

Pulling Together: Dedicated Geneticists, Wheat Researchers, Agencies & Commissions Moving Forward to Limit 'Falling Number' Risks

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Measuring Time, Gathering Numbers & Grading the Results with a Test 'Long in the Tooth'

The Hagberg-Perten (H-P) test that grades wheat by measuring the effects of the alpha-amylase (AA) enzyme was developed a long time ago. Its original purpose was not to measure grain quality but to help bakers wanting to know how much AA to add when making loaves of bread.

Back then Dwight Eisenhower was President, Hawaii and Alaska had just been admitted to the Union, and folks were worried about the Soviet Sputnik satellite they'd seen in the night sky a few years earlier. I was in fifth grade at the two-room country school at Hooper where we measured time by the big wall clock above the teacher's desk, studied numbers by writing them on the chalkboard and were graded by how well we did on the 'Think and Do' tear out page at the back of our workbooks. I remember one of my fellow students addressing the uncertainty of the test results by 'borrowing' the teacher's handbook and copying the answers verbatim including the clause 'any answer substantially similar is acceptable.' That was not a wise approach. The year was 1960.

Technology has advanced rapidly on so many fronts since those halcyon days. But for half a century the H-P test has remained the tool used to measure starch degradation effects on grain quality. We've known since the pioneer days that substantial rains on ripe grain can cause losses—and the H-P test measures them. Our typical hot, dry summers fortunately limit the risk. The test also is sensitive to late maturity alpha-amylase (LMA), caused by extreme temperature variation and cold weather during late grain filling, 25-30 days after pollen shedding. A French wheat researcher doing work in Australia recently described LMA as *"the tip of the*

iceberg" and preharvest sprouting (PHS) as more formidable—the full obstacle, above and below the water line. *"Does LMA cause declines in quality?"* he asks, and concludes more tests need to be done. PHS is clearly bad for sponge cake. There is likely some correlation, probably not as strong, between LMA and cake quality. Craig Morris, Alecia Kiszonas and the staff at the Western Wheat Quality Lab in Pullman, WA have been busy doing baking studies to learn more, with funding assistance from the Washington Grain Commission (check out Episode 86 of *'Wheat All About It'* podcast from the Washington Grain Commission).

The real-world consequences of low Falling Numbers (FN) caused by LMA can be severe. It was the primary culprit in '16 when losses were staggering, in the tens of millions of dollars. Kudos to grain companies who, this year, were able to handle the crops with minimal, if any, discounts and to our good fortune of not facing a temperature roller coaster as severe as a couple of years ago.

We can't find a 'Think and Do' book with the answers inside when a weighted plunger falls to the bottom of a wheat slurry in less than five minutes—300 seconds. Holding onto low FN wheat can help, if the number isn't 'too low' and the days are 'hot enough'. Only an expert can mix 'high' and 'low' numbered wheat because it takes only a little low FN wheat to lower the reading for the whole batch. As Washington State University researchers put it, the *"Falling Numbers test is a test [that] farmers love to hate."* The reason is inconsistency both from physical factors in test taking and biological variation.

A Team Making Big Strides Forward

We are fortunate to have our three land grant schools, USDA/Agricultural Research Service (USDA/ARS), and the Western Wheat Quality Lab in Pullman, WA working to get answers. David Weller, of the USDA/ARS, says of the group he leads-- *"we are*

"We are all working as a team in a seamless effort to address all aspects of this particular problem."

-David Weller, USDA/ARS

all working as a team in a seamless effort to address all aspects of this particular problem. We are working night and day, as hard as we can, to find solutions."

David Weller, Camille Steber and Craig Morris of ARS, Michael Pumphrey, Arron Carter and their colleagues at WSU, Jim Moyer, the recently retired Associate Dean of CAHNRS, the agricultural school at WSU—all have been active in meeting the challenge. I've pitched in whenever I could be of help.

WSDA Director Derek Sandison has been a stalwart ally along the way. New technology, he has stated, "would go a long way toward mitigating the current problem" by allowing better segregation of lower FN wheat. The Washington Grain Commission (WGC), ARS and WSU were already working on the challenge before the crisis of '16 hit like a sledge hammer—focusing on developing more resistant varieties and finding a better way to assay the presence of proteins—with quicker, more reliable results. The Pacific Northwest (PNW) wheat industry sought and obtained from Congress \$1 million in new funding to USDA/ARS that makes possible the hiring of a researcher to do full time background work on seed varieties. Likewise, efforts of the WGC working in collaboration with USDA/ARS have helped provide \$1 million worth of new equipment to replace the tools already well-worn when Craig Morris took the lead at the USDA/ARS Western Wheat Quality Lab thirty years ago.

Putting the Pedal to the Metal: A Fast Pace after the '16 Debacle

Just over two years ago, in September '16, as the dismal FN results came in, growers, scientists, agencies, state legislators and Representative Cathy McMorris Rogers met in our office to chart a path ahead. My biggest concern at the time was to make sure the FN challenge didn't fall off the radar screen down the road as other issues came to the fore. It hasn't.

