#### Causes of low Falling Numbers in wheat

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

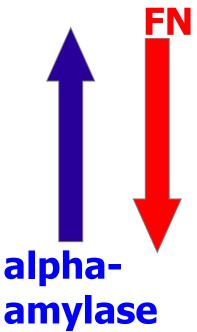


#### **Preharvest Sprouting**

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)

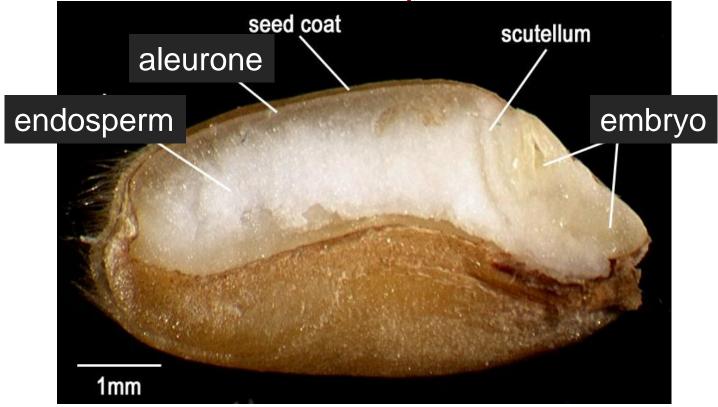
- Weather events cause low FN/high alpha-amylase in susceptible varieties.
- The FN test can protect millers and bakers, but farmers suffer serious losses when Falling Numbers are lower than 300 seconds.
- In 2013, there was a discount of 25 cents/bushel for every 25 seconds below 300.
- Degree of problem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





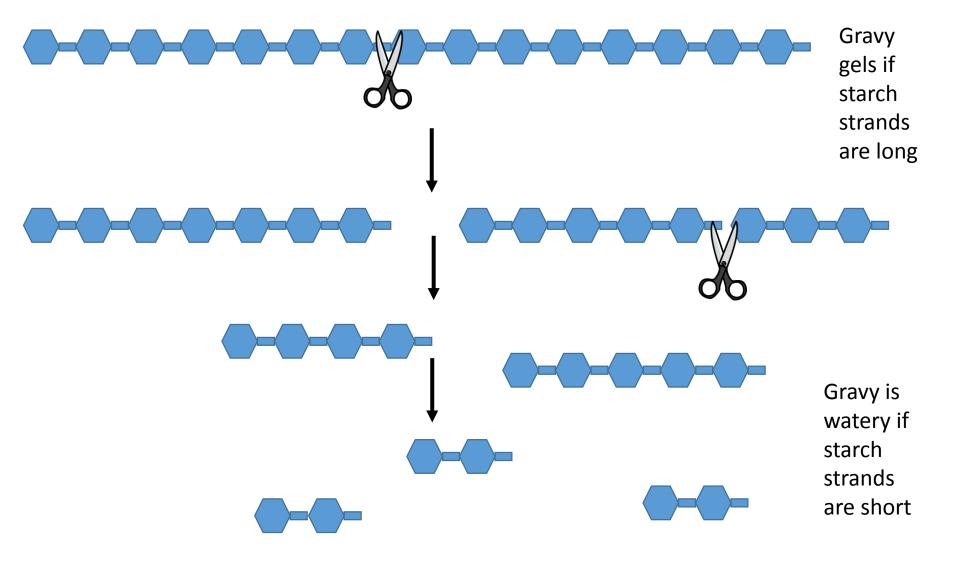
www.perten.com

### The aleurone layer produces alphaamylase, 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.



#### The Hagberg-Perten Falling Number Test



1. Grind grain to meal





2. Weigh 7g (adjusted for 14% moisture).



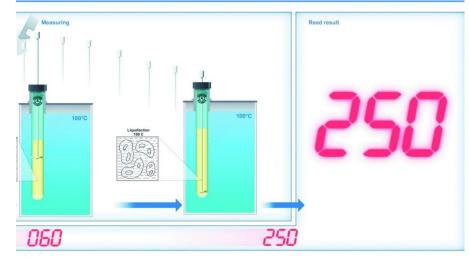
5. Place stirrers into 6. Place tubes in FN machine, tubes in white holder. stirs and heats for 60 sec



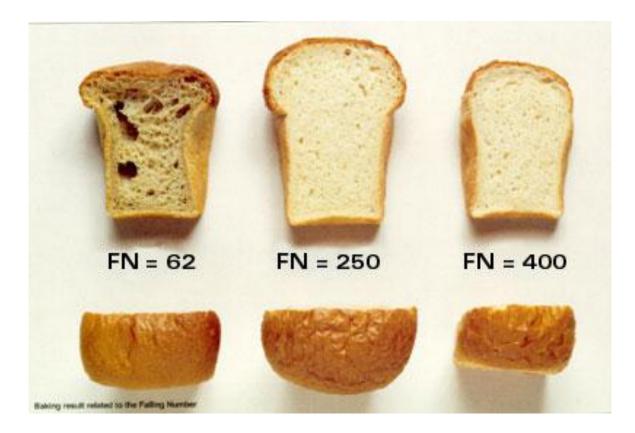
3. Add 25 mL water (amylase digestion starts).



