U. S. WHEAT & BARLEY SCAB INITIATIVE

Fusarium Focus

Volume 10 Issue 1 Winter 2010

Highlights from 2009 National FHB Forum

Approximately 175 crop scientists, growers and wheat and barley industry representatives participated in the 2009 National Fusarium Head Blight Forum, held on December 7-9 in Orlando, Fla. The 12th FHB Forum took place at the Wyndam Orlando Resort.

The 2009 annual event featured stakeholder and scientific speaker presentations, along with focused group discussions and evening breakout sessions. Numerous scientific posters also were displayed during the Forum, with their authors in attendence to discuss the highlighted research.

Organized and hosted by the U.S. Wheat & Barley Scab Initiative (USWBSI), the Forum serves as a venue for the reporting of the latest research findings on Fusarium Head Blight (scab) and deoxynivalenol (DON), the mycotoxin produced by scab infection in grain.

The following pages feature photos and narrative describing highlights from the 2009 FHB Forum. The event's entire proceedings can be found on the USWBSI website: www.scabusa.org. ◆

Mark Your Calendar!

2010 National Fusarium Head Blight Forum

December 7-9

Hyatt Regency Milwaukee
Milwaukee, Wisc.

Above: USDA and university scientists, grain industry personnel, commodity group representatives and growers from several states were in attendance at the 2009 FHB Forum in Orlando.

Right: Co-chairs of the U.S. Wheat & Barley Scab Initiative are Dave Van Sanford (left), wheat breeder with the University of Kentucky, and Minnesota producer Art Brandli (right).

— 2009 FHB Forum —

Carl Schwinke

Carl Schwinke, vice president for grain supply at Siemer Milling Company, used the title "12 Days in May" for his plenary talk on a miller's view of 2009 soft red spring wheat quality. Siemer, a family and employee-owned company, purchases about 15 million bushels of locally grown wheat each year for its flour mills at Teutopolis, Ill., and the western Kentucky community of Hopkinsville.

Schwinke reported that above-normal rainfall and humidity in the region contributed to significantly higher levels of DON in 2009. The average in 1,000 loads of wheat received at the company's Hopkinsville mill last year was 2.89 parts per million. That compared with an average of less than 0.5 ppm in 2008 and about 0.3 ppm in 2007.

Alluding to the title of his presentation, Schwinke noted that weather and grain quality records for the years 2002 through 2009 underscore the strong "cause and effect" correlation. During that eight-year period, the 2003, 2004 and 2009 seasons stood out for their high DON levels (averaging 3.95, 4.07 and 2.89 ppm, respectively). One contributing factor to the higher incidence of DON in those years, Schwinke suggested, was the use of strobilurin-class fungicides rather than a triazole product. The increased production of wheat in a no-till system (well over half of Kentucky's wheat is now no-till) is another contributor, Schwinke observed.

What has been the economic impact? Schwinke estimated the net loss to Kentucky from scab in 2009 to be more than $29 million. That figure is based on an estimated yield loss of nearly 5.5 million bushels and quality discounts averaging eight cents per bushel.

The scab incidence and effect over the past decade has required Siemer Milling to, in some years, purchase wheat from outside its normal trade area, Schwinke added.

While acknowledging the progress that has been made with varietal resistance, fungicide management, disease forecasting and other scab-fighting tools, "I challenge this group to continue to do more" to address the scab and DON issue, Schwinke concluded. ◆

Chidozie Amuzie

Chidozie Amuzie, postdoctoral scientist with Michigan State University's Department of Pathobiology and Diagnostic Investigation, discussed the use of biomarkers as a risk assessment tool for deoxynivalenol (DON).

Upon exposure, DON is rapidly distributed in animal tissues and induces proinflammatory cytokines, Amuzie noted. This occurs in less than two hours. Over the coming weeks and months, DON can reduce weight gain in many animal species for reasons that are not well understood. This then invokes uncertainties when it comes to human safety assessment and establishing DON regulatory limits.

Amuzie reviewed how DON's risk to animals and humans is currently determined. He spoke about the 100-fold safety factor that is commonly used (*i.e.*, the assumption that humans are 100 times more sensitive to toxins than laboratory animals); outlined various countries' regulatory thresholds for DON in human diets (the European Union is significantly more strict than the United States — particularly with infant diets); and the relationship between tolerable daily intake and NOAEL (*n*o *o*bserved *a*dverse *e*ffect *l*evel).

