Causes of low Falling Numbers in wheat

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WASHINGTON STATE UNIVERSITY

USDA
Two causes of low Falling Numbers (FN), high alpha-amylase

Preharvest Sprouting
Germination of mature grain on the mother plant induced when cool rainy conditions occur before harvest

Late Maturity Alpha-amylase
Induced by cold or heat shock during late maturation of wheat grain.
The Problem: Low Hagberg-Perten Falling Numbers (FN)

- Weather events cause low FN/high alpha-amylase in susceptible varieties.
- The FN test can protect millers and bakers, but farmers suffer serious losses when Falling Numbers are lower than 300 seconds.
- In 2013, there was a discount of 25 cents/bushel for every 25 seconds below 300.
- Degree of problem varies – even on one farm, depending on the timing/distribution of rain or temperature shock.
The aleurone layer produces alpha-amylase, which digests the starchy endosperm.

Wheat the big picture (www.wheatbp.net), Barrero et al., 2009
As alpha-amylase cuts, the starch chains get smaller and provide less structural integrity.

Gravy is watery if starch strands are short.

Gravy gels if starch strands are long.
The Hagberg-Perten Falling Number Test

1. Grind grain to meal
2. Weigh 7g (adjusted for 14% moisture).
3. Add 25 mL water (amylase digestion starts).
4. Place into shaker and hit start.
5. Place stirrers into tubes in white holder. Stirs and heats for 60 sec.
6. Place tubes in FN machine, stirs and heats for 60 sec.
7. Measure the time in seconds needed for the stirrer to fall through the “gravy”. Gels better if starch is undamaged. Correct for altitude of 2500ft.
Too much alpha-amylase enzyme activity results in poor end-use quality in bread baked from hard red wheat grain.
Increasing alpha-amylase (lower FN) in soft white wheat leads to cakes that fall.

Effect of increasing $\alpha$-amylase from PHS on sponge cake – image from WWQL, USDA-ARS, Pullman.
Grain maturation

Induce Dormancy & Desiccation tolerance

Synthesis of seed reserves

Embryo Development

Germination

Preharvest Sprouting

Amylase

Yamaguchi et al., 2007 In “Seed Development, Dormancy, and Germination” pp. 224-247
LMA is a developmental defect leading to elevated alpha-amylase.

Grain maturation

Late Maturity
Alpha-amylase

Embryo Development

Temperature stress

Yamaguchi et al., 2007 In “Seed Development, Dormancy, and Germination” pp. 224-247, Barrero et al., 2013 Plant Physiology vol 161, pp. 1265-77.
http://biology.kenyon.edu/courses/biol114/Chap12/Chapter_12A.html,
LMA may be associated with seeds staying green longer.

Figure from Barrero et al., 2013
Fairfield, WA 2013, a strong PHS event.

Daily Max Temperature
35°C
15.5°C

Days before harvest

Rain event, 1.8 cm/0.7 in

Falling Numbers, soft white winter

FN of WSU Cereal Variety Trials, steberlab.org
Pullman, WA, a mild PHS event

Daily Max Temperature
35°C

Daily Min Temperature
18.2°C

Rain event, 0.38 cm/0.15 in

Tracy Harris, Xavian Thompson, Rehana Parveen
St. Andrews 2013
-- no low FN in spite of plenty of rain.
-- If it is hot when it rains, wheat is less likely to sprout.

Daily Max Temperature
35°C

Rain event,
0.8 cm/0.3 in
Walla Walla 2013, likely an LMA event. No FN below 300 in Anatone.

Other LMA events
2011: Mayview, Bickleton, Walla Walla
2013: Bickleton, Franklin County, Walla Walla
2014: Bickleton, Connell, Lind, Ritzville, Walla Walla
Sprout-damage in wheat, a spoil-sport problem

Not “sprouted”

“Sprouted”

α - Amylase Activity

Starch Degradation

Falling Number

Not “sprouted”

“Sprouted”
Ways to measure alpha-amylase in grain

Alpha-amylase enzyme assays

Hagberg-Perten Falling Number (FN) test

www.perten.com
The Phadebas alpha-amylase enzyme assay

1. Grind grain to meal

2. Extract enzyme from 1.7 g of meal.

3. Mix 1.7 g meal with 40 mL extraction buffer. Incubate at 50°C for 10 min. Centrifuge.

5. Add ½ Phadebas tablet and mix well.

6. Incubate on 50°C block for 15 minutes. Shake occasionally.

7. Place tubes in centrifuge so that it’s balanced. Spin for ~30 seconds.


Start here. 4. Place 1.8 mL of extract into 2 mL tube
Alpha-amylase activity correlates to FN in Soft White Winter wheat

Phadebas substrate is an insoluble dye cross-linked potato starch that becomes soluble and blue upon cleavage of the starch by α-amylase.
Is alpha-amylase the cause of the problem?

