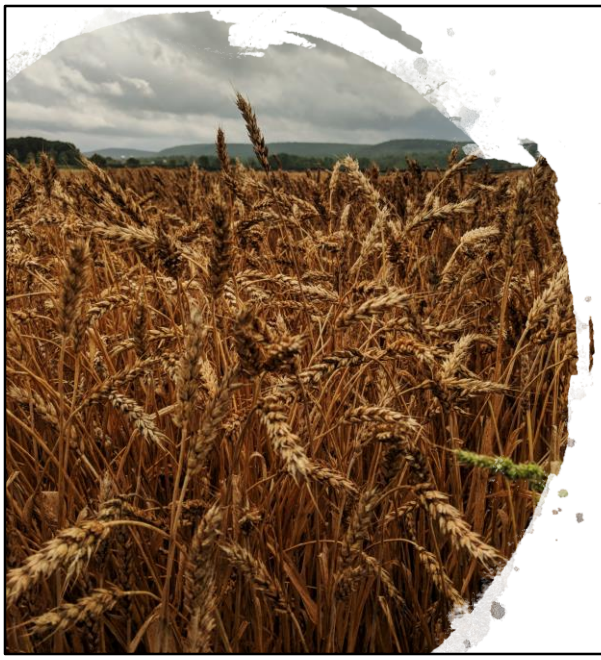

The First Step to Tackling the FN Problem:
Identifying PHS Tolerant Genes/QTL in PNW Germplasm

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FN Workshop | Jan 30th, 2019

PNW Quality Council

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github.com/shantel-martinez/FNWorkshop2019

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The majority of the research shown today is published at doi: 10.3389/fpls.2018.00141
Slides are publicly available along with other resources or data on github.com/shantel-martinez/FNWorkshop2019

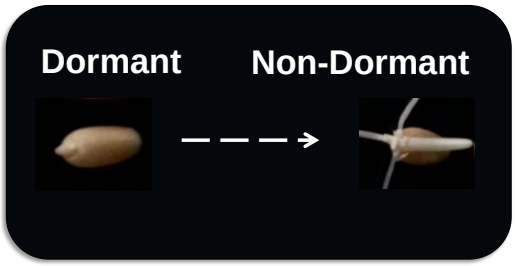
Preharvest Sprouting

Germination of mature seed on the mother plant when cool and wet conditions occur before harvest

