USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY06 Final Performance Report (approx. May 06 – April 07)
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Cover Page

PI:	Stephen M. Neate
Institution:	North Dakota State University
Address:	Department of Plant Pathology 353 Walster Hall Box 5051 Fargo, ND 55105-5012
E-mail:	stephen.neate@ndsu.edu
Phone:	701-231-7078
Fax:	701-231-7851
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USDA-ARS Agreement Title:	Management and Resitance Sources for Control of FHB in Barley.
FY06 ARS Award Amount:	$ 77,115

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Award Amount
CBCC	Preharvest Management Strategies in Barley to Reduce FHB and DON.	$ 21,657
EEDF	Quantification of Spores of Fusarium Graminearum Using a Quick and Accurate ELISA Method.	$ 22,948
HGR	Resistance Screening of Elite Barley Germplasm Selections from ND, MN, ICARDA/CIMMYT & Canada.	$ 21,036
VDUN	Screening Barley Lines for Scab Resistance in Uniform Nurseries.	$ 21,036
	Total Award Amount	**$ 77,115**

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Principal Investigator Date

* CBCC – Chemical, Biological & Cultural Control
EEDF – Etiology, Epidemiology & Disease Forecasting
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GET – Genetic Engineering & Transformation
HGR – Host Genetics Resources
HGG – Host Genetics & Genomics
PGG – Pathogen Genetics & Genomics
VDUN – Variety Development & Uniform Nurseries

Project 1: *Preharvest Management Strategies in Barley to Reduce FHB and DON.*

1. What major problem or issue is being resolved and how are you resolving it?

This project is farmer initiated. Over several seasons, farmers in the North Central region of North Dakota have approached extension agronomists for information on the impact of preharvest management on FHB and DON. Many had experienced crops that they believed were low in visual disease symptoms as they approached maturity, but after swathing or other pre-harvest management, the same crops had registered high DON levels at the elevator. Those farmers and agronomists had petitioned for research on the impact of preharvest management strategies on FHB disease in barley.

Weather conditions in the upper mid-west during barley harvest often result in non-uniform crop maturity within a field and also the development of green weeds interspersed with the ripe crop. In most years barley producers choose to use windrowing or pre-harvest herbicides as desiccants to accelerate crop maturity and drying, and to kill the green weeds. Swathing is the cutting of the crop at the base and lying it down in windrows as the crop starts to turn from green to buff in color and when the grain is at about 30-35% moisture. The crop is then in the windrow in contact with the soil for 7-14 days before combining. Current pesticide registration in North Dakota permits preharvest application of 2,4-D ester, metsulfuron and glyphosate as desiccants to aid drying. In addition paraquat and dicamba have been used experimentally to aid drying.

The objectives of this project are to determine, in a range of environmental conditions, the effect of two common preharvest management strategies, swathing and use of desiccant herbicides on the development of FHB and DON accumulation in barley.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishment:

The mean daily temperature in Fargo in July 2006 during grain ripening and harvest was 4.5^0 above the 30 year average and the monthly rainfall was 2.7 inches below the average. Under these environmental conditions crops ripened evenly and dried faster than average and FHB levels were very low. The use of two rates of 2-4-D ester, carfentrazone-ethyl, dicamba dimethylamine salt, glyphosate, metsulfuron methyl or paraquat dichloride on both Robust and Conlon barley at 30-35% moisture did not significantly affect moisture at harvest, DON accumulation or yield compared to the untreated control.

In the swathing experiment where the treatments were un-irrigated to represent the natural conditions, swathing or harvesting standing Robust or Conlon did not affect DON levels, however swathed crop resulted in a significantly lower yield compared to the crop harvested while standing. Where the crop was irrigated to represent a high rainfall season with rain on the swathed crop and higher levels of FHB, swathed Robust barley had significantly higher DON than Robust harvested while standing. Again swathed crop resulted in a significantly lower yield compared to the crop harvested while standing.