The following February a Falling Numbers symposium in Spokane brought together all facets of the PNW wheat industry to identify the research being done, knowledge gaps, and short- and long-term needs. Craig Morris and I were asked to take the lead in looking for ways to "use or modify existing or new technology to develop a rapid and simple test for elevators and growers that is quick, repeatable and accurate."

Glen Squires of Washington Grain Commission (WGC) points out that "one of the needs raised at the symposium was to improve the accuracy of the current test, including updating testing procedures and protocol and ensuring uniformity of processes.

"Factoring in barometric pressure does in fact show a linear relationship and improves accuracy of the current test."

-Glen Squires, WGC

WSDA's grain inspection division provided this type of information to the Federal Grain Inspection Services. At the same time, USDA/ARS in Beltsville, Maryland has been doing work looking at the influence of barometric pressure on FN test results. Factoring in barometric pressure does in fact show a linear relationship and improves accuracy of the current test. To date, the FN testing directive has called for an altitude adjustment at 2,000 feet to compensate for elevation changes. FGIS [Federal Grain Inspection Service] is planning to implement an updated directive next May, including barometric pressure."

Resistant Varieties are Clearly the Long-Term Answer

Breeding resistant wheat varieties is a complex balancing act. Though low AA readings can cause painful FN results when the crop heads to market, AA is vitally important to a growing crop, too, in fueling initial plant growth and for healthy plant leaves. Dr. Daolin Fu's group at the University of Idaho is working on wheat AA genes with funding support from the Idaho Wheat Commission. They are using CRISPR technology to edit six AA genes and will find out how specific alpha-amylases attribute to grain falling numbers and their effect on plant growth. Camille Steber explains that the problem comes with production of AA during the wrong time in development. As Steber, Pumphrey, and Arron Carter put it in '13: wheat breeders need to strike a balance between sufficient dormancy at maturity to prevent sprouting without sacrificing the ability of the seed to lose its dormancy quickly enough to obtain good emergence when planted. As they put it last year, "if we do our job right farmers will no longer notice when we have LMA vulnerable weather because their wheats will be resistant."

Andrew Ross of Oregon State University (OSU) has done extensive FN work for many years—including studying the impact of storage time and temperature on low FN wheat and the role of starch and protein in wheat flour noodles. His OSU colleague Bob Zemetra has made big strides in building resistant varieties. The germ plasm in Zemetra's program has shown real promise in reducing vulnerability to PHS

and LMA. Together our land grant schools and USDA/ARS are moving toward fewer susceptible releases.

Work has already been done to get a sense of how vulnerable current varieties are to FN. Camille Steber has shared results from several years of testing on line, funded by the Washington Grain Commission <http://steberlab.org/project7599data.php>. McGregor research leader Cat Salois has shared a quick reference chart. The FN test equipment is expensive--\$30,000+--slow, and not convenient to use. Wheat researchers at WSU have had to develop new separate sets for screening, one for PHS, one for LMA. In 2016, 10,000 'spikes' from 500 'lines' of wheat were on the docket. In 2017, WSU technicians took to calling the 40,000 spikes from 1,200 lines "the falling number mountain."

Despite the obstacles, significant progress has been made. Michael Pumphrey of WSU states: *"Genetics is paramount. I believe in genetics. This is one area where genetics is the solution."* But, he adds, *"Screening is an extremely expensive project. Grading grain doing FN's. We need to make it cheaper somehow."* If such an inexpensive solution can be found, Cat Salois points out, it could help farmers monitor fields for trouble. It would be less costly and more practical for public and private wheat researchers to monitor the potential of a wide array of potential varieties and cast aside those with low FN resistance. WSU researchers emphasize that it is *"absolutely critical that growers identify the presence of PHS/LMA as early as possible so that it can be isolated appropriately."* As Craig Morris puts it *"if there was some quick, easy antibody test"* it could be a major breakthrough.

Search for a Practical & Inexpensive Tool to Help in the Field

The H-P test will remain the international standard for the foreseeable future—overseas millers are understandably wary of sprouted wheat that could produce collapsed cakes or spongy noodles. Developing resistant varieties takes a considerable amount of time—for the research itself and for passing Foundation and Certified standards. We can't copy a 'Think and Do' instruction book in the interim but perhaps we could come up with a different test for growers and grain companies to get quick results in the field or at the elevator. AS WSU researchers put

it, a modern assay *"could have a transformative impact on Pacific Northwest wheat growers."*

The Potential for a New Test: ELISA

Enzyme linked immunosorbent assays (ELISA's) are widely used in science and medicine. Might they be adapted to detect the presence of AA proteins in cereals? Kudos to Bayer Crop Science and their international wheat lead, Ed Sousa, for giving us permission to proceed with testing an ELISA formula they had patented but decided not to pursue commercially. Once a research scientist at the University of Idaho, Sousa is familiar with our Inland Northwest wheat world and has worked with us for two years as we've gone through a very complicated process to get ELISA testing underway. Jim Moyer has worked with Ed Sousa, Craig Morris, and me in the search for a tool that could measure AA and limit risk.