PHS Tolerant ⟷ PHS Susceptible

Wheat Seed Dormancy

The inability to germinate even under **favorable environmental conditions**



After-ripening
Cold Imbibition

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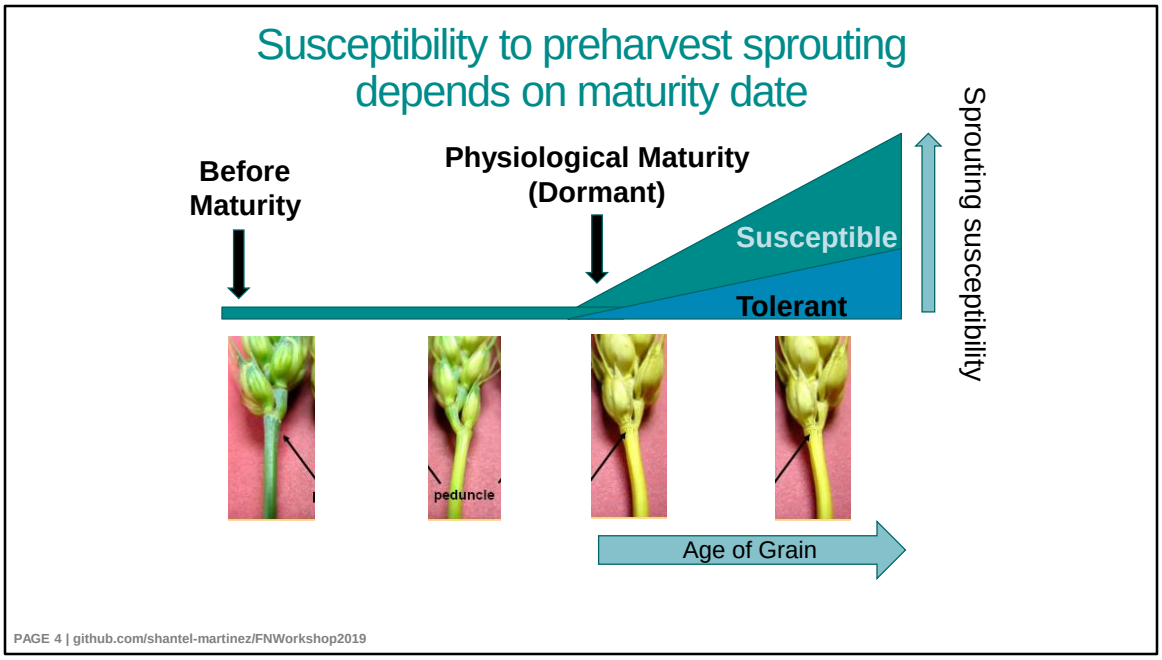
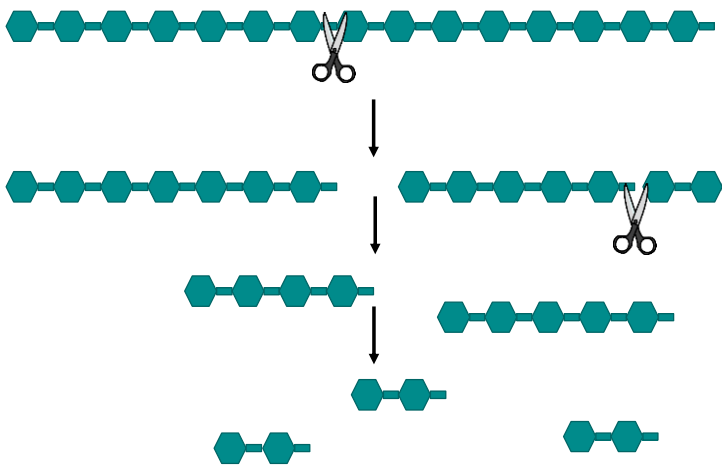
-PHS is the germination of mature seed on the mother plant when cool and wet conditions occur before harvest
-If these conditions occur, wheat varieties may show tolerance, with no visible germination or they may have a higher rate of germination on the right
-The large variation in wheats' genetic tolerance to PHS is largely due to insufficient seed dormancy at maturity.
-Wheat seed dormancy is the inability to germinate even under favorable environmental conditions
-Dormancy can be lost many ways, one of which is a period of dry storage, called after-ripening



Seeds have their highest dormancy at physiological maturity and lose dormancy in the field through after-ripening.
A tolerant variety loses dormancy slower than a susceptible variety
This is important when you're assessing PHS susceptibility in the field because maturity date also impacts whether or not the wheat sprouts after rain

PHS is a result of a-amylase activity breaking down starch chains

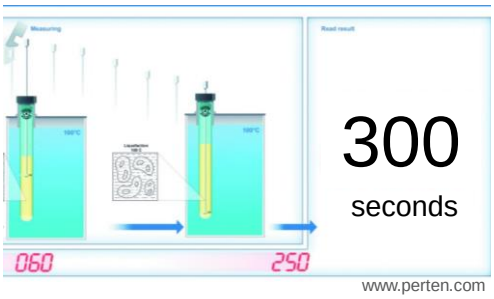


Sound

Severely Sprouted

In addition to dormancy, PHS is also a result of a-amylase activity breaking down starch chains