4. Place into shaker and hit start.

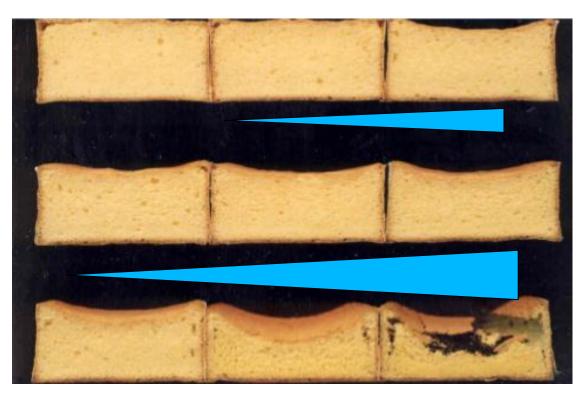


7. Measure the time in seconds needed for the stirrer to fall through the "gravy". Gels better if starch is undamaged. Correct for altitude of 2500ft. Too much alpha-amylase enzyme activity results in poor end-use quality in bread baked from hard red wheat grain.



# Increasing alpha-amylase (lower FN) in soft white wheat leads to cakes that fall

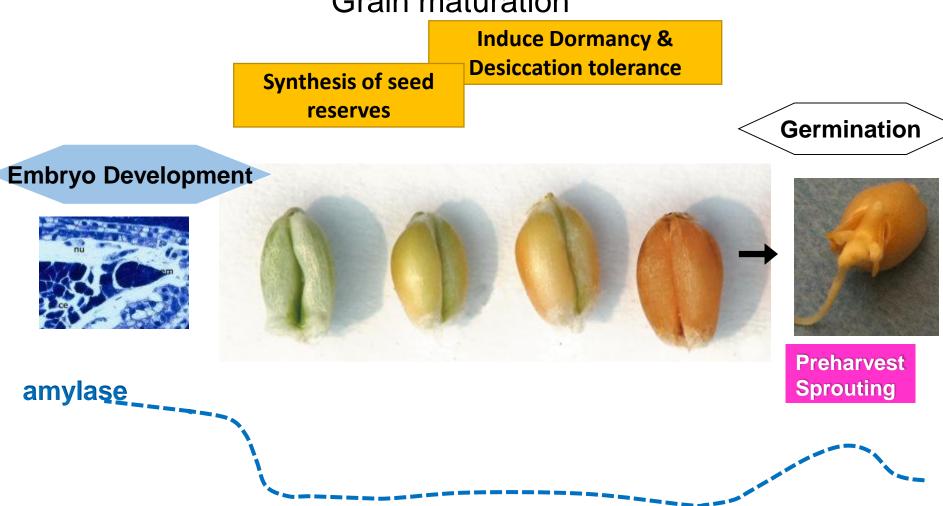




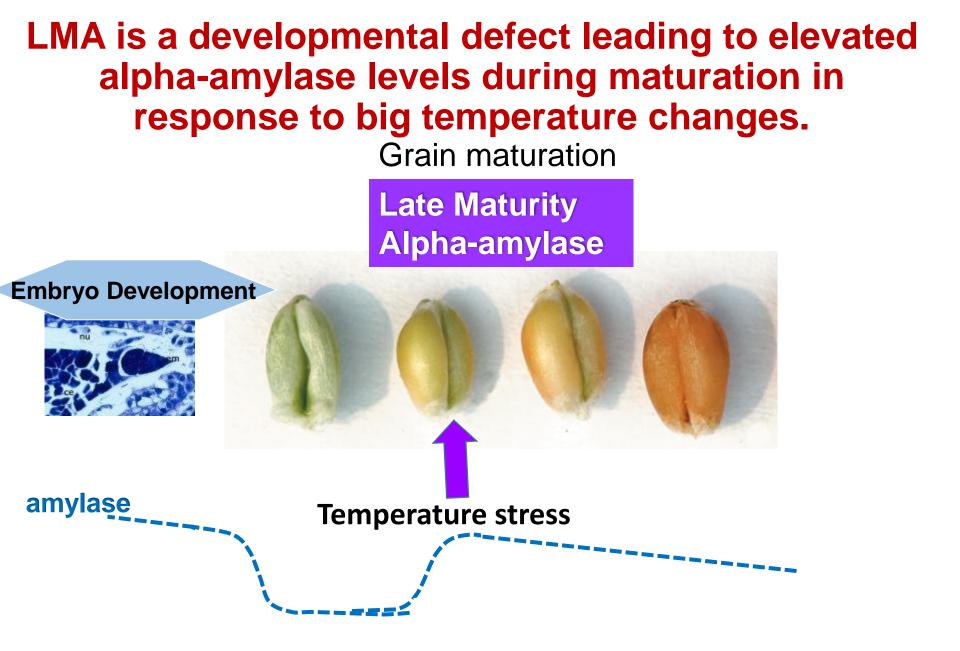
Effect of increasing  $\alpha$ -amylase from PHS on sponge cake – image from WWQL, USDA-ARS, Pullman

#### Normally alpha-amylase is produced during development and during germination to turn starch into fuel for growth.

Grain maturation



There should be no amylase activity during grain maturation.



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.