"Risk assessment of natural food-borne toxins is a delicate balance between 'human health' on one side and 'food supply' on the other," Amuzie pointed out. "The use of biomarkers can help make the assessment process more precise and accurate," he added — which, in turn, can be of great assistance to regulators in their decision-making processes.

Biomarkers can serve as indices of toxicity and/or injury in both animals and humans. That information subsequently can aid in diagnosing and monitoring disease, predicting outcomes — and measuring the effectiveness of treatments.

A potential DON biomarker currently being developed is based on IGFALS (*i*nsulin-like *g*rowth *f*actor *a*cid *l*abile *s*ubunit), which is a growth-related protein. "We now have tools that (a) tell us when mice are exposed to DON, and (b) tell us about the effect of DON on the body," Amuzie noted. "When validated in humans, these markers will assist regulators in assessing the human impact of dietary DON (if any) and make precise protective decisions." ◆

— 2009 FHB Forum —

Right: DON testing laboratory leaders participated in a panel discussion on proper sample selection, preparation techniques and submission protocol. Pictured, left to right, are: Yanhong Dong, University of Minnesota DON Testing Lab (wheat and barley); Paul Schwarz, North Dakota State University DON Testing Lab (barley); Kelly Benson, North Dakota State University DON Testing Lab (wheat); David Schmale, Virginia Tech DON Testing Lab (wheat).

In summing up the group's recommendations to researchers, Schmale offered the following suggestions to those using the DON labs' services:

• Review the University of Kentucky Plant Science Department's referral website: www.uky.edu/Ag/Wheat/wheat_breeding/USWBSI/DON.

• Prioritize your samples, as each of the labs is running many samples.

• Separate your samples into smaller batches and Excel worksheets.

• Be realistic when estimating the number of samples you'll be submitting — and then stay within your allocation.

• Consider bulking reps. Have the smaller batches tested first.

• Work with the labs. Communicate your needs and deadlines.

Above: Christina Cowger, USDA-ARS plant pathologist at North Carolina State University, discussed infection timing and moisture effects on DON and Fusarium-damaged kernels (FDK) in wheat. She said that field experiments conducted to clarify the relationship between DON, infection timing and postharvest moisture indicated that the window of maximum FHB susceptibility of winter wheat in North Carolina is "generally less than or close to 10 days after mid-anthesis."

Below: North Dakota State University extension plant pathologist Marcia McMullen updated the Forum audience on the features of the "ScabSmart" website (www.scabsmart.org). She urged Forum participants to help promote the website and its use, adding that there's still room for its improvement, e.g., providing (1) an expanded section on integrated FHB management, and (2) more uniformity in the formats in which the individual states' data are presented.

Above: "No single management approach" provides the answer to FHB and DON, emphasized Pierce Paul during his update on integrated management. The Ohio State University plant pathologist spoke to the enhanced levels of control that can be attained when combining multiple management methods — including the disease forecasting models. The estimated net value of the disease prediction system to U.S. wheat growers exceeds $47 million, he stated.

Talks on USWBSI Website

The following presentations from the 2009 Fusarium Head Blight Forum are available on the U.S. Wheat & Barley Scab Initiative's website: www.scabusa.org.

PLENARY SESSION —

- *DON Impact on the 2009 Wheat Crop*
  Carl Schwinke, Siemer Milling Co., Teutopolis, Ill.

FOOD SAFETY, TOXICOLOGY & UTILIZATION OF MYCOTOXIN-CONTAMINATED GRAIN —

- *Risk Assessment & Biomarkers for Deoxynivalenol*
  Chidozie Amuzie, Michigan State University, East Lansing

FHB MANAGEMENT —

- *Infection Timing & Moisture Effects on DON & FDK in Wheat*
  Christina Cowger, USDA-ARS, Raleigh, N.C.
- *Integrated Management of FHB & DON: A 2009 Update*
  Pierce Paul, Ohio State University, Wooster