Hagberg-Perten Falling Number: The Industry Standard to Measure PHS



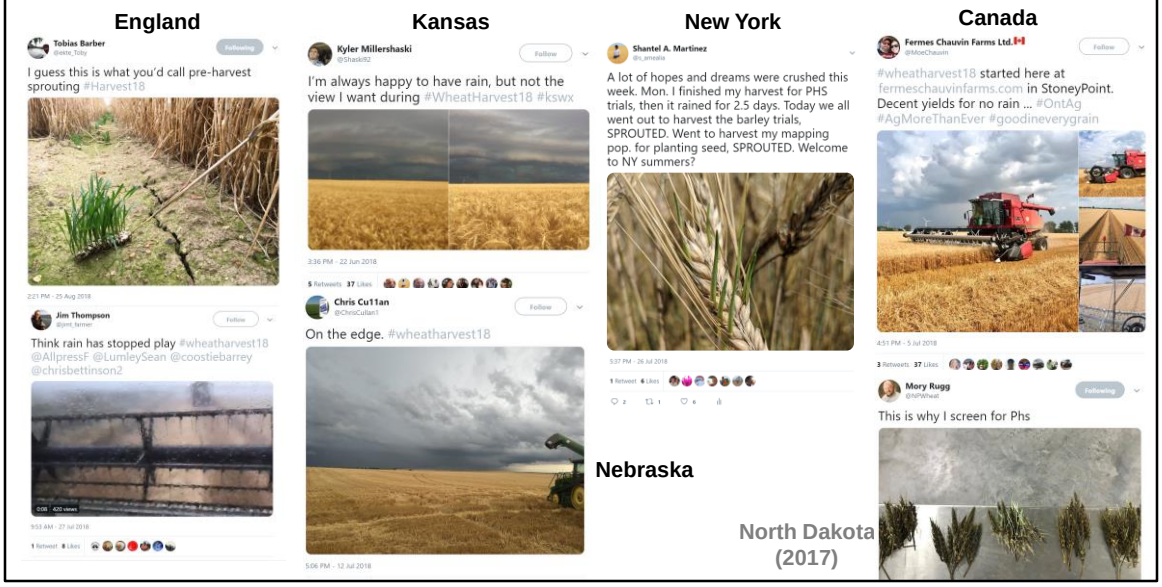
www.perten.com

Farms.com

Low FN is associated with low end use quality

This process of a-amylase activity is measured by the Falling Number (FN) test
FNs below 300 seconds result in discounts to farmers, as much as 25 cents per 25 seconds below 300 seconds
The PNW had FN problems in 2016, but you are alone

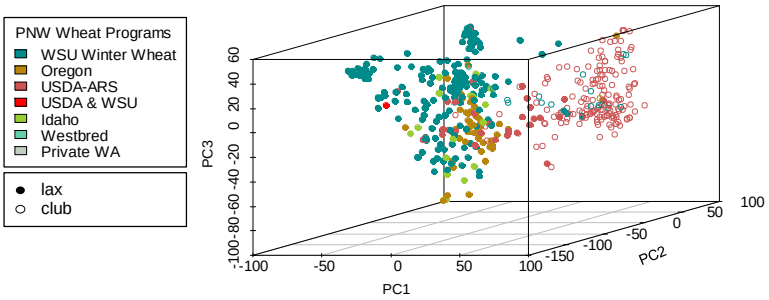
You are Not Alone - 2018 Preharvest Sprouting



By just watching the wheat twitter in 2018, there were many farms or breeding programs with PHS events or extremely close calls

I started out my PHS genetic story here in the PNW with the WSU and ARS crew in Pullman, now I have moved to NY to work with a breeding program were PHS is their 2nd priority to yield, which you can see why here in this ridiculous PHS event after 2 days of rain.

Improving Preharvest Sprouting in the PNW: Understanding the Genetic Tolerance that Exists in the Current Breeding Programs