Time of cold shock

LMA resistant

LMA prone

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

LWW10-1018

AP Legacy

Xerpha

Bruneau 99-06202A

AP 700 CL

Bruchl WB-Junction

WA 8173

WA 8154

WA 8172 WA 8171

WA 8155

ARS990077-1C

ARS-Crescent

ARS97230-6C 03-29902A

ARS010729-1L

02-10606A Tubbs 06

ARS-Chrystal

LWW-04-4009

Puma (WA 8134)

Ladd (OR2070870)

OR2071071 (Rosalyn)

Bobtall (OR0807P94)

ARS010746-2C

WA 8142

WA 8151

WB 523

WA 8175

WA 8174 ARS-Amber

Trifecta

Eltan ARS010780-3C

ID01108

WB-528

Masami

Mary

OR2080641

SY Ovation

WB-1070CL

ARS98X402-1C

WA 8176

Stephens **ORCF-103** 

WA 8153

WB 456

Cara Legion 349

363

363

FIII

Skiles

Coda Madsen 111

122

123 142

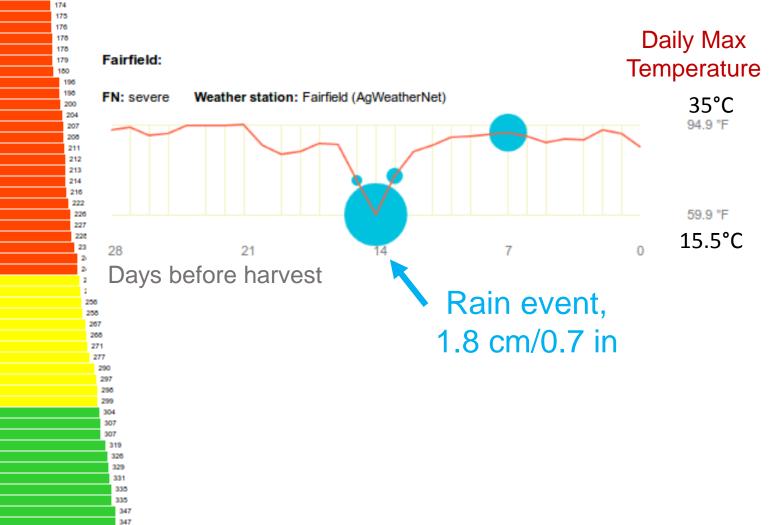
146

165

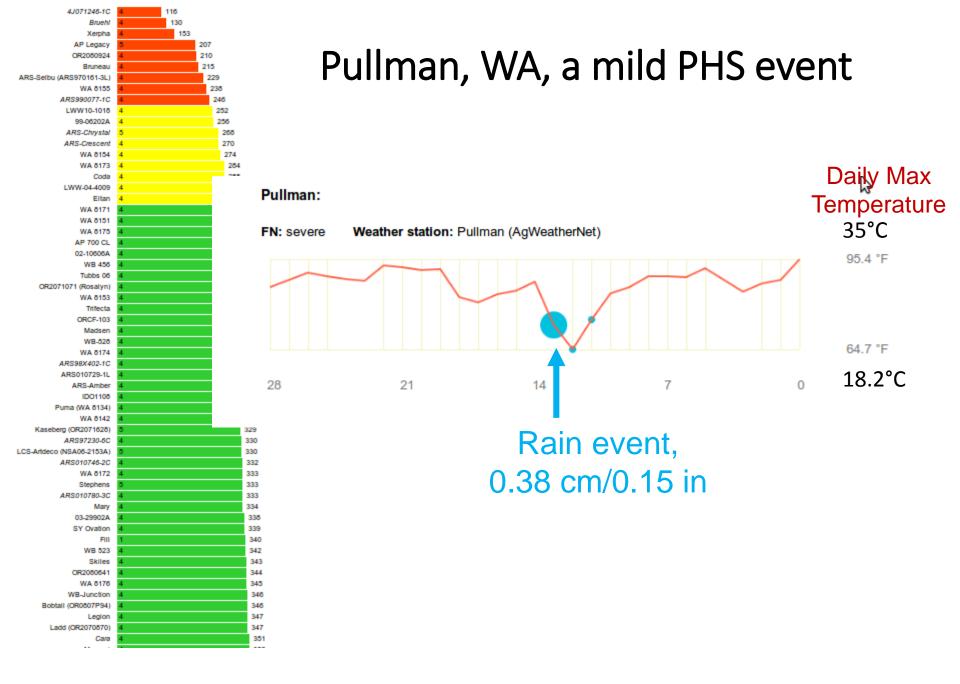
Sec <250 Z

250<FN<300 Artdeco (NSA06-2153A) >300 Kaseberg (OR2071628) ЧĽ

#### Fairfield, WA 2013, a strong PHS event.



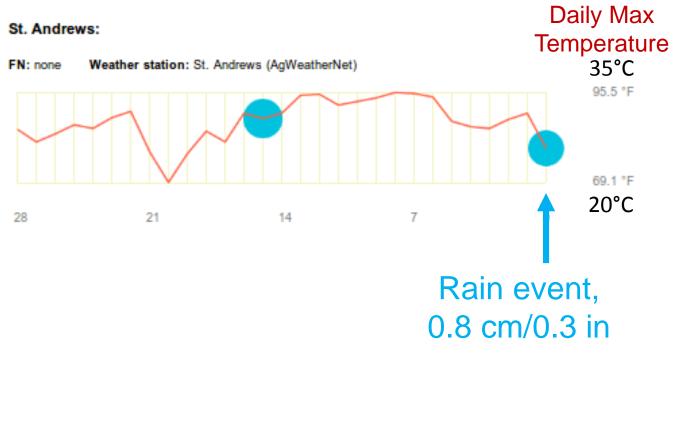
FN of WSU Cereal Variety Trials, steberlab.org