VARIETY DEVELOPMENT & HOST PLANT RESISTANCE —

- *Recent Progress in Breeding for FHB Resistance in Canadian Barley*
  Bill Legge, Agriculture & Agri-Food Canada, Brandon, Manitoba
- *Successes in Development of Fusarium Head Blight-Resistant Soft Red Winter Wheat Varieties Using Phenotypic Evaluation*
  Fred Kolb, University of Illinois, Urbana
- *Family-Based Association Analyses in Plant Populations*
  Clay Sneller, Ohio State University, Wooster

GENE DISCOVERY & ENGINEERING RESISTANCE —

- *Molecular & Genetic Studies on Fusarium Ear Blight Disease of Wheat*
  Kim Hammond-Kosack, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom
- *Unraveling the Triticeae-Fusarium Graminearum Interaction*
  Gary Muehlbauer, University of Minnesota, St. Paul
- *Genetic Manipulation of Susceptibility to Fusarium Head Blight*
  Michael Lawton, Rutgers Univ., New Brunswick, N.J.

Above: This barley contingent was one of the commodity-based focus groups that met during the 2009 Fusarium Head Blight Forum. Other focus groups included durum, hard winter wheat, spring wheat, northern soft winter wheat, southern soft winter wheat, FHB management, gene discovery and engineering resistance, pathogen biology and genetics, and food safety, toxicology and utilization of mycotoxin-contaminated grain. Each group reviewed its standing action plan, evaluated progress and discussed new needs. Leaders from the various groups then reported back to the USWBSI Steering Committtee during its meeting the following day.

Below: Following adjournment of the 2009 FHB Forum, the U.S. Wheat & Barley Scab Initiative Steering Committee gathered on the afternoon of December 9 for its semi-annual meeting. The committee heard a report on the status of USWBSI within the federal budget; reviewed and approved the Executive Committee's fiscal year 2010 recommended plan and budget; and heard reports from the various focus groups.

Differential Accumulation of DON In Three Winter Wheat Cultivars

*By Stephen Wegulo, John Hernandez Nopsa, and P. Stephen Baenziger**

One of the objectives of this three-year study was to determine whether three winter wheat cultivars accumulated DON differentially. The cultivars were Harry (moderately resistant to FHB), 2137 (susceptible) and Jagalene (moderately susceptible).

The cultivars were planted following soybean in the fall of 2006 (Harry and 2137) and following corn in the fall of 2007 and 2008 (Harry, 2137 and Jagalene) at the University of Nebraska Agricultural Research and Development Center near Mead, Neb. In addition to natural inoculum, plots were inoculated with 1×10^5 spores/ml of *Fusarium graminearum* at early anthesis in May/June 2007, 2008 and 2009 and were not irrigated. Cultivars were arranged in a randomized complete block design with three replications.

FHB severity was determined 21 days after inoculation on 20 heads tagged in each of 11 disease severity categories in each plot — 0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50% (2007 and 2009); or 13 disease severity categories in each plot — 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 70 and 90% (2008). After harvest, DON concentration in grain from each severity category was determined at the North Dakota Veterinary Diagnostic Laboratory.

In all three years, Harry, with a moderately FHB-resistant phenotype, consistently accumulated more DON than the susceptible 2137 *(Fig. 1)*. In 2008, Jagalene accumulated more DON than 2137 at all FHB severities. Regression lines showed that in 2008, Jagalene accumulated less DON than Harry at severities less than 40% and more DON at severities higher than 40%.

In 2009, DON accumulation in Jagalene was less than that in Harry but similar to that in 2137. A test for differences between regression slopes (DON regressed on FHB severity for each cultivar) was significant in 2007 ($P = 0.0008$) and 2008 ($P < 0.0001$), but not in 2009 ($P = 0.8976$).

The reason for the consistently higher DON in Harry compared to 2137 is not known. One hypothesis is that Harry matures later than 2137, which allows more time for DON accumulation. However, it is possible that differences between the two cultivars in the way they interact with *F. graminearum* at the cellular and molecular levels are responsible for the differential accumulation of DON. It is also possible that stress to the fungus resulting from the resistance mechanism in Harry may cause the fungus to produce more DON.

This work clearly shows that winter wheat cultivars differ in the levels of DON they accumulate.

