Falling Number update, Western Wheat Workers, Corvallis OR, May 31, 2017

At the Falling Number Summit in Feb. 2017, the following goals and action items were adopted. This is a progress report on those goals as of the Western Wheat Workers meeting that occurred on May 31, 2017. Additional progress that has been made in some areas will be included in a progress report developed in November 2017.

Goal 1. Improving the Hagberg-Perten Falling Number (FN) test

Problem: The Hagberg-Perten Falling Number test is the industry standard for measuring starch digestion by alpha-amylase enzyme in grain damaged by preharvest sprouting (PHS) or late maturity alpha-amylase (LMA). Differences in altitude and atmospheric pressure, or in the way the test is performed can cause variability in the FN test. Because the grain market uses FN as a standard, our goal is to optimize the FN test to reduce the variability.

Proposed Approaches from FN summit:

A. **Revise FGIS Rule** (v 9180.38(5/2013)) and ensure that revisions become the standard across all testing labs. Test the effectiveness of the revised rule and training materials

B. Benchmark: The FGIS rule is revised and followed and the FGIS rules for sampling are followed routinely.

Progress and New Comments.

A. Don Potts of the Washington State Dept. of Agriculture was leading the effort to propose and implements the improvements to the FGIS Rule. Since he has passed away, Pat McCluskey of the Federal Grain Inspection Service was recommended to lead this effort. It may be physically impossible to reduce the error of the FN test to a coefficient of variation less than 5%.

B. Stephen Delwiche of the USDA ARS Food Quality Lab has requested access to a U.S. Army hypobaric chamber to perform the experiments required to correct more accurately for altitude differences. He is on a waiting list, and has been told that he'll likely be given access to the facility in August.

C. Sampling methods should accurately reflect the average FN of a load. The current FGIS protocol for sampling a load is quite good. Delwiche has requested that researchers in Washington, Oregon, Idaho, Montana, or Utah contact him as soon as they know that there is a low FN problem in 2017. The sampling process will be monitored to identify areas for improvement.

Goal 2. Examine alternatives to the FN test.

Problem: Financial losses in 2016 were more severe because low FN grain was mixed with high FN grain before elevators realized there was a problem. Because alpha-amylase is a catalyst, low FN grain lowers FN far more than an average of the two lots mixed. A rapid testing technique is needed when grain is unloaded to preserve the value of the high FN wheat and segregate the likely problem wheat grain. Alternative approaches will also be tested to determine if accuracy can be improved. The current FN assay cannot differentiate between PHS and LMA as causes of low FN.

Proposed Approaches from FN summit:

A. Alternative to the FN test may be less dependent on barometric pressure and other sources of variation. These include the stirring number measurement from the rapid viscoanalyzer (RVA), the Amylograph, and the Testogram (Chopin). Craig Morris agreed to compare these methods to the Hagberg-Perten FN machine.

B. NIR. Risius et al., (2015) claimed to have developed an NIR calibration that could predict the FN of an intact grain sample

C. Enzyme assays. The alpha-amylase enzyme assay manufactured by Phadebas is a tablet that is dropped into a meal/water slurry. The degree of blue is read by a spectrophotometer and compared against a standard curve to determine the amount of alpha-amylase activity in the

sample. This approach could potentially be developed for use at the elevator. An enzyme may differentiate between PHS and LMA because LMA induces only alpha-amylase whereas PHS induces alpha-amylase plus many other hydrolytic enzymes including proteases, Beta-glucanases, and lipases. New enzyme assays that allow differentiation of PHS and LMA will be developed in collaboration with Megazyme, the manufacturer of the an alpha amylase assay using the Ceralpha method.

D. ELISA for alpha-amylase. The ReadRite(TM) is a patented ELISA for alpha-amylase that was distributed by Bayer Crop Science in Canada and Australia. This product was intended for rapid analysis of a meal/water slurry. It is no longer marketed. Development of an ELISA test could allow differentiation of low and high FN at the elevator to distinguish LMA from PHS.

Progress and New Comments.

A. Craig Morris and Alecia Kiszonas of the USDA ARS Western Wheat Quality Laboratory compared the FN and stirring number from RVA on samples from FGIS and from the WSU variety trials. They found a low correlation between the two. RVA cannot serve as a replacement for FN.
B. Delwiche has been unable to find an acceptable NIR calibration for FN. He will generate a report for the grain commissions, and will consider publishing his negative results.
C. Art Bettge of ADB Wheat Consulting discussed the potential to generate enzyme assays to differentiate between LMA and PHS with Barry McCleary of Megazyme. Doug Engle provided samples for preliminary experiments.
D. Alex McGregor of the McGregor Company and Morris contacted Bayer Cropscience. Bayer intends to drop the ReadRite product and does not intend to maintain patent rights. PNW researchers will have to raise antibodies for this purpose, in close collaboration with an industry partner interested in manufacturing an ELISA as an easy-to-use product.

Goal 3. Improve preharvest sprouting resistance.

Problem: The soft white wheat cultivars that show a high degree of susceptibility to preharvest sprouting when it rains before harvest include Bruehl, Xerpha, Bruneau, and Brundage. PHS susceptibility tends to be associated with excellent emergence from deep planting. Selection in breeding programs for improved genetic resistance to PHS without compromising emergence is needed.