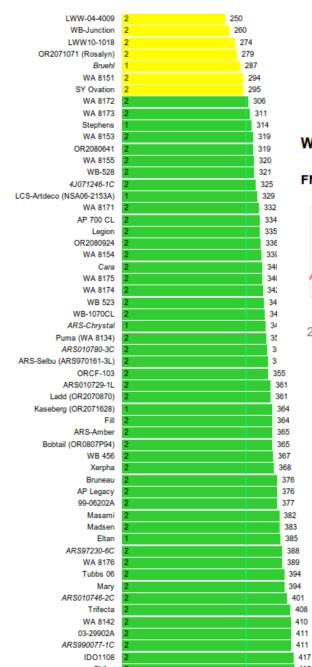


Tracy Harris, Xavian Thompson, Rehana Parveen

# 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.







#### Walla Walla 2013, likely an LMA event. No FN below 300 in Anatone.

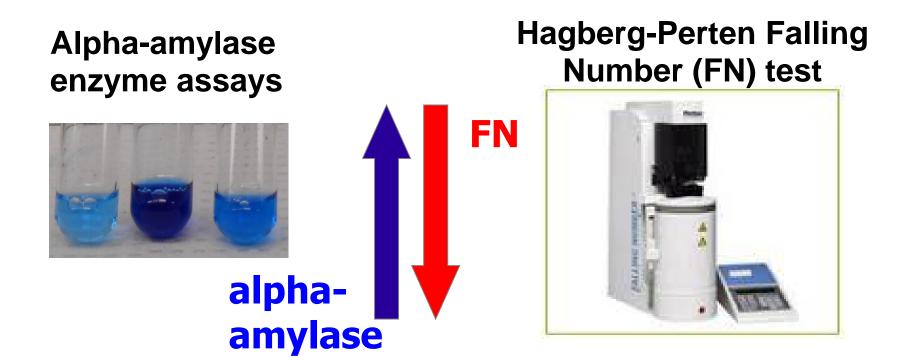
#### Walla Walla:



#### **Other LMA events**

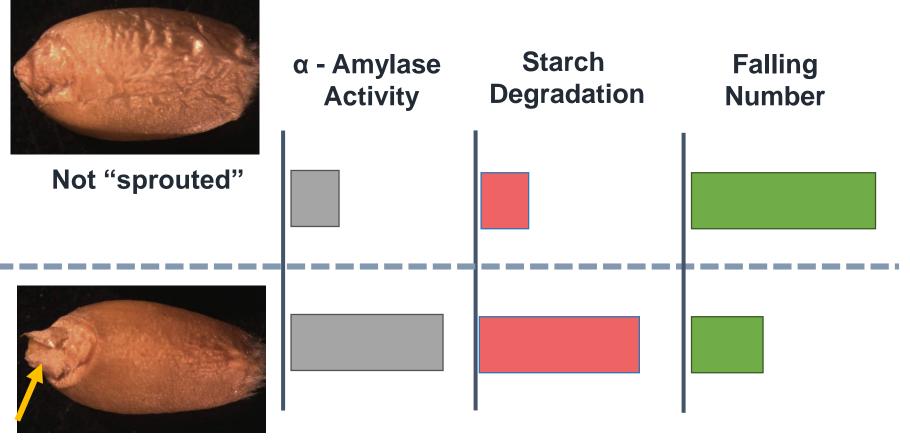
2011: Mayview, Bickleton, Walla Walla2013: Bickleton, Franklin County, Walla Walla2014: Bickleton, Connell, Lind, Ritzville, Walla Walla

#### Ways to measure alpha-amylase in grain



www.perten.com

Sprout-damage can be hard to see without Falling Number or alpha-amylase testing



"Sprouted"

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.



Start here. 4. Place 1.8 mL of extract into 2 mL tube



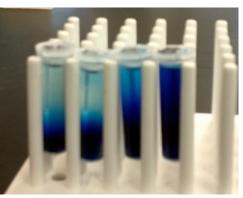
5. Add ½ Phadebas tablet and mix well.



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



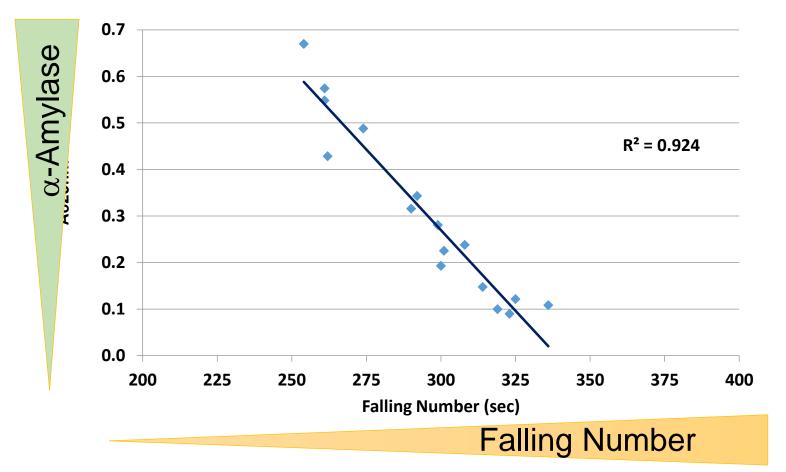
7. Place tubes in centrifuge so that it's balanced. Spin for ~30 seconds.



8. Compare colors. Darker blue means more alpha-amylase.

#### Alpha-amylase activity correlates to FN in Soft White Winter wheat

SWW Pullman 2013



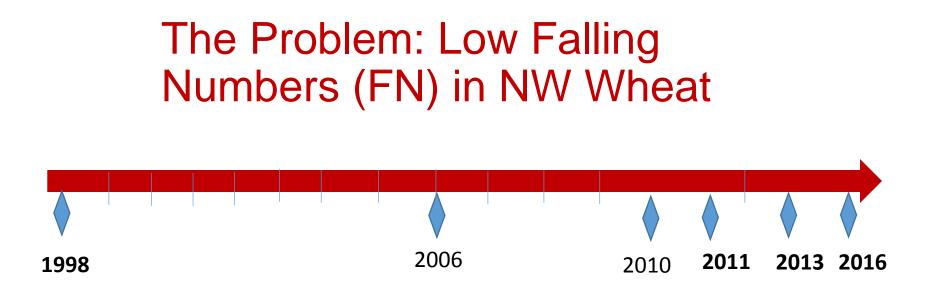
Phadebas substrate is an insoluble dye crosslinked potato starch that becomes soluble and blue upon cleavage of the starch by  $\alpha$ amylase

#### Is alpha-amylase the cause of the problem?

- 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 alphaamylase 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.