This work clearly shows that winter wheat cultivars differ in the levels of DON they accumulate, and that a cultivar with a resistant phenotype does not necessarily accumulate less DON than a cultivar with a susceptible phenotype and vice versa.

Growers should consider resistance/susceptibility to both FHB and DON when selecting cultivars. To reduce losses from DON (discounts at the elevator or rejection of grain) and FHB (yield and grain quality reduction), cultivars with resistance to both parameters should be selected whenever available. ◆

** Stephen Wegulo is assistant professor and extension plant pathologist at the University of Nebraska-Lincoln. John Hernandez Nopsa is a UNL graduate student. P. Stephen Baenziger is UNL small grains breeder.*

Acknowledgement and Disclaimer:
This material is based upon work supported by the U.S. Department of Agriculture under Agreement No. 59-0790-7-080. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

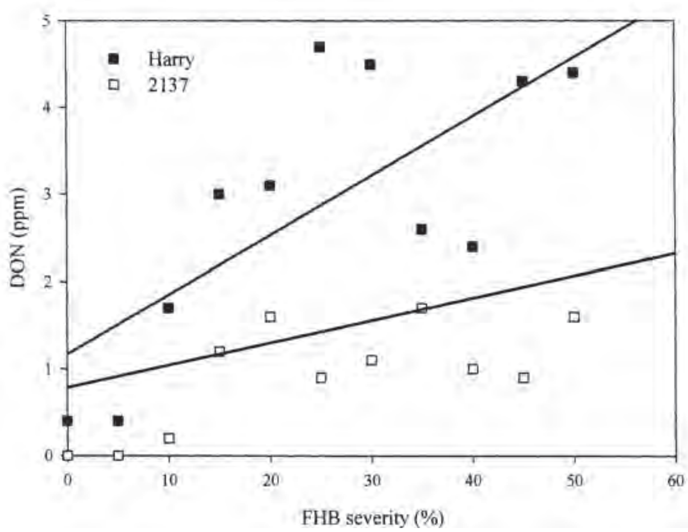


Fig. 1. Regression lines showing that Harry, a winter wheat cultivar with an FHB-resistant phenotype (filled squares, red line), accumulated more DON than the FHB-susceptible 2137 (open squares, blue line) in 2007. Similar results were obtained in 2008 and 2009.

‘Quest’

A New Barley Variety with Improved Scab Resistance

By Kevin Smith*

Quest is the first malting barley variety release from the University of Minnesota Agricultural Experiment Station with improved Fusarium Head Blight resistance.

Formerly known as experimental line M122, Quest is a spring, six-rowed malting barley that accumulates about half the level of deoxynivalenol (DON) compared to other varieties grown in the Midwest. (See *Fusarium Focus* Volume 7, Issue 1, Spring 2007.)

One of the parental sources of resistance in Quest, Zhedar 1, a two-rowed variety from China, was introduced through a line selected from an early generation population created by Richard Horsley at North Dakota State University. The sharing of germplasm in this way has been extremely important to scab resistance breeding efforts.

The other source of resistance comes from the variety MNBrite, which derives its resistance from a Swiss landrace called Chevron.

Quest accumulates half the level of DON compared to the varieties Tradition, Lacey and Robust, which together currently occupy nearly 70% of the barley acreage in the Midwest. Quest has yields similar to Lacey and Tradition.

Quest is currently being evaluated in the American Malting Barley Association (AMBA) plant-scale brewing program. Seed is available through the Minnesota Crop Improvement Association. ◆

** Kevin Smith is barley breeder and associate professor with the University of Minnesota.*

Use of Air-Assist Sprayers for Spraying Small Grains for Suppression of FHB

By Scott Halley, Vern Hofman and Kevin Misek

Langdon Research Extension Center, North Dakota State University

Field studies with air-assist sprayers to suppress Fusarium Head Blight were conducted during 2007, 2008 and 2009 at the Langdon Research Extension Center. These studies were inoculated and misted and were completed on both hard red spring wheat (HRSW) and barley. Prosaro fungicide was applied at 6.5 fl oz/acre with 0.125% v.v Induce adjuvant at the recommended growth stages on barley, Feekes 10.5, and HRSW, Feekes 10.51.