Impact:

Farmers and extension personnel now have a second year of data showing that swathing can influence both visually infected kernels and DON accumulation in some years, but that desiccant herbicides at recommended rates are unlikely to cause changes in disease or DON accumulation. For preharvest swathing, farmers can make the decision to utilize this management technique knowing that it could increase disease. For preharvest desiccants, farmers can make decisions on whether to spray an herbicide based on the need to facilitate harvesting without needing to consider potential interactions between the management techniques and disease.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?

Extension personnel can begin to advise farmers about the impact of preharvest management techniques on FHB and DON accumulation. With a final year of trials in 2007 in a wider range of environments it should allow more definitive advice to farmers that will allow them to reduce disease in their crops.

Project 2: *Quantification of Spores of Fusarium Graminearum Using a Quick and Accurate ELISA Method.*

1. **What major problem or issue is being resolved and how are you resolving it?**
Fusarium can be isolated from the atmosphere by simple systems such as exposed trapping surfaces which are largely qualitative and of variable efficiency, or volumetric spore traps which are both quantitative and efficient. Traditionally a Burkhard volumetric spore sampler has been used to collect spores but this sampler is expensive, heavy and stands more than a meter high and a meter wide. We are testing a small 15cm diam x 20cm high volumetric spore sampler that can be precisely sample different locations relative to the crop canopy and combining it with a more accurate and operator independent method of quantification.

In all spore collection systems the collected spores are either inspected for morphological characters to tentatively identify them as a *Fusarium* which has limited value due to the great number of *Fusarium* species, or they can be cultured and identified. Culturing is extremely time consuming and requires highly trained technical staff. Newer methods of identification have been proposed including chemical profiling of metabolites, immunological methods, carbohydrate and protein fingerprints, and a range of molecular techniques. All are technically complex and relatively costly. One method that has great potential due to its low cost and simplicity is immunology. ELISA has potential to offer a system that is sensitive, affordable, quick and specific. Currently a test for DON, the toxin produced by *F. graminearum*, is widely used by the grains industry. Development of a similar test for spores of the pathogen would be useful for researchers attempting to quantify the effects of control strategies on the pathogen, to determine spore loads in work areas and would also have use for modeling disease epidemics. Dr Nick Hill at University of Georgia has developed a sensitive and specific ELISA test to detect and quantify *F. graminearum* hyphae (Hill et al., 2006). A technical difficulty with collecting spores for quantification is preventing the spores from germinating which would rapidly and uncontrollably increase the antigen detected in the ELISA test as the antigen is likely to be pathogen cell wall material. Furthermore many of the chemicals like formaldehyde that are traditionally used to stop germination have the potential to interfere with the ELISA reaction. We are investigating effects of chemicals on ELISA.

The short term aim of this project is to develop an ELISA system of quantification of macroconidia and ascospores of *Fusarium graminearum.* A longer term aim is to develop an in-situ microtiter ELISA based spore trap in that can quantify spore numbers in the field.

2. **List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):**

Accomplishment:
To our knowledge this is the first time that ELISA has been used to quantify spores of *F. graminearum* and in previous experiments we have demonstrated that while the technique is sensitive, spore collection and preservation can impact on the results. In 2006 between the 25th June and the 24th July the heading and flowering period, the time when spores are

released, temperatures were exceptionally hot and dry with no rainfall recorded in Fargo and only 0.5" recorded in Langdon. As a result, the volumetric spore samplers placed and monitored throughout our trials at different spatial locations in different treatments did not collect sufficient spore numbers to be used for the proposed collection and preservation ELISA experiments. Barley heads were collected from multiple random locations within our trials and in the fall underwent a lengthy washing and spore extraction process so that spores could be use in collection and preservation ELISA experiments. The head extraction experiments also did not yield useful numbers of spores and experiments had to be abandoned. To compensate for the lack of data collected in 2006 the experiments are being repeated in 2007 after the project has officially ceased.