- 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



#### Germination

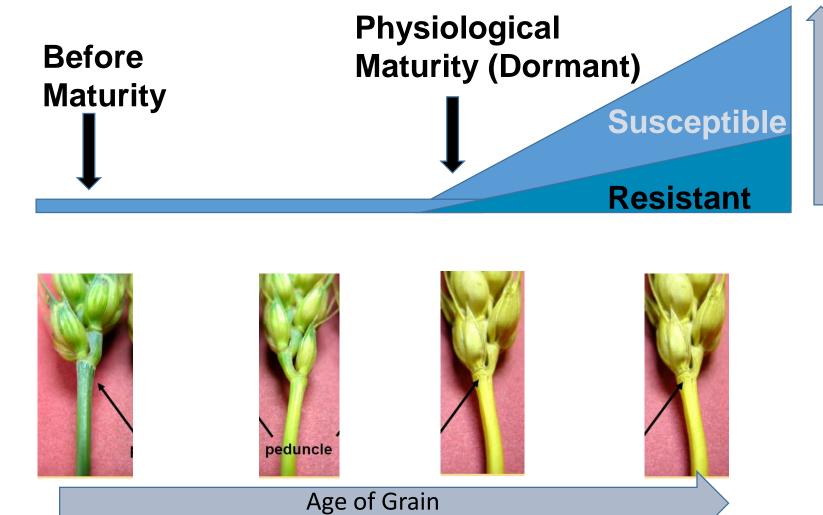


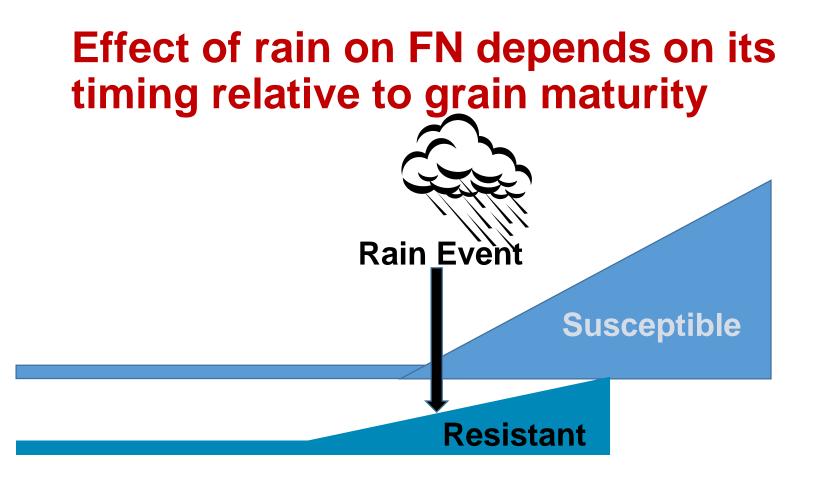
After-ripening Cold Stratification



- 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



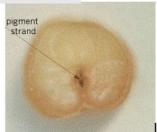


If we base our conclusions about FN on a single rain event, then an early-maturing resistant line may seem "worse" than a late maturity sprouting susceptible line.

#### Physiological Maturity in Wheat



Not mature Mature



- 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 a higher temperatures (~85 degrees F).

Minnesotafarmguide.com



#### The spike wetting test Anderson et al, 1993

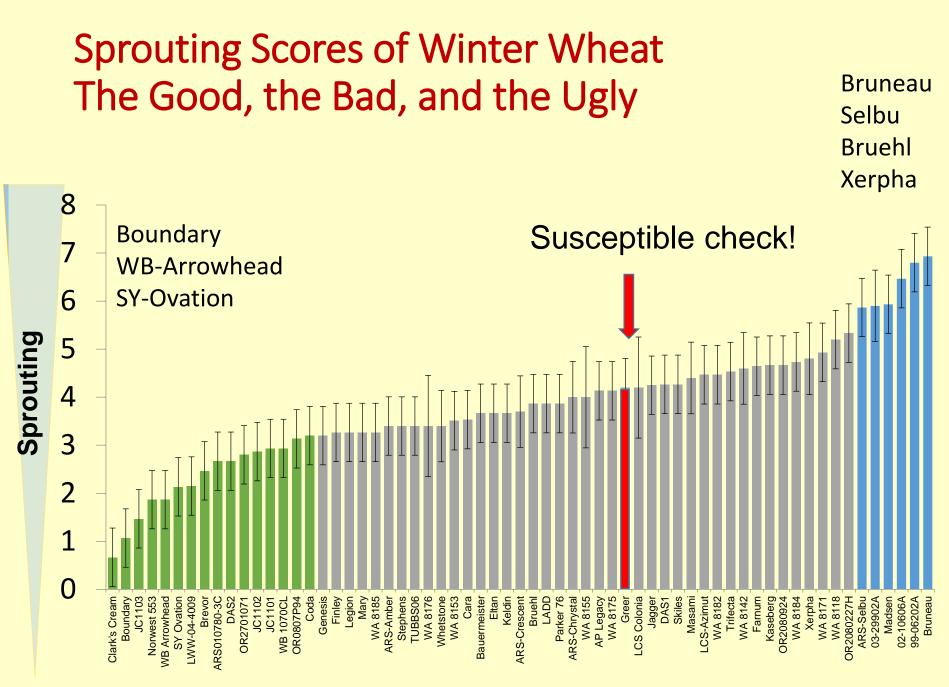
#### Sprouting Score McMaster & Derera et al, 1976



Evaluating PHS Tolerance in the greenhouse reduces problems by making sure all wheat if 5 days past physiological maturity.