Three different air stream flows, ranging from 24 to 72 miles per hour, were tested with a spray application rate of 10 gallons per acre. The air flow was measured with the use of a digital wind meter held about two inches from the air orifice. Flat fan spray nozzles were mounted outside the air stream and angled to direct the spray into the air stream so the air flow would carry spray to the target.

The most effective result with both wheat and barley was to direct the air stream forward at about 30 to 45 degrees downward from horizontal. Thirty degrees down is preferred, but some air-assist sprayers are hard to adjust to that angle.

The best air velocity was found to be about 50 miles per hour. Use 80 degree flat fan nozzles operating at a pressure of about 40 to 50 pounds per square inch (to obtain a dropsize in a range of 300-350 microns), directing the spray into the air stream. Adjust operating pressure, travel speed and nozzle size to apply 10 gallons per acre. A drop size of about 300 to 350 microns tends to move past the awns, so the drops deposit on the kernels.

The spray boom should be mounted about 10 to 12 inches above the grain heads or the spray target when angled 30 degrees downward from horizontal. This will cause the air velocity of the sprayer to push the heads forward so they are as close to perpendicular as possible to the air stream and spray.

An NDSU extension publication, now being prepared, will provide more information on the use of air-assist sprayers. It will be found at www.ag.ndsu.edu. ◆

Brueggeman Joins NDSU As Barley Pathologist

Dr. Robert Brueggeman has joined North Dakota State University as assistant professor and barley research pathologist.


Robert Bruggeman

Raised in the Pacific Northwest, Brueggeman received his B.S. in genetics and cell biology, and M.S. and Ph.D. in crop sciences, all from Washington State University. His professional experience began in the lab of Dr. Andris Kleinhofs in1997 as an undergraduate research assistant, and he continued working in Kleinhofs' barley molecular genetics lab for 12 years. The major focus of his research has been the cloning and characterization of stem rust resistance genes of barley.

Brueggeman's responsibilities at NDSU include developing a strong research program on barley diseases of current and/or potential economic importance to North Dakota. "In addition to my basic research, I will work with the NDSU barley breeding program to assist the development of disease-resistant germplasm and varieties for the state," he indicates. "Currently, my research program is investigating Fusarium Head Blight, stem rust (Ug99) and the two forms (spot type and net type) of net blotch." ◆

Rotation, Fungicide, Seed Treatment & Cultivar as Mgmt. Tools to Control Disease on Two- & Six-Row Barley

By S. Halley & K. Misek*

Efforts have been initiated and funded by the U.S. Wheat & Barley Scab Initiative to communicate some of the research progress made in developing and identifying strategies that will reduce or minimize the negative effect on small grains from the disease Fusarium Head Blight (FHB) or scab. Seed treatment was added to this study with support from the North Dakota Barley Council.

Two of these efforts are reported here, comparing the use of crop rotation, a foliar fungicide treatment, a seed treatment and cultivars with different levels of resistance or tolerance to FHB. The study utilized a common regional crop rotation — barley after canola — as a comparison to a small grain rotation (barley after hard red spring wheat). The theory was that inoculum quantity would be lower when the prior crop was not susceptible to FHB.

The second strategy researched was an application of Prosaro fungicide to minimize the effects of FHB. The third strategy researched would be the selection of a cultivar with less susceptibility to FHB. An additional strategy was also tested comparing a broad-spectrum seed treatment with untreated seed.

Factors evaluated:

- Previous crop canola.
- Previous crop hard red spring wheat
- Prosaro fungicide and Induce adjuvant applied at Feekes growth stage 10.5.
- Untreated.

- Charter F2 (triticonazole/metalaxyl) applied at rate of 5.4 fl oz/cwt, Stamina (pyraclostrobin) at rate of 0.4 fl oz/cwt and the BASF insecticide Axcess (imidicloprid) applied at a rate of 0.2 fl oz/cwt.
- No seed treatment.

- Two-row cultivars AC-Metcalfe, Conlon, Merit, Pinnacle, Rawson, Scarlet.
- Or six-row cultivars Excel, Legacy, Quest, ND20448, Robust and Tradition.

