Causes of low Falling Numbers in wheat

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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.
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 α-amylase from PHS on sponge cake – image from WWQL, USDA-ARS, Pullman**
Normally alpha-amylase is produced during development and during germination to turn starch into fuel for growth.

Grain maturation

- Synthesis of seed reserves
- Induce Dormancy & Desiccation tolerance

Embryo Development

amylase

There should be no amylase activity during grain maturation.
LMA is a developmental defect leading to elevated alpha-amylase levels during maturation in response to big temperature changes.

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
The next series of slides shows weather conditions associated with PHS and LMA in the past.

• PHS tends to occur when rain is associated with cooler temperatures.

• LMA can explain low FN in Washington wheat when there was no sprout-inducing rainfall.
Falling Numbers, soft white winter

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

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

Rain event, 0.38 cm/0.15 in

Daily Max Temperature
35°C

18.2°C
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
20°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
Ways to measure alpha-amylase in grain

**Alpha-amylase enzyme assays**

**Hagberg-Perten Falling Number (FN) test**

www.perten.com
Sprout-damage can be hard to see without Falling Number or alpha-amylase testing

<table>
<thead>
<tr>
<th>Sprouted</th>
<th>α - Amylase Activity</th>
<th>Starch Degradation</th>
<th>Falling Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not “sprouted”</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>“Sprouted”</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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Alpha-amylase is present, even when all you see the seedling root barely poking out of the grain. After the kernel dries, the embryo retracts into the grain leaving a crack or pit at the end. The embryo may fall out of the damaged grain leaving a germless kernel.
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.

4. Place 1.8 mL of extract into 2 mL tube

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.

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. 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. Spit contain amylase. Don’t accidentally spit on your FN experiments!

Identifying low FN/high amylase grain samples:

1. Highly sprouted grains has 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.
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 -> Physiological Maturity (Dormant) -> Susceptible/Resistant

Sprouting susceptibility

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.
Physiological Maturity in Wheat

- Physiological Maturity is when the maximum amount of dry matter has accumulated in the developing kernel. The kernel still needs to dry down before harvest.
- When the green color is in the process of disappearing from the peduncle
- When about half of the spike is dry
- When a pigmented strand appears in the grain.
- Grain transitions from the soft dough to hard dough stage.
- Dormancy is highest at physiological maturity and then drops off during dry storage through the process of after-ripening.
- Note: Dormancy loss by after-ripening is faster at higher temperatures (~85 degrees F).
The spike wetting test
Anderson et al, 1993

Sprouting Score
McMaster & Derera et al, 1976
Evaluating PHS Tolerance in the greenhouse reduces problems by making sure all wheat if 5 days past physiological maturity.

- Harvest spikes from field at physiological maturity
- 5 spikes per plot, 3 plots per line
- Allow to after-ripen in a dry place at room temperature for 5 days (longer if grains are very dormant).
- Wrap spikes in plastic, store at -15C until experiment, when all of the spikes have been harvested and AR’d.
- Misted: 6 sec / 1 min under a fine misting system
- Score for 7 consecutive days
Sprouting Scores of Winter Wheat
The Good, the Bad, and the Ugly

Susceptible check!
**PHS score versus Falling Number**

- **Xerpha**
- **Bruehl**
- **4J071246-1C**
- **Selbu**
- **99-06202A**
- **Madsen**
- **Masami**
- **Coda**
- **ARS010780-3**
- **Ovation**

- $r^2 = 0.22$
- $p$-value = 0.089

- $r^2 = 0.32$
- $p$-value = 0.017
Two Causes of low Falling Numbers (FN)

Based on weather data, some of the low FN is due to LMA
Late Maturity Alpha-amylase (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
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)

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

- Grow to maturity
- Assay all grains from one spike for alpha-amylase

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

1. Harvest spikes at about 26 days past anthesis.
2. Control cut spikes left outside without cold shock.
3. Cold shock spikes in vases for 7 days at 64°F day/45°F night.
4. Allowed to senesce.
5. 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

**Bruehl**

- Colton: 1 (154)
- Fairfield: 1 (109)
- Farmington: 2 (250)
- Pullman: 4 (130)
- Dayton: 2 (307)
- Mayview: 1 (62)
- Reardan: 1 (146)
- St. John: 1 (143)
- Walla Walla: 2 (336)
- Almira: 2 (335)
- Anatone: 2 (358)
- Creston: 2 (358)
- Dusty: 1 (143)
- Lamont: 1 (143)
- Connell: 1
- Harrington: 2 (335)
- Horse Heaven: 1 (291)
- Lind: 3 (322)
- Ritzville: 4 (360)
- St. Andrews: 1 (378)
- Moses Lake: 1

**Jasper/WA8169**

- Colton: 1 (321)
- Fairfield: 1 (385)
- Farmington: 1 (386)
- Pullman: 1
- Dayton: 1 (331)
- Mayview: 1
- Reardan: 1
- St. John: 1
- Walla Walla: 2 (385)
- Almira: 1
- Anatone: 1
- Creston: 1
- Dusty: 1
- Lamont: 1
- Connell: 1
- Harrington: 1
- Horse Heaven: 1
- Lind: 1
- Ritzville: 1
- St. Andrews: 1
- Moses Lake: 1
Field testing of winter elite breeding lines: 42 plots, 42 geno; 5 spikes/plot for control & cold treated

Jasper has LMA

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

Jasper/WA8169

Most low FN problems in 2014 were due to LMA (but for Almira and Fairfield). Jasper had low FN due to LMA in multiple locations. If we’d had the field LMA testing method sooner, we might have caught the Jasper problem before it was released. Now the choice is up to the farmer...
Protein content and starch composition also influence FN. Amylopectin 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 (200-300 sec), it can’t hurt to store it for awhile (2-3 months) to see if your FN rises.
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