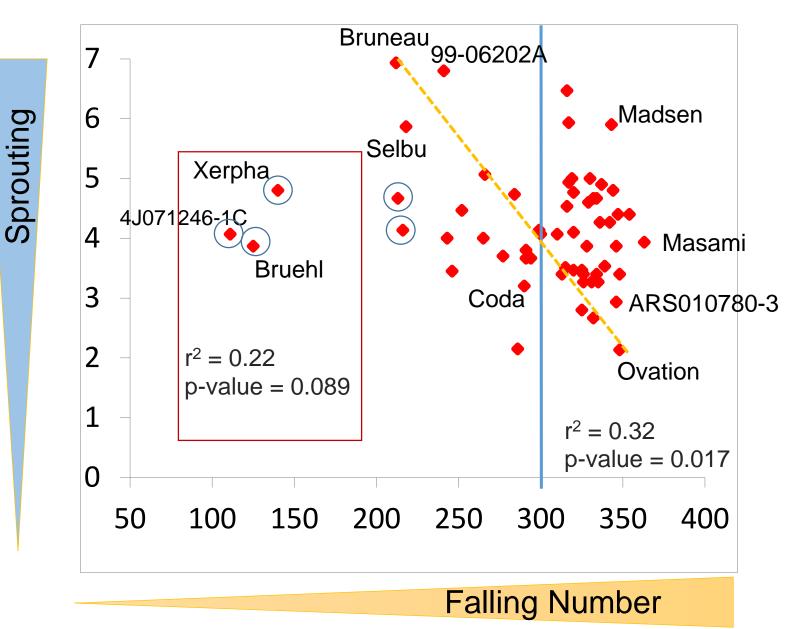


- Harvest spikes from field at physiological maturity
- 5 spikes per plot, 3 plots per line
- Allow to after-ripen in a dry place at room temperature for 5 days (longer if grains are very dormant).
- Wrap spikes in plastic, store at -15C until experiment, when all of the spikes have been harvested and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

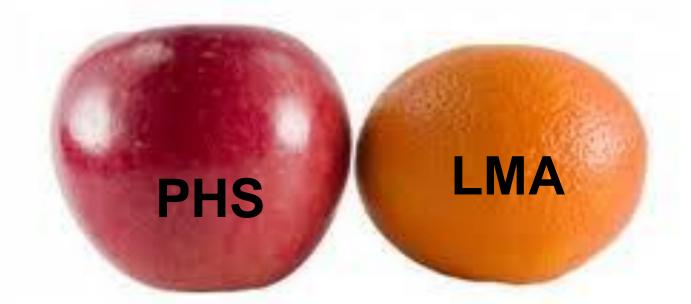


#### **Shantel Martinez**

#### PHS score versus Falling Number

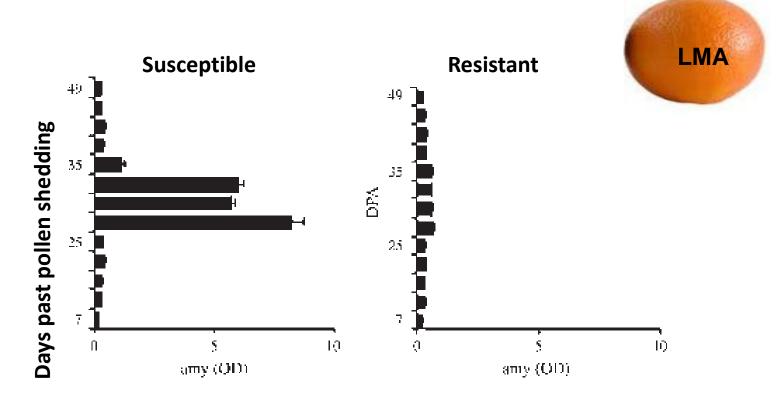


# Two Causes of low Falling Numbers (FN)



# Based on weather data, some of the low FN is due to LMA

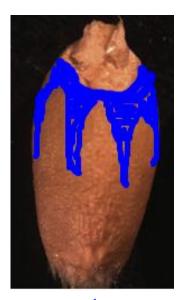
### Late Maturity Alpha-amylase (LMA)

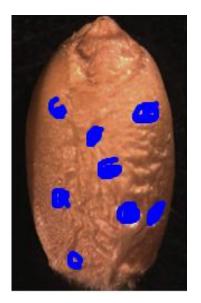


Alpha-amylase induced by cold shock during grain maturation (25-35 days past pollen-shedding) in susceptible and resistant Australian cultivars.