Differences in significant effects should be additive. For example, the increase in yield from a seed treatment on two-row barley (8.3 bu/ac) can be added to the increase from planting into canola residue (14.7 bu/ac) and planting cultivar Scarlet versus AC-Metcalfe (33.3 bu/ac). So total expected yield increase due to select management options was 56.3 bushels for two-row barley raised in an environment similar to Langdon in '09.

The full reports can be viewed on the NDSU-Langdon site —www.ag.ndsu.edu/langdon/plantpathology.html — and include Fusarium Head Blight disease assessment, additional quality parameter evaluations, and disease assessments from the sub-crown internode section from the roots. ◆

Support for this study was provided by the North Dakota Barley Council. This material also is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-8-069 and the North Dakota Barley Council. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of USDA.

Effect of Disease Management Strategies on DON Levels, Yield & Test Weight in Two-Row & Six-Row Barley, Langdon, N.D., 2009

Two-Row Barley

Main Effects	DON*	Yield	Test Wt.
Seed Treatment	*(ppm)*	*(bu/ac)*	*(lbs/bu)*
Untreated	0.44	92.8	49.2
Treated	0.37	101.1	49.8
LSD (P=0.05)	0.07	4.2	0.4
Residue			
Canola	0.37	104.3	49.7
HRSW	0.44	89.6	49.3
LSD (P= 0.05)	NS	9.5	NS
Fungicide			
Untreated	0.49	97.5	49.3
Prosaro	0.32	96.4	49.7
LSD (P=0.05)	0.14	NS	NS
Cultivar			
AC-Metcalfe	0.27	78.0	48.5
Conlon	0.30	95.5	50.7
Merit	0.42	100.6	49.1
Pinnacle	0.50	103.0	49.5
Rawson	0.59	93.2	48.2
Scarlet	0.36	111.3	51.0
LSD (P=0.05)	0.12	4.8	0.6

Six-Row Barley

Main Effects	DON*	Yield	Test Wt.
Seed Treatment	*(ppm)*	*(bu/ac)*	*(lbs/bu)*
Untreated	1.3	100.9	48.3
Treated	1.2	102.2	48.5
LSD (P=0.05)	NS	NS	NS
Residue			
Canola	1.2	114.8	48.8
HRSW	1.3	88.3	48.0
LSD (P= 0.05)	NS	5.7	0.5
Fungicide			
Untreated	1.9	99.2	48.2
Prosaro	0.7	103.9	48.6
LSD (P=0.05)	0.6	NS	0.4
Cultivar			
Excel	1.6	111.5	47.8
Legacy	2.0	98.9	47.5
Quest	0.7	104.2	47.8
ND20448	1.0	91.2	48.0
Robust	1.1	103.6	49.9
Tradition	1.1	100.0	49.3
LSD (P=0.05)	0.3	4.8	0.3

*Deoxynivalenol accumulation in the seed. Data presented from two similar studies. Data should not be compared between the two studies, but only within each study.

* The authors are, respectively, crop protection scientist and research specialist with North Dakota State Univ., Langdon.

Fighting *Fusarium* Through Molecular Genetics

Editor's Note: *The following article appeared in the February issue of* Agricultural Research, *the magazine of the USDA Agricultural Research Service. It was authored by Alfredo Flores and Stephanie Yao of the ARS Information Staff.*

In Madison, Wisc., as part of the U.S. Wheat and Barley Scab Initiative (USWBSI), ARS plant molecular biologist Ron Skadsen is working to understand and improve barley's resistance to the fungal pathogen *Fusarium graminearum*, which causes the devastating disease commonly known as "scab." Scab reduces yield by causing sterility and shrunken kernels and contaminating the grain with mycotoxins.

Skadsen, who is in the Cereal Crops Research Unit, first sought to identify the barley tissues that *Fusarium* most readily attacks. He infected barley seed spikes with *Fusarium* transformed to contain a green fluorescent protein that makes the fungus glow neon green when examined under a fluorescence microscope. Skadsen found that *Fusarium* attacks the protruding seed tip of the developing seed, the soft tissue connected with it (just under the hull), and, to a lesser extent, the seed's outer hull.

Based on his findings, Skadsen's team developed gene promoters that can be used to "turn on" genes that defend against *Fusarium* in these susceptible tissues. The promoters — from barley genes Lem1, Lem2 and Ltp6 — are attached to barley antifungal genes to turn them on at specific locations.