1. Perform an FN test or alpha-amylase test for each of the following.
2. Sprouted sample with and without amylase inhibitor AgNO₃.
3. Sound sample with and without fungal alpha-amylase (or human saliva).
Can you spot the sprouted grain?

1. On the table, we have four different grain samples.
2. Look at each one and write down which one(s) you think are sprouted.
3. We will compare visual inspection to FN and alpha-amylase enzyme assays.
Which of the four grain samples has low FN or high alpha-amylase levels?

1. Perform an FN test on grain samples 1, 2, 3, and 4. Write the results on the wipe board.
2. Perform an alpha-amylase enzyme assay on the same four samples using the blue Phadebas tablets.
3. Compare visual inspection to FN and alpha-amylase enzyme results.
Is alpha-amylase the cause of the problem?
1. The alpha-amylase can cause lower FN in a sound sample - the enzyme is enough to cause a problem.
2. The low FN of sprouted wheat can be raised by alpha-amylase inhibitor – the low FN is partly due to enzyme digestion DURING the test.
3. Don’t accidentally spit on your FN experiment!

Identifying low FN/high amylase grain samples:
1. Highly sprouted grains have a protruding embryo, a dent, or discoloration at the embryo end of the grain.
2. The FN and Phadebas test can detect damage we can’t see with visual inspection.
3. The Phadebas test is faster than the FN test.
Project 7599 – FN Database can tell you how a cultivar behaved after PHS in 2013 and LMA in 2014

url: steberlab.org/project7599.php

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<th>Falling Numbers Data</th>
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<td><strong>Falling Numbers for grain from the 2014 WSU Cereal Variety Trials</strong></td>
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Project 7599 – FN vs Yield Tool

Pullman 2013

116FN

354FN

156yield

113yield

Yield bushels/acre
The Problem: Low Falling Numbers (FN) in NW Wheat

- An infrequent problem in the inland NW
- Difficult to select against it by running FN on breeding lines every year.
- Need to use greenhouse screening and molecular markers to prevent susceptibility from creeping up in breeding programs
Preharvest sprouting (PHS)
• Associated with lack of seed dormancy
• Associated with open flower morphology, low epicuticular wax.
• Losses to farmer and miller
• Induction of $\alpha$-amylase digests starch causing poor end-use quality

Seedling emergence
• Seed dormancy can lead to poor seedling emergence.
• Negative impact on yield.
• Selection for good emergence can inadvertently lead to preharvest sprouting susceptibility
Seeds are dormant when they fail to germinate under conditions that normally stimulate germination. Seed dormancy gives higher resistance to sprouting, accounting for 60-80% of the variation (McCaig and DePauw, 1991). Dormant seeds acquire the capacity to germinate through
- After-ripening (AR), period of dry storage
- Cold stratification, imbibing water in the cold
Susceptibility to preharvest sprouting depends on maturity date.

Before Maturity → Sprouting susceptibility

Physiological Maturity (Dormant) → Susceptible

Resistant → Age of Grain

peduncle
Effect of rain on FN depends on its timing relative to grain maturity

If we base our conclusions about FN on a single rain event, then an early-maturing resistant line may seem “worse” than a late maturity sprouting susceptible line.
Evaluating PHS Tolerance in the greenhouse

The spike wetting test
Anderson et al, 1993

Sprouting Score
McMaster & Derera et al, 1976
Sprouting Scores of Winter Wheat
The Good, the Bad, and the Ugly

Sprouting

Boundary
WB-Arrowhead
SY-Ovation

Susceptible check!

Shantel Martinez
Sprouting

PHS score versus Falling Number

- Bruneau
- 99-06202A
- Madsen
- Masami
- Ovation
- Coda
- ARS010780-3

$\text{r}^2 = 0.32$
$p\text{-value} = 0.017$

$\text{r}^2 = 0.22$
$p\text{-value} = 0.089$

Xerpha
Bruehl
4J071246-1C
Two Causes of low Falling Numbers (FN)

Based on weather data, some of the low FN is due to LMA
Alpha-amylase induced by cold shock during grain maturation (25-35 days past pollen-shedding) in susceptible and resistant Australian cultivars.

