of both data sets as well as validation of the GBS data. We have used the 384-primer set and have grown up and extracted DNA from a set of 418 historic barley lines for PCR-GBS genotyping. We expect to have all the genotyping finished and association mapping using BLUP analysis completed by the end of 2014.

Impact: Significant progress is being made toward developing FHB resistant barley cultivars. All North American barley breeders have access to the data collected in this project. The breeders are able to use the relative performance data to make decisions about continuing or dropping particular breeding lines. Breeders now have: 1) tests of the resistance stability of their breeding lines across a range of environments and disease pressures; 2) a measure of the resistance in their advanced lines compared to those of the other barley breeders in North America; 3) access to unique germplasm with resistance to FHB and DON accumulation. Record numbers of FHB resistant lines are being submitted for AMBA testing and these lines underwent evaluation in the NABSEN.

The 13 years of FHB data and SNP genotyping of the NABSEN entries will provide the opportunity to conduct the association mapping analysis to identify additional FHB resistance QTL present in the breeders elite materials. These analyses should provide markers for further MAS and GWS.

Project 2: *Field Tests of Transgenic Barley Lines.*

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

Commercially accepted barley lines grown in the upper Midwest are susceptible to FHB and have DON accumulation levels exceeding those acceptable by the malting industry. Breeding programs have made consistent progress to bring DON levels down and some of these lines have been approved for malting quality. However, DON accumulation may still be above acceptable industry standards in these new lines when environmental conditions are conducive for a major FHB epidemic. Transgenic barley lines with novel or synthetic sources of resistance will allow for a boost in resistance and lower DON accumulation that is not available through endogenous genes currently available from the primary barley germplasm pool.

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 NDSU barley pathology program is cooperating with Dr. Lynn Dahleen from the USDA-ARS facility in Fargo, to field screen transgenic materials she has developed or has acquired from collaborators. The NDSU barley pathology project planted the material, applied corn based inoculum, set up the misting system and maintained the nursery, all in Langdon, ND. Dr. Dahleen evaluated the nursery for disease and harvested the grain for DON evaluation. See Dr. Dahleen's project report for the accomplishments she has achieved with this project.

Impact:

See Dr. Dahleen's report on this project.

FY13 (approx. May 13 – May 14)
PI: Brueggeman, Robert
USDA-ARS Agreement #: 59-0200-3-002

FY13 Final Performance Report

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.

Poster Abstract:

P. L. Gross, P. Tamang, J. LeBoldus and **R. Brueggeman**. 2013. “Association Mapping of FHB resistance in Barley Utilizing Historic NABSEN Data and Genotype-By-Sequencing.” In: S. Canty, A. Clark, Y. Salat, and D. Van Sanford (Eds.), *Proceedings of the 2013 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. p. 23.