Impact:

Due to exceptionally hot and dry weather during the typical spore release period, experience was gained in operating sampling equipment and spore extraction but no progress was made toward the major aims of the project. Experiments are being repeated in 2007 when it is expected that average environmental conditions will result in successful collection of spores for experiments. When the project is complete it is anticipated that a robust and functional ELISA based spore sampling and quantification system will be available to researchers and industry.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?

Due to exceptionally hot and dry weather during the typical spore release period, no progress was made in 2006 toward the main aims of the project, but experiments are being repeated in 2007 after the official end of the project.

Project 3: *Resistance Screening of Elite Barley Germplasm Selections from ND, MN, ICARDA/CIMMYT & Canada.*

1. **What major problem or issue is being resolved and how are you resolving it?**

   Cultural and chemical controls for Fusarium head blight (FHB) in barley have given less than the desired reduction in disease severity or are not widely adopted for reasons of reduced flexibility in farm management, or economics. Use of cultivars with disease resistance is most favored by farmers because the control obtained does not have an immediate financial cost and does not require changes in crop management practices. There are four barley breeding program in the upper Midwest of the U.S.; the six-rowed breeding program at North Dakota State University, the two-rowed breeding program at North Dakota State University, the University of Minnesota barley breeding program and the Busch Agricultural Resources barley breeding program. All are attempting to develop cultivars with FHB resistance. However development of resistant FHB cultivars by all of these breeding programs requires access to new and better sources of resistance to FHB incidence and severity, as well as resistance to toxin accumulation.

   Although resistance is the most economic means of managing FHB in barley, few sources of resistance better than Chevron, the 6-rowed resistant standard, and CI 4196, the 2-rowed resistant standard, have been identified to date. A significant problem for the Midwest breeding programs is that the best resistance found is in two-rowed barley which is only a small proportion of the barley grown in the region. In previous work, sources of moderate levels of resistance to FHB incidence and severity were identified in about 0.01% of the barley accessions screened from the US National Small Grains collection. To date the major sources of resistant barley are in the accessions Chevron, CIho4196, Zhedar 1, Imperial and Svanhals and similar resistance QTL's have been identified in all accessions. Both the barley breeders and industry are desperate to identify new and better sources of resistance that can be incorporated into six-rowed barley in particular.

   Over several years germplasm has been screened from the USDA small grains collection, the Vavilov collection in Russia, the Centre for Genetic Resources in the Netherlands, the ICARDA barley collection in Syria and the Canadian Germplasm collection. From these screenings of landraces, unadapted and wild barley, elite germplasm with good resistance has been identified by the two US, one Canadian and one CIMMYT group doing the screening.

2. **List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):**

   <u>Accomplishment:</u>
   In 2006 156 elite wild barley accessions, sourced from the four screening programs, were screened in replicated, irrigated and inoculated nurseries in Fargo, ND and Langdon ND for their resistance to FHB and DON accumulation and 703 lines from the Centre for Genetic Resources in the Netherlands were screened in Hangzhou China for their disease resistance

to FHB. These resistant lines represent germplasm that has shown good resistance from repeated testing by Agriculture Canada, University of Minnesota, CIMMYT/ICARDA or the NDSU Barley pathology program.

Of the 79 2-rowed and 77 six-rowed accessions tested, at Langdon, 17 2-rowed lines had disease severity less than CI 4196 the resistant check and 4 6-rowed lines had disease severity less than Chevron the resistant check. In addition 29 2-rowed lines had DON accumulation less that CI 4196 the resistant check and 5 6-rowed lines had DON accumulation less that Chevron the resistant check. At Fargo, where DON levels were less than at Fargo, 65 2-rowed lines had DON accumulation less that CI 4196 the resistant check and 12 6-rowed lines had DON accumulation less that Chevron the resistant check.