Mrva et al., 2006
Can we identify PHA vs LMA event based on the location of alpha-amylase activity in the grains?

Preharvest sprouting alpha-amylase strongest at the embryo end

LMA randomly spaced patches of alpha-amylase

Mrva et al., 2006
Alpha-amylase (A620)

FN

Embryo

Brush end

Sound grain

PHS

LMA

EMBRYO

Brush end

PHS

LMA

Embryo

Brush
Greenhouse screening for LMA

Grow plants until 26 days past anthesis (pollen shedding)

Cold shock, 7 days at 64°F day/45°F night

Control, no cold treatment (72-77°F/64°F)

Grow to maturity

Assay all grains from one spike for alpha-amylase

LMA-susceptible lines will have higher alpha-amylase after cold treatment.

Sindhu Nair, Method based on Mares and Mrva, 2008
Problem: The greenhouse LMA test is slow because we can only run a limited number of tests at a time. Need to develop a higher throughput field LMA test so that we can screen elite breeding lines BEFORE they’re released.
Field “wheat bouquet” test for LMA

- Harvest spikes at about 26 days past anthesis.
- Cold shock spikes in vases for 7 days at 64°F day/45°F night.
- Control cut spikes left outside without cold shock.
- Allowed to senesce.
- Assay all grains from one spike or bulked spikes for alpha-amylase.

Testing its ability to predict field LMA issues, using to test breeding lines.

Keiko Tuttle, Method based on Mares and Mrva, 2008
PHS problems in 2013 were more severe in Bruehl than Jasper/WA8169.
Field testing of winter elite breeding lines: 42 plots, 42 geno; 5 spikes/plot for control & cold treated

* denotes NO statistical difference
LMA problems in 2014 were most severe in Jasper – the one the got away...

If we’d had the field testing method sooner, we might have caught the Jasper problem before it was released. Now the choice is up to the farmer – to grow it or not to grow it...
Protein content and starch composition also influence FN. Amylo-pectin in “waxy” wheat is more sensitive to alpha-amylase than amylose.
Varieties with low FN, low \( \alpha \)-amylase:
- Alturas
- IDO851
- WA8195
- Nick

Variety with high FN, high \( \alpha \)-amylase:
- WA8162

- Alturas is a known “partial waxy” wheat, meaning that it has a lower ratio of amylose to amylopectin due to a mutation in one of the three GBSS genes needed for amylose synthesis.

- Maybe the cultivars that give lower FN than expected for the alpha-amylase content all have lower amylose giving a “partial waxy” trait.
Amylose content measured with Megazyme’s amylose/amylopectin test kit:

IDO851, Alturas, WA8195, Nick are partial waxy wheats. They have more amylopectin vs amylose. They are more susceptible to lower FN from PHS or LMA.
What we’ve learned about Falling Number problems in wheat

• Preharvest sprouting in “rained on“ wheat gives low FN/high alpha-amylase because alpha-amylase is induced during seed germination to mobilize starch as a food source for the growing wheat seedling.

• LMA is a developmental defect due to inappropriate expression of alpha-amylase during embryo maturation. Results from large temperature changes.

• Wheat cultivars with a lower ratio of amylose to amylopectin are more sensitive to the effects of preharvest sprouting and LMA.

• If breeders choose to select for the “partial waxy” trait, then they will need to also select for better resistance to preharvest sprouting and LMA.
Suggestions for Reducing Risk

• Harvest wheat quickly after it reaches harvest maturity to reduce the risk of getting rained on.
• Avoid harvesting green plants since green kernels have higher alpha-amylase.
• Avoid cultivars known to be PHS or LMA susceptible.
• If a susceptible favorite is tempting, grow two cultivars (in separate fields) with different flowering/maturity dates. It is less likely an isolated cold shock or rain event will result in low FN of both varieties.
• Blending low FN and high FN wheat will give a lower FN than you expect because the alpha-amylase is still active after milling.
• If you have moderately low falling numbers (200-300sec), it can’t hurt to store it for awhile (2-3 months) to see if your FN rises.
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