PHS

Falling Number

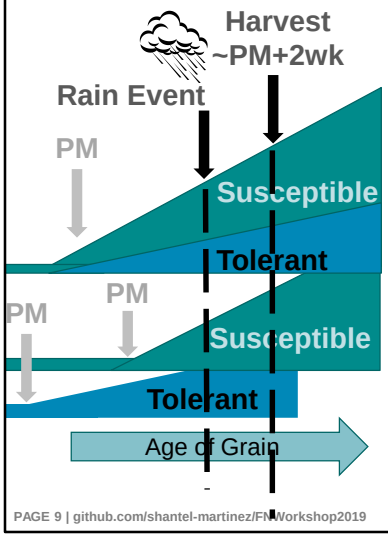
Spike Wetting Test

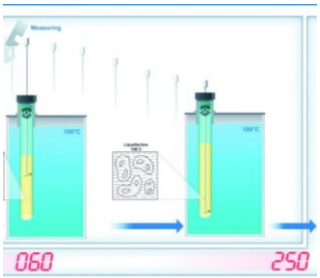
The panel is derived from at least six white winter wheat breeding programs.

Jernigan and Godoy et al., 2018 | Martinez et al., 2016

However today, I want to focus on my PhD work here in the PNW
I set out to conduct an AM study with 480 lines representing WSU OR ID and private germplasm.
We chose this panel, because it was designed to represent all of the soft white breeding programs of the NW that includes club and lax wheat
We tested preharvest sprouting traits through two methods, Falling Numbers Test and Spike-wetting test.

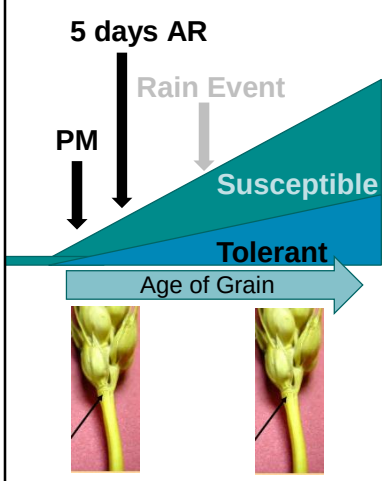
Falling Numbers Test:
Samples are Harvested at "Harvest Maturity"





When a breeding program tests FN, all of the varieties/lines are harvested at the same time, typically 2wk after physiological maturity, regardless of when the lines reach maturity or the age of after-ripening. And if a breeding program is "lucky" enough to get a natural rain event, then we can test samples for low FN specifically due to PHS events on a large scale.

