

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY05 Final Performance Report (approx. May 05 – April 06)
July 14, 2006**

Cover Page

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Fiscal Year:	2005
FY05 ARS Agreement ID:	59-0790-3-083
Agreement Title:	Management and Resistance Sources for Control of FHB in Barley.
FY05 ARS Award Amount:	\$ 73,582

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
EDM	Preharvest Management Strategies in Barley to Reduce FHB and DON.	\$ 19,512
EDM	Quantification of Spores of Fusarium graminearum Using a Quick and Accurate ELISA Method.	\$ 24,390
GIE	FHB Resistance Screening of Unique Barley Germplasm from the Dutch Centre for Genetic Resources.	\$ 18,537
VDUN	Screening Barley Lines for Scab Resistance in Uniform Nurseries.	\$ 11,143
	Total Award Amount	\$ 73,582

Principal Investigator

Date

* BIO – Biotechnology
 CBC – Chemical & Biological Control
 EDM – Epidemiology & Disease Management
 FSTU – Food Safety, Toxicology, & Utilization
 GIE – Germplasm Introduction & Enhancement
 VDUN – Variety Development & Uniform Nurseries

(Form – FPR05)

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 disease at 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 North Dakota during barley harvest can result in non-uniform crop maturity within a field and 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 laying 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 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. In addition paraquat and dicamba have been used experimentally. Swathing and preharvest herbicides allow threshing with reduced grain loss and contamination.

The objectives of this project are to determine the impact of two common preharvest management strategies, swathing and use of desiccant herbicides on control 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:

From preliminary trials in 2004 Fargo ND with a factorial design of two cultivars by two simulated rainfall treatments and two swathing treatments, both DON and percent visually infected kernels were higher in the straight combined treatment than the swathed treatment, however there were no differences between swathing treatments for kernels infected by *Fusarium graminearum* which suggests that other pathogens are involved in the increased visually infected kernels. In contrast in 2005, a much wetter year than 2004, there was no difference between treatments for any of the measurements. It appears that season influences the effect of swathing treatments and further trials should be conducted in different seasons and environments.

In 2004 in preliminary experiments, 3 preharvest herbicides at 1x and 2x rates had no effect on DON accumulation, but glyphosate at both rates significantly reduced

the amount of disease compared to the untreated control. In 2005 the first full year of the project 6 different preharvest herbicides at 1x and 2x rates had no effect on DON accumulation at either Fargo or Minot ND, but application of glyphosate, paraquat and the control resulted in less kernels infected with *Fusarium graminearum* than the other herbicide treatments. In summary, in both 2004 and 2005, desiccant herbicides at recommended rates had no effect on DON or *F. graminearum* infected kernels compared to the untreated control and it appears that concern about these herbicides influencing disease or DON is unfounded.

Impact:

Farmers and extension personnel now have information from a limited number of environments 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 increased disease or DON accumulation. This will allow farmers to make preharvest management decisions about swathing knowing that it could increase disease. For preharvest desiccants, farmers can make decisions on whether to spray a 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 confirmatory trials in a wider range of environments it should allow 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 volumetric 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

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 in non-scientific settings. 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., in press), but it is unclear if this can be used to quantify the spores of the pathogen as the antigen on which it was based was sterile hyphae of the fungus.

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. 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:

A series of experiments testing the impact of light intensity, wavelength and duration, temperature and relative humidity have been undertaken to optimize and make more consistent the production of perithecia and ascospores of *F. graminearum* both invitro and on organic material colonized by the pathogen. Sporulation ability appears to be partially genetic and we are using isolates chosen for their ability to produce spores in-vitro. We have now developed a reliable system of producing spores.

Perithecia of *F. graminearum* were produced in Petri plates and spores collected on the lids. Spores were concentrated by centrifugation and then made into a dilution series for ELISA testing. There was a good positive relationship between absorbance reading and spore number. It was clear from these experiments that various chemicals used in collection and for preservation of the spores can have an impact on the ELISA readings. Spore dilutions sent from North Dakota to Georgia frozen arrived with the minimal mycelial contamination. Untreated spore dilutions in DW show mycelial growth which interferes with an accurate ELISA reading.