A range of elite germplasm previously identified by the four international screening programs as resistant or more resistant than the current resistant checks, have been shown to be consistent across environments which is crucial if they are to be used in breeding programs. When resistant germplasm was tested in these combined trials all four international screening groups had found material that may be useful for future breeding and as expected more resistant germplasm was contributed by the groups that had screened the most germplasm over the most years. As each program has worked with unique material from different germplasm collections there is hope that the basis of resistance in some of the identified germplasm will be unique. Haplotyping is underway to determine which material is likely to be unique.

Impact:
Putatively new resistant lines are now available for further testing to determine their stability in a wider range of environments, as environmental stability is often a problem found with resistance to FHB in barley. The unique material is also available for haplotyping, genetic mapping and pre-breeding. Ultimately the research gives barley breeders access to new and unique sources of FHB resistance and introgression of new resistance from these sources into barley will reduce the impact of the FHB pathogen and its toxins.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?
The scientific community has data from a series of trials in which barley germplasm identified as resistant to FHB by four international screening programs over the last 10 years was compared at sites in the upper Midwest. Through these trials we have identified a number of both two-rowed and six-rowed accessions from each screening program that are better than the current six-rowed and two-rowed resistant standards, CI 4196 and Chevron.

Project 4: *Screening Barley Lines for Scab Resistance in Uniform Nurseries.*

1. What major problem or issue is being resolved and how are you resolving it?

Resistance is the most economic means of managing Fusarium head blight (FHB) in barley. There are four national and five international barley breeding programs in North America that are breeding for FHB resistance; the six-rowed breeding program at North Dakota State University, the two-rowed breeding program at North Dakota State University, the University of Minnesota barley breeding program, the Busch Agricultural Resources barley breeding program, the four combined Agriculture Canada barley breeding programs and the CIMMYT/ICARDA barley breeding program. Each program uses unique breeding material and breeds for different environments in North America and as a result creates its own elite breeding material with FHB resistance.

The objective of this project is to screen for resistance to *Fusarium* head blight (FHB), in uniform nurseries in North Dakota, Minnesota, China, Canada and Mexico, the current elite barley germplasm from the North American barley breeding programs. This series of uniform nurseries allows, 1) tracking of progress toward breeding for resistance 2) side by side comparison of the elite germplasm in a wide range of environments to ensure that the material being advanced in each breeding program is resistant in the widest range of environments 3) the most resistant germplasm to be shared through the germplasm sharing agreements between participants.

Both mist irrigated and non-irrigated sites are sown to represent the range of environments and disease pressures that may be experienced in different years. Mist-irrigated nurseries that are inoculated with *Fusarium graminearum* are used so that data can be collected in years such as 2006 when environmental conditions are less conducive for natural infection. Dryland nurseries are grown so that lines can be grown under conditions similar to those experienced by producers. Agronomic notes, *Fusarium* head blight severity and deoxynivalenol (DON) accumulation are determined for each entry, and each entry is replicated at least twice per location. Results from the NABSEN nursery including weather summaries for the sites are circulated to all nursery participants as well as barley researchers who desire them.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishment:

The 2006 North American Barley Scab Evaluation Nursery (NABSEN) was grown in a diverse range of high FHB environments including Fargo, Langdon, Osnabrock and Bottineau, ND; St. Paul and Crookston MN, Brandon, Manitoba, Hangzhou China and El Batan Mexico. Nurseries at Crookston, Fargo, Langdon, Brandon, St Paul, Hangzhou and El Batan were irrigated, and nurseries at Osnabrock, Bottineau and Crookston were unirrigated (dryland). In 2006, 54 lines were evaluated in replicate in the NABSEN trials. In 2006 the upper mid-west experienced a severe drought with the result that no data was able to be

collected from Osnabrock and Bottineau, and disease levels in Fargo, St Paul and Crookston were very low even with irrigation. Good disease however was seen in El Batan, Langdon and Brandon.