Greenhouse Spike Wetting Test: Samples are Harvested at Physiological Maturity



Misted 6 sec / min

Scored every 24 hrs for 7 days

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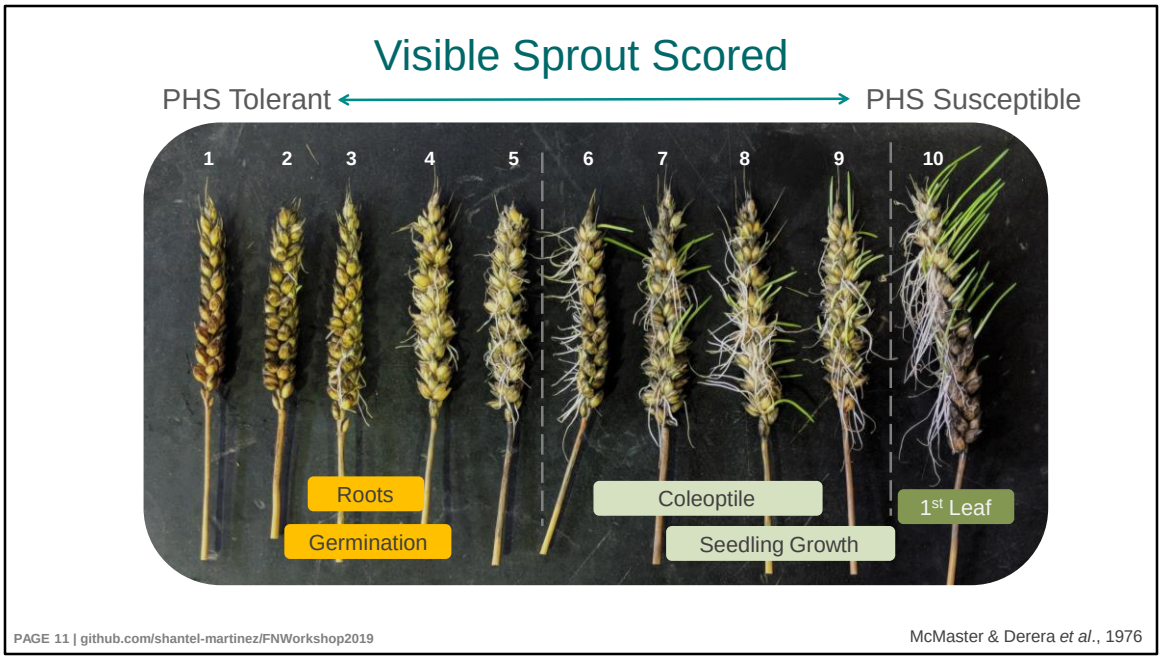
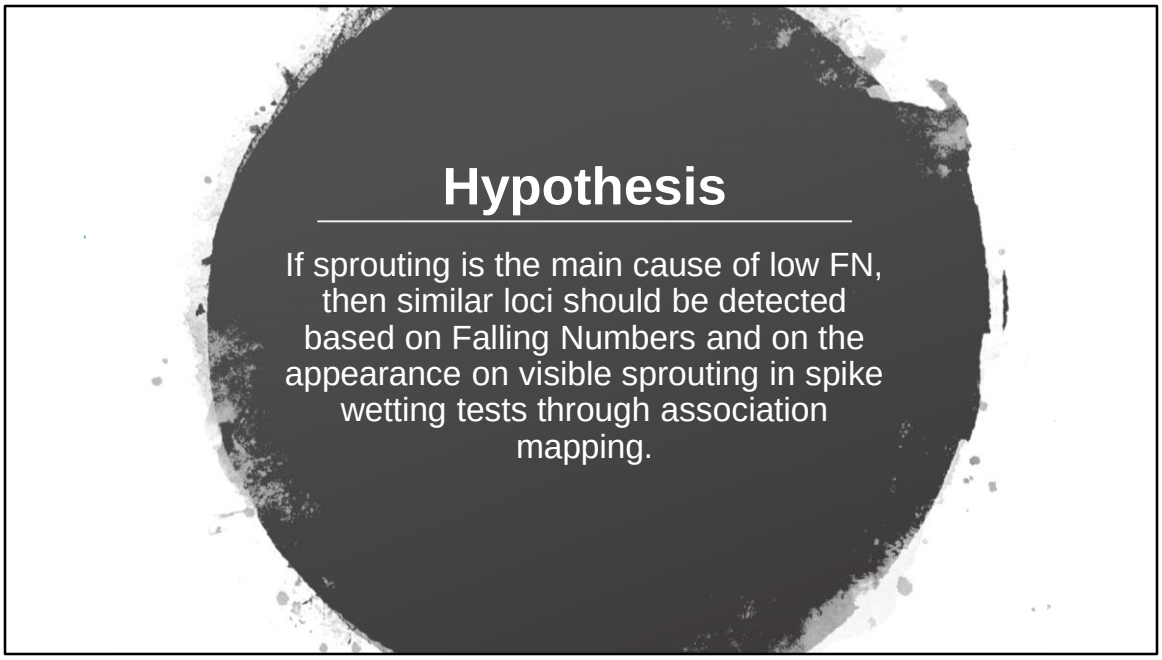
Another method to measure PHS tolerance is the spike wetting test. Typically, you harvest each individual line at physiological maturity, followed by 5 days of aging/after-ripening, and then we induce sprouting by bringing the lines into the greenhouse misting chamber that mists for 6sec/min. The lines are then scored based on a visible sprouting scale every 24 hours.