Proposed Approaches from FN summit:

A. Camille Steber of the USDA-ARS Wheat Health, Genetics and Quality Unit will screen current varieties for PHS resistance using the spike wetting test
B. Determine if preharvest germination/sprout can be blocked by inhibiting GA biosynthesis or signaling or by increasing ABA hormone sensitivity.
C. Identify genetic loci for PHS tolerance that are currently segregating in PNW wheat using genome wide association mapping.
D. Investigate the effects of the starch, protein and lipid composition of the grain on FN, independently of or in spite of preharvest sprouting or LMA.

Progress and New Comments.

A. Steber is screening USDA, and WSU breeding lines using funding from the Washington Grain Commission
B. Steber has found that ABA hypersensitive wheat carrying the ERA8 gene has increased preharvest sprouting tolerance. The ERA8 as part of a NIFA-funded project.
C. Arron Carter or WSU, Kim Garland-Campbell of the USDA-ARS Wheat Health, Genetics and Quality Unit, and Steber are conducting a genome-wide association study of PHS on a winter wheat population representing PNW breeding programs.
D. The Idaho Grain Commission funded Amy Lin of the Univ. of Idaho to examine if differences in starch composition affect FN.

E. The Eastern Wheat Workers requested that Steber could include varietal sources of PHS resistance genes and the associated molecular markers on 7599 Falling Number website. (http://steberlab.org/project7599.php)
F. Deven See , director of the USDA-ARS Western Regional Small Grains Genotyping laboratory, will develop and assay breeding lines with molecular markers linked to preharvest sprouting genes in wheat germplasm from the PNW and elsewhere.
G. The Idaho Grain Commission funded Daolin Fu of Univ of Idaho to examine the pattern of alpha-amylase expression during PHS and LMA.
H. Steber is conducting research to identify the timing of amylase expression during germination and its correlation with FN.

4. Breeding for LMA resistance.

Problem: LMA can cause low FN, even when there is no rain. It is triggered when developing wheat experiences a major temperature change between 25 and 30 days after flowering. This problem was first recognized in PNW wheat in 2013. LMA caused widespread problems in 2016. Little in known about the genetics of this problem in PNW wheat. Identification and selection for resistant cultivars is the best way to prevent the problem. The published literature says that increased nitrogen application increases FN by increasing protein content . There have also been reports of fungicide interactions with low FN.

Proposed approaches from FN summit:

A. Categorize current wheat varieties for FN stability.
B. Conduct analysis of currently existing recombinant inbred line populations to identify genes associated with LMA resistance in Pacific Northwest wheat.
C. A comprehensive LMA-screening program requires that a more rapid screening method for field screening be developed. An LMA screening assay that can be conducted in controlled environments is also needed
D. Identify the timing and temperature ranges associated with LMA in the PNW.
E. Determine if fungicide application effects LMA
F. Determine if specific agronomic conditions support higher FN.

Progress.

A. Garland Campbell analyzed three years of FN data for winter wheat to examine which varieties show stable FN, and to characterize the heritability of the trait. Extending this study to include FN data from Idaho and Oregon can be done once data is shared. The list of more stable varieties has been shared in grower talks in 2017.
B. Carter compiled information on available mapping populations in Washington, Idaho, and Oregon. Michael Pumphrey of WSU gathered seed for parents of spring wheat mapping populations. This screening was initiated during the 2017 crop season. Jianli Chen of Univ. of Idaho and Steber are screening the parents of the spring wheat mapping populations. See will collect the winter wheat mapping populations for planting in the fall of 2017 and screening in the 2018 crop season.
C. Steber and Chen are examining temperatures and developmental windows for LMA induction in winter and in spring wheat, respectively.
D. Juliet Marshall of Univ of Idaho and Carter will investigate the effects of fungicide on LMA.
E. Kurt Schroeder of Univ of Idaho and Marshall examined the effects of nitrogen and manure treatment on FN. They found that manure caused decreased FN, likely because manure treatment caused the wheat to lodge so that it stayed wet longer and was more susceptible to PHS.

Goal 5. Improving Communication.

Proposed approaches from FN summit:

A. Distribute summary of FN summit via multiple venues
B. Create a summary of FN over all years for the 7599 website

C. Create a modeling system to help farmers choose a variety.
D. Meet regularly to discus progress and strategy.

Progress.

A. The FN summit was described in Wheat life magazine, and a White Paper summarizing the recommendations of the summit was shared with stakeholders.
B. The summary of FN over all years will be posted as soon as the statistical analysis is completed for 2017 (Goal 4A).
C. The modeling project requires that the data analysis for LMA and PHS resistance described above be finished first.
D. Progress was discussed at the Western Wheat Worked meeting in Corvallis on May 31, 2017. Progress will also be discussed at the Tristate Growers' meeting in November.

Input from Utah and Montana participants in the Western Wheat Workers Meeting.

Dr. David Hole of Utah State University had low FN problems in SE Utah in 2016. He said that SW Colorado had low FN problems, especially in the organic production nursery. FN values ranged from 270 to 444. He is not sure why farmers having low FN problems. Low FN is an issue in organic wheat production.

Dr. Robert (Bob) Stougaard of Montana State University said that Montana farmers have been suffering from discounts for low FN for several years, even when there is no rain and in red wheat. Stougaard provided a report of low FN values in Montana field trials. The lowest FN value was 180 sec for SY Teton, a value that would lead to a $1.50/bushel discount. Dr. Mike Giroux of Montana State Univ is also working on the low FN problem with a goal to map the genes for resistance to low FN.

Compiled and submitted by C. Steber, C. Morris, A. Kiszonas, and K. Garland Campbell, August 14, 2017