The resistance of the cultivars relative to the resistant and susceptible checks was more consistent in 2006 than in 2005. Cultivar resistance at the different sites was significantly correlated with the exception of Fargo and Langdon with El Batan. When DON accumulation rankings of the lines were compared between the different sites, in general, the best correlations were between Langdon Brandon and Crookston, all sites with a latitude of greater than 47^0 N. This variation between sites is seen each year and indicates that at least part of the resistance is influenced by environment and that cultivars are bred to perform well in a more narrow range of environments than we use for this screening.

Each year the breeder's lines get closer to the resistance exhibited by the resistant checks CIho 4196 and Chevron. In 2006, M128 from the University of Minnesota, TR05285 from AARFC Brandon Canada and ND20493 from the six-rowed program at NDSU had resistance as good as the resistant checks. As in 2005 the majority of the elite lines being tested were when averaged over all sites, better than the susceptible checks Robust and Stander.

In 2005 resistance to DON accumulation was comparable to the increases in resistance to FHB severity. In 2006 while the majority of lines accumulated much less DON than Stander the susceptible check, the differences from Robust, a moderately susceptible check, were smaller.

Impact:
This information is important to breeders who rely on it for making decisions about which elite material should be retained and used for crossing in the following season. The importance of this information to the breeders who participate in the evaluation nursery is clear from the phone calls and emails that we get asking when the data will become available. Many want the data weeks before the DON data is able to be analyzed so that we find it necessary to create interim reports containing partial data sets to cater to their needs.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?
As a result of this research the plant breeders and scientific community have information about the relative resistance to FHB severity and DON accumulation of the best elite lines in the public and private North American barley breeding programs and they have access to the genetic resources which created those lines or the lines themselves for incorporation into their own breeding programs.

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.

Hill, N.S., Neate, S.M., Cooper, B., Horsley, R.D., Schwarz, P.B., Dahleen, L.S., Smith, K.P. and Dill-Macky, R. (2006) ELISA Analysis for Fusarium in Barley: Application in Field Nurseries. ASA-CSSA-SSSA International Annual Meetings, Indianapolis, November 12-16, 2006.

N.S. Hill, S. Neate, B. Cooper, R. Horsley, P. Schwarz, L.S. Dahleen, K.P. Smith, R. Dill-Macky, K. O'Donnell and J. Reeves (2006) Is there Value in Quantifying Fusarium Mycelium for Breeding FHB Resistance? p.98. 2006 National Fusarium Head Blight Forum, December 10-12, 2006, Durham, North Carolina.

Hill, N.S., Schwarz, P., Dahleen, L.S., Neate, S.M., Horsley, R., Glenn, A.E., O'Donnell, K. (2006) ELISA Analysis for *Fusarium* in Barley: Development of Methodology and Field Assessment. Crop Science 46: 2636-2642.

Horsley, R.D, Franckowiak, J.D., Schwarz, P.B. and Neate, S.M. (2006) Registration of 'Stellar-ND' Barley. Crop Science 46:980-981.

Manoharan, M., Dahleen, L.S., Hohn, T., Neate, S.M., Yu, , X.-H., Alexander, N.J., McCormick, S., Schwarz, P., and Horsley, R. (2006) Expression of 3-OH trichothecene acetyltransferase in barley (*Hordeum vulgare* L.) and effects on Fusarium head blight. Plant Science 161:699-706.

Neate, S.M. (2006) Barley diseases. Joint Meeting of NCERA-184 and WCERA-97 Small Grain Pathologists: Fargo, June 2006.

Neate, S.M. (2006) Barley diseases. Farmer/industry talk, ND State Barley Show, Osnabrock ND, March 23th 2006.

Neate, S.M. (2006) New strategies for management of plant pathogenic soil microorganisms - genetically modified plants. Session 2.3P World Congress of Soil Science, Philadelphia July 2006.

Stein, J.M., Osborne, L.E., Neate, S. and Hollingsworth, C. (2006) Environmental factors influencing Fusarium head blight of barley in the northern great plains. p.51. 2006 National Fusarium Head Blight Forum, December 10-12, 2006, Durham, North Carolina.