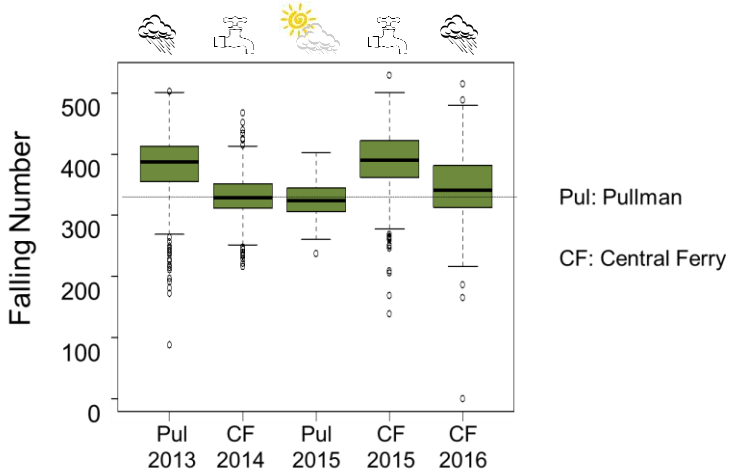
Visible sprout is scored using the scale developed by McMaster and Derrera
1 being no visible sprouting, 10 being 100% sprouting and 1st leaf growth.
However what you're essentially seeing in a 1-5 score is the initial germination whereas a 6-10 score measures seedling growth.

Historically, breeding programs have use the spike wetting test (SwT) to screen for PHS tolerance whereas the wheat industry uses the FN test to screen for a-amylase activity
Previous mapping studies have all focused on results from either just the SWT or just the FN test. I wanted to focus on both PHS traits from the same set of germplasm, because the FN is what matters for the farmers getting paid, and the breeders still need an efficient quantifiable method to screen for PHS tolerance



But you will see as the story unfolds, this may not be a fair assumption.

The FN Trait Was Tested Over 5 Environments, 3 Different Events



We looked at the FN trait across 5 environments: 2 Natural Rain evenst, 1 No rain event used as a control, and 2 artificially rain induced with sprinklers to mimic PHS out in the field.

You can see clearly that during the rain events, there is a large number of lines that fall below that 325 sec FN threshold, which is when we start to see a-amylase activity affect quality.

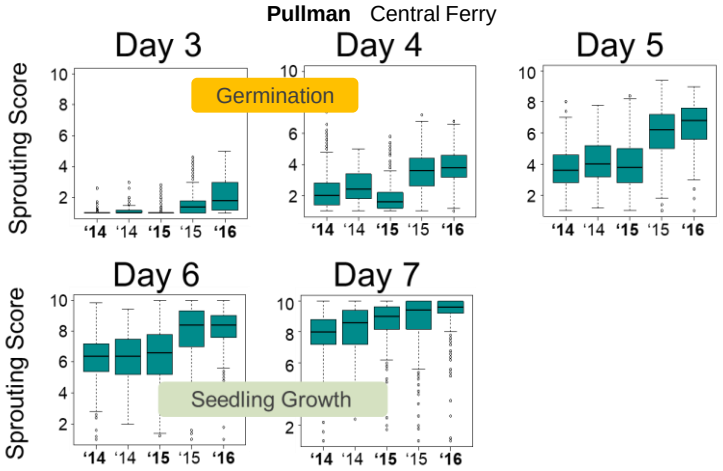
The environment has a large affect on the FN response

	Pul13	CF14	Pul15	CF15
CF14	0.29**			
Pul15	0.23**	**0.42****		
CF15	0.23**	0.29**	0.29**	
CF16	0.33**	**0.46****	0.30**	0.34**

**: $p \leq 0.001$ * : $p < 0.05$

When you compare FN environments to one another, we see *some* positive correlations and the remainder have quite low correlations. This lack of correlation makes sense since we were comparing different types of rain events which can affect the FNs.