Craig Morris and his fellow research scientist, Alecia Kizonas, began by studying three currently available ELISA kits, including one that measures protein in human saliva and another used for measuring proteins in the pancreas. The pancreatic test, they found, successfully measured AA and *"provides a potential 'off the shelf' avenue to use ELISA to evaluate and detect AA in wheat grain samples."* *"We consider it highly serendipitous,"* they wrote, that the pancreatic kit *"appears to be detecting increased AA levels associated with PHS and LMA."*

While this work is underway, researchers are also evaluating other possibilities, including a step beyond near infrared. As Camille Steber puts it: *"I am also excited because Dr. Zhiwu Zhang, the quantitative geneticist in the Department of Crop and Soil Science, thinks he can make NIR or something like it work using fancy mathematics. He said that if I can give him many more samples with known FN (and I get these from the variety trials), that he should be able to use machine learning mathematics used for facial recognition to get a hyperspectral (further outside the visual range of light than near-infrared) calibration that can predict FN. I really hope this could give us a quick approximation at the elevator."* She's helping him with a grant proposal to try his idea out for size.

Steber and Michael Pumphrey are collaborating on ELISA with WSU professor Andy McCubbin, who did his postdoctoral work on alpha-amylase and has been a major contributor. Working together as WSU and USDA they are now taking the next step

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forward—studying antibodies raised to 3 amino acid sequences from the AA enzyme, one from the same string of amino acids in the Bayer patent, another a variant on that string, and a third of their own design. There is a lot of work ahead, fine tuning and selecting the best, most specific tool. As Steber puts it, *“luck is involved as an element in finding a good antibody that sees AA and nothing else.”* We’re working together to find a scientific laboratory familiar with grain production to make kits once the best ELISA tool is ready to go. Engineers at WSU have been working on the mechanics of instant delivery of an ELISA test via a smart phone chip.

The Road Ahead

Might it be possible to have a kit that growers could use in a couple of years? Yes, I believe it is. Researchers cannot commit to it—there could be unforeseen obstacles ahead. But knowing them and the hard work underway, I like our odds. The Washington Grain Commission and USDA/ARS set a goal five years ago of a highly sensitive, easy to use tool to help growers reduce risks. There have been big strides made since then.

We’re fortunate to have remarkable people and resources devoted to meeting the AA challenge. The Western Wheat Quality Lab in Pullman, WA—one of four in the nation and the only one west of Kansas—with Craig Morris, Alecia Kiszonas, Doug Engle and a half dozen ARS staff. Camille Steber and the USDA/ARS team, including a soon to be hired ARS scientist specifically dedicated to solving the AA/FN issue. The University of Idaho, Oregon State University (Pendleton and Corvallis), Washington State University—three land grant schools with scientists working to meet the challenge right next door here in wheat country. A new assay that can have a transformative impact? Who better to make it happen!

We’ve got a tradition, dating back more than a century, of growers, scientists, and local businesses working together and getting results, a remarkable relationship that few regions can match. As Pullman farmer Girard Clark put it way back in 1909, *“in the world of cereal improvement, there is no limit to the possibilities, hence no stopping place.”* Kudos to Mike Miller and the WGC, Jim Moyer of WSU, our legislators and the PNW wheat industry for getting \$1 million in new money to the ARS for FN research to help meet the challenge—with many others helping make it possible, among them Mary Palmer Sullivan, Glen Squires, Michelle Hennings, Nicole Berg, Marci

Green and Gary Bailey. Pulling together we get results. Always have. Always will.

I am encouraged by progress made so far by the outstanding team of USDA/ARS and land grant researchers. Great strides toward a reliable AA test and promising new varieties with improved resistance in the works, even with a slow, complex tool and a mountain of samples. As David Weller put it, a seamless effort, going as hard as they can, night and day—it’ll make a difference and be a big help in challenging times. The challenge will be met, the battles won, and we’ll no longer be stuck with only a test we all love to hate. The motto of the Western Wheat Quality Lab in Pullman—bringing solutions to agriculture—says much about the road ahead. We’ll all see the day, sooner rather than later, where warm spring days and cold nights will be a lot less risky and much more enjoyable. You have my word on it.

A follow up FN summit is being organized for late January 2019, prior to the Pacific Northwest Wheat Quality Council meeting in Portland. It will be a time to compare progress reports from all the research work underway around the region. We’re looking at other grant possibilities, too, to fill any gaps in the work underway and to continue to expedite this important work. We’ll keep you posted.

Alex McGregor is Chairman of The McGregor Company, a farm supply business with 360 employees and offices in nearly four dozen rural communities of the Inland Northwest. He also serves as Managing General Partner of McGregor Land & Livestock, a 136-year-old eastern Washington family wheat, alfalfa, barley and livestock ranch.

Alex currently serves on the boards of the Association of Washington Business, the Washington State Historical Society and LaCrosse Community Pride. He is Chair of the Rural Job Task Force for Association of Washington Business and was a long-time member of the Regulatory Performance Advisory Group of the Washington Department of Ecology. Alex has served a tour of duty on the Governor’s Global Competitiveness Council and the Governor’s Higher Education Task Force.