Mrva et al., 2006

#### Can we identify PHA vs LMA event based on the location of alpha-amylase activity in the grains?



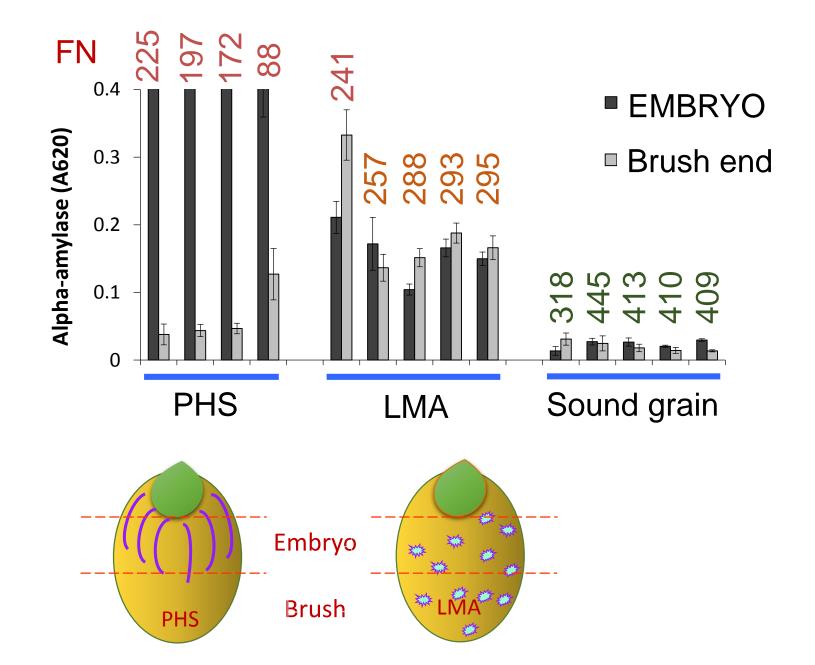


#### LMA

Preharvest sprouting alpha-amylase strongest at the embryo end

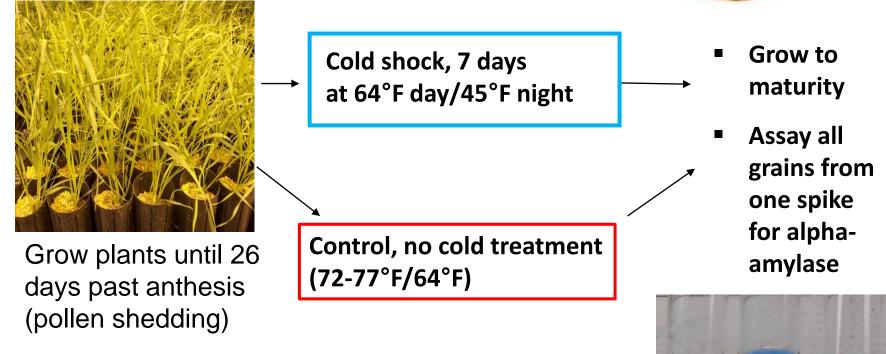
randomly spaced patches of alpha-amylase

Mrva et al., 2006



#### Greenhouse screening for LMA

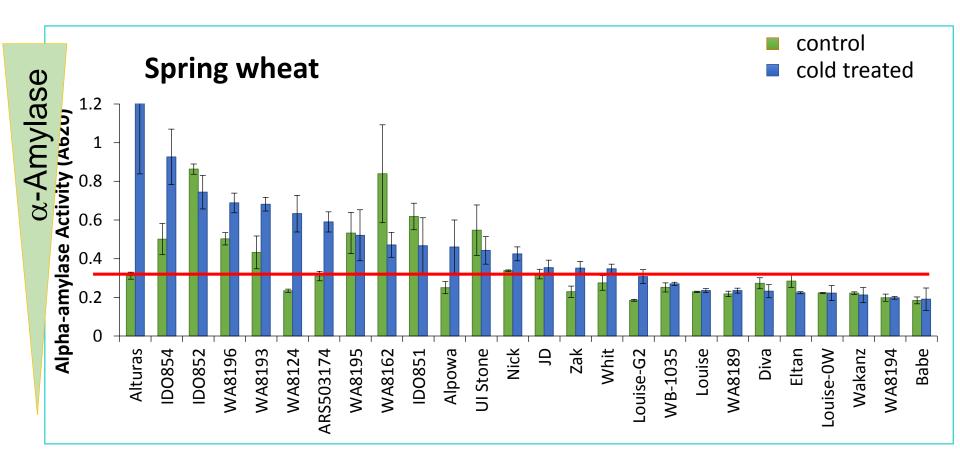




LMA-susceptible lines will have higher alpha-amylase after cold treatment.

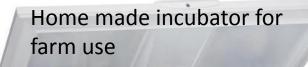
Sindhu Nair, Method based on Mares and Mrva, 2008

### First LMA induction trial



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



**Cold shock spikes in vases** for 7 days at 64°F day/45°F night

Harvest spikes at about 26 days past anthesis

**Control cut spikes left** 

outside without cold shock

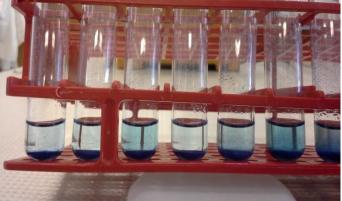
Allowed to senesce.

Assay all grains from one spike

LMA

or bulked spikes for alphaamylase

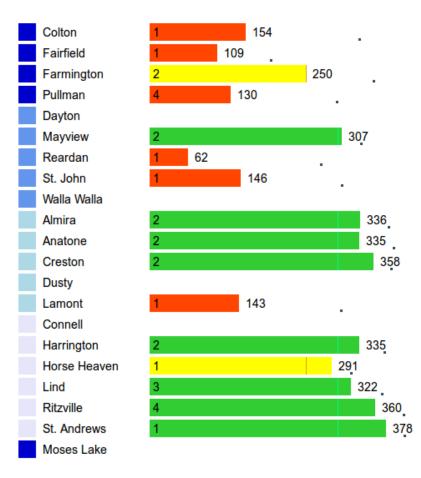
Testing its ability to predict field LMA issues, using to test breeding lines.