"Knowing which parts of the barley plant *Fusarium* attacks gives us insight into how the infection process works," says Skadsen. "We now know where to knock out *Fusarium* in the early stages of infection, which will aid targeted breeding and biotechnology strategies for making barley resistant."

Previous studies examining *Fusarium* infection found that the fungus will liquefy the starchy part of the seed within five days after infection. But these studies used detached seeds. Skadsen found that, even 16 days after infection, *Fusarium* does not penetrate the starchy endosperm when the seed remains attached to the spike. This means breeders can focus on looking for traits that will prevent or head off the fungus's early penetration.

Skadsen and research leader Cynthia Henson are also looking to understand the biochemistry of susceptible barley tissues through metabolic profiling during the first three days of infection. They found that there is a shift in sugar levels, especially the appearance of and rapid increase in the sugar alcohol ribitol, a metabolite that has not been extensively studied. The scientists are next looking to see whether the shifts in various sugar levels are caused by the plant's mobilizing sugar away from the infection point or by the fungus's taking specific sugars and metabolites away from the plant through feeding. They hope these results will inform researchers how *Fusarium* alters the metabolism of barley tissues to optimize its own nutrition.

Marker-Assisted Breeding Of Scab-Resistant Barleys

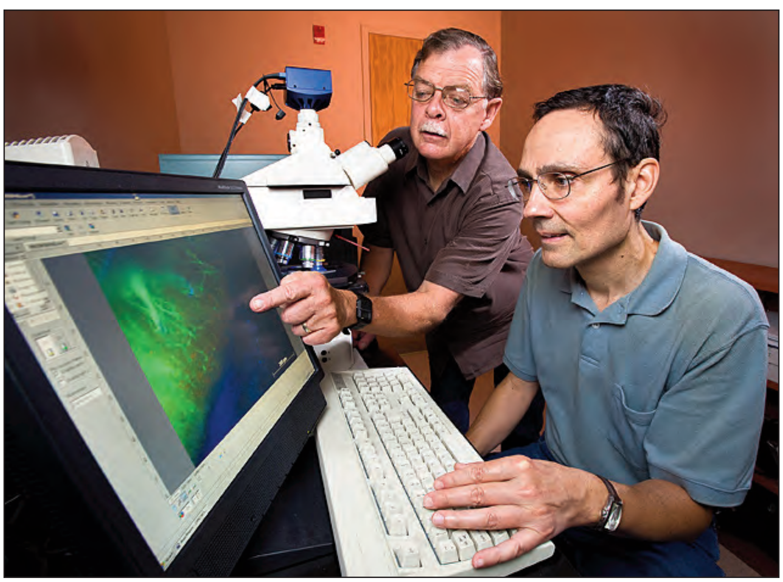
Shiaoman Chao and her team at an ARS genotyping laboratory in Fargo, N.D., work closely with small grains breeders in the Northern Plains region to use marker technologies to improve crops. Chao, a molecular geneticist, uses current genomic information to develop DNA markers tagged to important agronomic traits. Once appropriate markers are identified, they can be used in breeding populations to increase the efficiency of selection.

"At Fargo, we provide genotyping service to all small-grains breeders located in the region," says Chao, who is in the ARS Cereal Crops Research Unit.

Rich Horsley at North Dakota State University (NDSU) and Kevin Smith at the University of Minnesota (UM) are key collaborators in Chao's work, providing breeding lines for the ARS Fargo team to do the genotyping.

To accelerate the rate of deployment of genes for resistance to scab using marker technologies, the Fargo lab has developed a sample preparation protocol and high-throughput genotyping procedures that are both efficient and cost effective for carrying out marker-assisted breeding.

Breeders send samples generated in



USDA-ARS molecular biologist Ron Skadsen (left) and technician John Herbst use a fluorescence microscope to view a thin cross-section of a barley seed.

their breeding programs by inserting leaf clippings into 96-well plates provided by the Fargo lab. Much of the genotyping process has been semi-automated through use of robotic instruments.