In 2005 a barley crop was sown on *Fusarium* infected residue to ensure moderate levels of disease. At different times from flowering through to maturity Burkhard portable air samplers modified to run for periods of up to 8 hours were placed under the canopy of the crop in the middle of each plot ensure that the majority of spores were freshly ejected and from within crop. The samplers were timed to run for different periods to determine typical numbers of spores collected. Spores from heads were washed off, the solution centrifuged to concentrate spores and spores have been stored frozen for future analysis when the ELISA system and germination inhibition treatments have been optimized.

Impact:

To our knowledge this is the first time that ELISA has been used to quantify spores of *F. graminearum* and we have demonstrated that while the technique is sensitive spore collection and preservation can impact on the results. With further research we will provide a cheap, reliable and sensitive method of quantification of spores that can sample from precise locations within a crop.

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?:

We have collected information that supports the viability of an ELISA based spore detection and quantification system, and identified some of the technological problems that impact on getting reliable data from the system. When the project is complete a robust and functional ELISA based spore sampling and quantification system will be available to researchers and industry.

Project 3: *FHB Resistance Screening of Unique Barley Germplasm from the Dutch Centre for Genetic Resources.***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 is perceived to be free and does not require any changes in crop management practices. Therefore the use of resistant cultivars is the favored method of preventing FHB. There are four breeding programs 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 barely 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 have been identified to date, and the best resistance found in barley is in two-rowed barley which makes up only a small proportion of the barley used by the major brewing companies in the US. 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, Clho4196, Zhedar 1, Imperial and Svanhals. Both the barley breeders and industry are desperate to identify new and better sources of resistance that can be incorporated into six-rowed barley.

The genetic material which I have targeted is from 1550 landraces collected in the period 1953 to 1981 in several countries located in the centers of diversity of *Hordeum*, such as Iran, India, Nepal, Pakistan and Ethiopia. The accessions are from the Centre for Genetic Resources in the Netherlands and are not represented in other collections in Europe, Japan or the US where the collections in those countries have been systematically screened for resistance. The first 350 accessions were tested in the greenhouse and field in 2004.

**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:**

Between April 2005 and August 2005, 650 barley accessions from the Centre for Genetic Resources were screened in replicated in a nursery in Fargo ND in an

irrigated and inoculated trial for disease resistance to FHB. Between October 2005 and May 2006 the same 650 accessions were screened in replicate in an irrigated and inoculated nursery in Hangzhou China.

In addition, in 2005 22 of the most resistant lines from screening programs of highly resistant unadapted material from Agriculture Canada, University of Minnesota, CIMMYT/ICARDA and the NDSU Barely pathology program were screened in replicated trials in Fargo and Langdon ND.

Due to severe flooding at the Fargo site in 2005, about 250 of the entries from the Centre for Genetic Resources were not able to be assessed for disease. Of the remaining 450 entries 25 gave disease scores better than the resistant checks Chevron and CI4196. Of the 25 highly resistant entries, 22 were from Ethiopia, one from India and two from Argentina. In comparison in 2004 of the ten highly resistant lines identified from 350 tested, five were from Ethiopia, two from Pakistan and one each from Turkey, Sudan and China. In 2005 a further 111 entries were better than Stander, the moderately resistant check. The 5.5% of isolates showing good resistance is higher than the 0.01% found in other screening efforts and higher than the 3 percent found in 2004. This is possibly due to the unusual conditions experienced in 2005 or the source of the genetic material being from areas more likely to have FHB resistance.

The elite unadapted trial at Fargo was destroyed by floods in 2005 however at Langdon five of the 22 elite unadapted lines had disease severity scores less than the six-rowed resistant check Chevron and four had disease severity scores less than the two-rowed resistant check CIho 4196. Twenty of the 22 were better than the moderately resistant check Stander.

Impact:

Putatively new resistant lines are now available for testing in a wider range of environments and comparison with resistant sources from the primary and secondary gene pools being screened in Minnesota, Canada and CIMMYT/ICARDA. With the comparative screening effort we have initiated, we can choose the best material and make it available to the plant breeders.

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 a further 22 six-rowed barley lines that can be further tested to determine if the resistance genes in these lines are the same as those in the currently know resistance sources. If any of these these lines contain new genes then they can be incorporated into the breeding programs.

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 barely 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. It is essential that the elite material from each site be tested in uniform nurseries in a wide range of environments that produce reliable FHB disease pressure to determine the stability of the resistance across environments and between years. In addition the uniform nurseries allow each breeding program to determine at each site the performance of their best material compared to the best material from the other breeding programs and it allows exchange of the best germplasm between breeding programs to ensure that all are advancing at similar rates toward high levels of resistance.