Keiko Tuttle, Method based on Mares and Mrva, 2008

#### PHS problems in 2013 were more severe in Bruehl than Jasper/WA8169

#### Bruehl





Colton

Fairfield

Pullman

Dayton

Mayview

Reardan

St. John

Almira

Anatone

Creston

Dusty

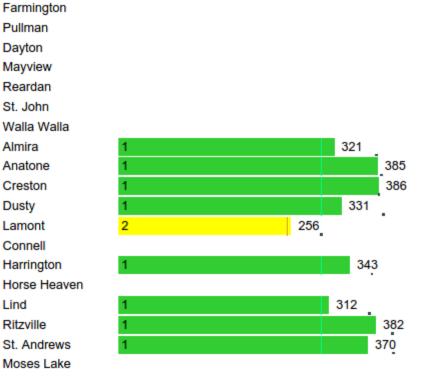
Lamont

Connell

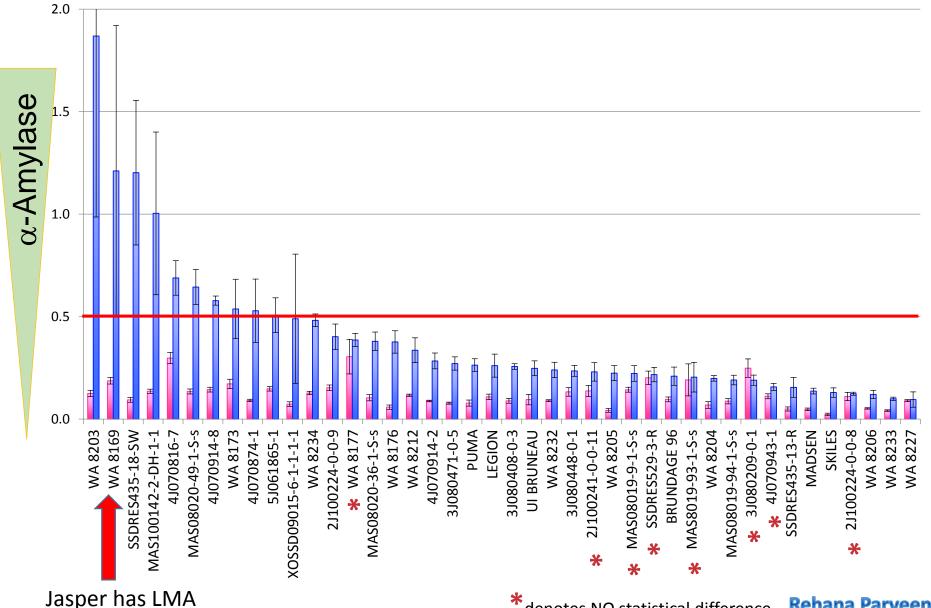
Lind

Ritzville

Harrington



#### Field testing of winter elite breeding lines: 42 plots, 42 geno; 5 spikes/plot for control & cold treated Control



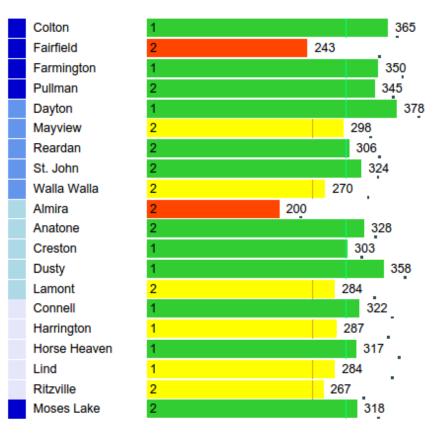
 $^{m{st}}$ denotes NO statistical difference

Rehana Parveen

Cold Treated

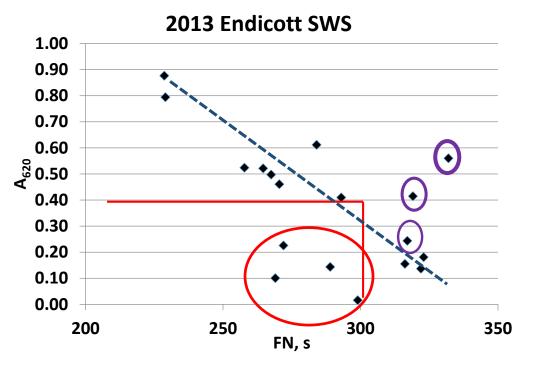
## LMA **problems** in 2014 were more severe in Jasper – the one the got away...

#### 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. Amylo-pectin in "waxy" wheat is more sensitive to alpha-amylase than amylose.



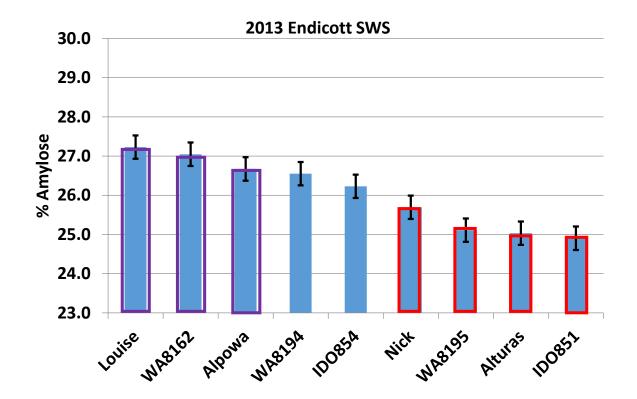
Varieties with low FN, low  $\alpha$ -amylase:

- Alturas
- ID0851
- 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 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 amylo-pectin 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.

### **Acknowledgements**

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