High-throughput DNA extraction and marker genotyping protocols can help scientists conduct genetic mapping studies with large populations. This has been important in mapping scab resistance in barley because three of the most important resistance genes in barley are located very close to undesirable genes, which creates a substantial problem for breeders. It can be overcome by screening very large populations with markers and identifying progeny in which the undesirable traits are unlinked from the resistance trait.

Chao screens breeding lines with resistance to scab using DNA markers previously identified as linked to the resistance genes in both wheat and barley. She also works on DNA markers for other traits, such as protein quality and resistance to leaf rust and tan spot in wheat and resistance to net blotch and Septoria speckled leaf blotch in barley.

"Creation of the ARS genotyping centers has dramatically changed the way small-grains breeders think about using DNA markers in breeding," says Smith, who runs the barley breeding project at UM. "There, the genotyping is done at a scale and speed that would not be possible if left to individual breeding programs."

USWBSI Projects in North Dakota Take Shape

There are a total of 145 USWBSI projects in all research categories, according to the initiative's manager, Sue Canty. Among USWBSI's top objectives is the reduction of mycotoxins. This is important to the producers, processors, and consumers of wheat and barley.

Rich Horsley, a professor and barley breeder at NDSU, has been using his USWBSI grant for development of improved six-rowed and two-rowed germplasm. Horsley is also coordinating a Fusarium Head Blight (FHB) nursery at Zhejiang University in Hangzhou, China. Highlights include advancement of the six-rowed barley breeding line ND20448 into the final stages of plant-scale malting and brewing evaluation by the American Malting Barley Association, Inc. (AMBA).

Horsley has collaborated with Chao on mapping quantitative trait loci that confer resistance to scab and reduce accumulation of the mycotoxin known as "deoxynivalenol." Identification of markers useful for screening across the multiple pedigrees and genetic backgrounds used by the NDSU barley breeding program has been unrealized until 2009. In the past, markers would work in some populations and not others. Using markers identified in research conducted by collaborators at UM, Chao was able to genotype all six-rowed lines used as parents in crosses the past four years and all six-rowed lines grown in yield trials in the summer of '09.

Data provided by Chao has allowed Horsley's program to be more efficient in selecting lines as parents for this fall's crossing block, planning which crosses to make, and determining which lines should be candidates for advancement to 2010's yield trials. The project's goal for 2010 is to submit to Chao's laboratory leaf tissue from early-generation lines so the research team can determine which lines to advance for further testing in the breeding program.

Scab is a major factor in the decline of malting barley production to historic lows in the Dakotas and Minnesota, key states for raw material for the U.S. malting and brewing industry. "Research outputs from the USWBSI are expected to help address the production decline, reducing the need to source malting barley long distances," says AMBA president Mike Davis.

CAP Key to Developing Elite Barley Germplasm

The focus of Coordinated Agricultural Project (CAP) research is to identify molecular markers that will speed up barley breeding efforts. The novelty of this approach is that mapping of important traits is done using contemporary breeding populations. The result is that breeding and mapping are done in parallel, accelerating translation of genetic information useful to plant breeding. Begun in 2005, CAP has already identified genetic markers associated with malting quality traits, winter hardiness, and resistance to scab.

The goal of CAP is to detect single-nucleotide polymorphism (SNP) variations located at 3,072 different positions in the genomes of 3,840 barley breeding lines studied over a four-year period. To detect these genetic variations, the Fargo lab is using a high-throughput genotyping system—a speedy and efficient way of analyzing SNPs—that's capable of analyzing SNPs located at 1,536 positions of 96 individuals in a single reaction assay.

Chao is ARS's lead scientist in CAP. Her team in Fargo carries out the high-throughput genotyping to generate 10 million data points. CAP participants mine this data and identify markers for barley improvement. The results have been published in scientific papers on evaluating the genetic diversity present among U.S. barley breeding programs. All findings in identifying SNP markers closely associated with various agronomic traits, such as scab resistance, are made publicly available to assist all barley breeders. ◆

Fusarium Focus

Fusarium Focus is an online newsletter published periodically by the U.S. Wheat & Barley Scab Initiative. The USWBSI is a national, multi-disciplinary and multi-institutional research system whose goal is to develop as quickly as possible effective control measures that minimize the threat of Fusarium Head Blight (scab), including the production of mycotoxins, for the producers, processors and consumers of wheat and barley. Contact information is as follows:

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