The objective of this project is to screen elite barley germplasm for resistance to *Fusarium* head blight (FHB) in uniform screening nurseries in North Dakota, Minnesota, Canada, Mexico and China. 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. Nurseries are also inoculated with *Fusarium graminearum* to ensure that data can be collected in years when environmental conditions are not conducive for natural infection. *Fusarium* head blight severity and incidence as well as deoxynivalenol (DON) accumulation and heading date are determined for each entry, and each entry is replicated at least twice per location.

**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 2005 North American Barley Scab Evaluation Nursery (NABSEN) was grown in a diverse range of high FHB environments including Fargo, Langdon, Osnabrock and Casselton, ND; St. Paul and Crookston MN, Brandon, Manitoba and Toluca Mexico. Nurseries at Crookston, Fargo, Langdon, Brandon, St Paul and Toluca were irrigated, and nurseries at Osnabrock, Casselton and Crookston were unirrigated (dryland). In 2005, 54 lines were evaluated in replicate in the NABSEN trials. Unfortunately some of the sites were abandoned due to severe weather

conditions in the upper mid-west destroying the crops, with Fargo and Osnabrock flooded, no disease data for dryland Crookston and missing information from Toluca Mexico.

The resistance of the cultivars relative to the resistant and susceptible checks varied by location. When DON accumulation rankings of the lines were compared between the different sites, in general, the best correlations were between Langdon and Crookston either irrigated or dryland and Langdon and Casselton. Brandon showed poor correlations with most of the other sites. 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 breeders lines get closer to the resistance exhibited by the resistant checks Cihó 4196 and Chevron. In 2005 M122 from the University of Minnesota, SM00599 from University of Saskatchewan and ND20481 from the six-rowed program at NDSU had resistance as good as the two resistant checks. In 2005 the majority of the elite lines being tested were when averaged over all sites, better than the susceptible checks Robust and Stander.

Eleven of the 50 breeding lines had mean DON levels of less than 2 ppm while the resistant check Chevron was 0.9 ppm and Cihó 2196 was 1.7 ppm. Among the 11 low DON lines were representatives from all the North American breeding programs. It is clear that resistance to DON accumulation is advancing at a rate comparable to the increases in resistance to FHB severity.

Impact:

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. It is clear from conversations with the breeders that they use this data for making crosses and to determine which material will be advanced in the next season.

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 plant breeders and scientific community have information about the relative resistance to FHB severity and DON accumulation of the best elite lines in all the 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.

Refereed Journal Papers

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 (accepted May 2006)

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

Books

Neate, S.M. and McMullen, M. P. 2005. Barley Disease Handbook. North Dakota State University, Fargo ND

Conference papers

Neate, S.M., Halvorson, M.A., Gross P.L. and Sun, Y. 2005. Impact of Preharvest Management Strategies in Barley on FHB, Seed Colonization and DON. p123. Proceedings of the 2005 National Fusarium Head Blight Forum, Milwaukee Dec , 2005.

Halley, S. and **Neate, S.M.** 2005 The Effect of Previous Crop Residue, Chaff Management, and Tillage System on Fusarium Head Blight of Barley. p198. Proceedings of the 2005 National Fusarium Head Blight Forum, Milwaukee Dec , 2005.

Neate, S.M. 2005. Barley Diseases in the West and Mid-west of the US. US Malt and malting Barley Buyers Conference, Portland Oregon Oct 30-Nov 2 2005.

Other Scientific presentations

Department of Plant Pathology Washington State University. Strategies for control of diseases of small grains in southern Australia and the mid-west of the US. Pendleton WA 6th September 2005

Invited lecturer 2nd International CIMMYT-McMaster Fellowship Soil-borne Diseases Masterclass, Henan, China May 2005. Fusarium headblight of small grains.

Departments of Plant Pathology and Brewing Science Zheijiang University, Hangzhou, China, May 2005. Fusarium head blight in The US and China. Invited lecture.

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Farmer/Industry Talks

American Malting Barley Association Industry Barley Tour, Dickinson, Minot and various field sites, July 11th-July 13th 2005

ND State Barley Show, Osnabrock, ND March 23rd 2006