Visible Sprouting Was Tested Over 5 Environments, Same GH Rain Event



The spike wetting tests was also conducted over 5 environments, however **no field rain events** separate each environment, since we harvested the samples at PM. Additionally, the days misting are looking at different points: germination and seedling growth

Visible Sprout Correlations Across Environments Were as Good as Other SWT Studies

Day 6 Seedling Growth

	CF14	Pul16	Pul14	CF15
Pul16	0.39**			
Pul14	0.39**	0.29**		
CF15	0.38**	0.30**	**0.40****	
Pul15	0.34**	0.16*	**0.46****	0.36**

**: $p \leq 0.001$ * : $p \leq 0.05$

Kulwal et al., 2012; Jaiswal et al., 2012; Ogbonnaya et al., 2008; Zhou et al., 2017

When you compare FN environments to one another, we see *some* positive correlations and the remainder have quite low correlations. Initially, this didn't make any sense since we controlled for the rain event in the GH. However it has been well published that the environment does effects on the degree of dormancy during seed development, and we could be seeing that across our different environments.
In fact, the correlations between our environments were as good as those in other spike-wetting test studies

The Correlations Between FN and Visible Sprout are Not Highly Negative

		Germination			Seedling Growth		
		3 days	4 days	5 days	6 days	7 days	SI
	**Pul13**	-0.16**	-0.24**	-0.17**	-0.18**	-0.20**	-0.21**
	**CF14**	-0.07	-0.09*	-0.06	-0.09	-0.10*	-0.10*
	**Pul15**	-0.07	-0.13*	-0.12*	-0.12*	-0.17**	-0.15**
	**CF15**	-0.09	-0.04	0.00	0.01	0.00	-0.01
	**CF16**	-0.17**	-0.19**	-0.18**	-0.17**	-0.17**	-0.19**

**: $p \leq 0.001$ * : $p \leq 0.05$

-0.80** -0.83**

Rasul et al., 2009; Jiménez et al., 2016

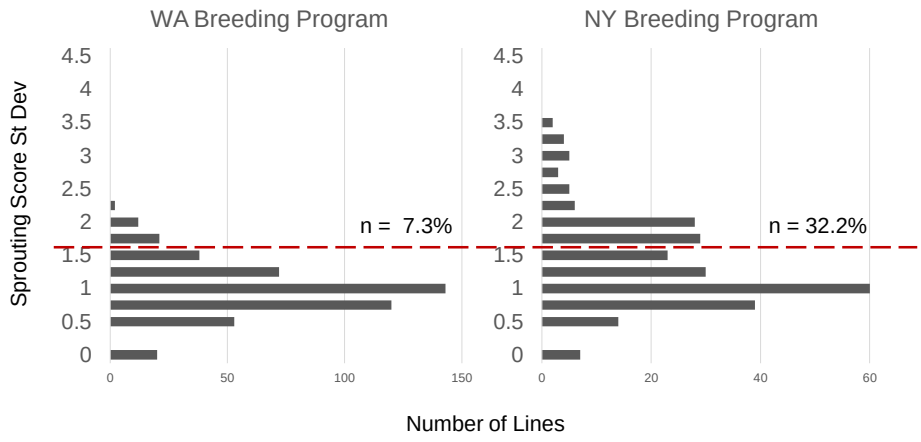
We have to remember that this study did look at 2 different stages of maturity: PM+2wk for FN and PM+5d for spike-wetting test

Previous published studies looked at samples of the same stage (PM vs PM), and observed an -.80 negative correlation

An improvement can potentially be made by increasing the artificial rain amount prior to HM for the FN samples, but needs to be tested.

Point: If you want to select for PHS via FN, you have to remember how much of an effect the environment will have on the FN response, and it may or may not be just PHS contributing to that response.

When Implementing Spike-Wetting Tests in a Breeding Program: The Germplasm / Environment Could Affect the Variance



Cornell University & Washington State University, unpublished

A) Different germplasm/resistance

B) Different environments could result in this large variation in technical reps

Point: Take note, when implementing SWT as a trait to test for PHS tolerance, its always possible the germplasm may have large variation due to the environment, especially prior to PM during seed development.

Genome-wide Association Study of FN and Visible Sprout

15,229 polymorphic markers | 21 chromosomes | 469 accessions

9 *QFN.wsu*

CF16	Pul15	CF15
3	3	0
Pul13		**CF14**
3		0

34 *QPHS.wsu*

Germination | Seedling Growth

Day 3 Day 4	Day 5	Day 6 Day 7	SI
12	6	13	3

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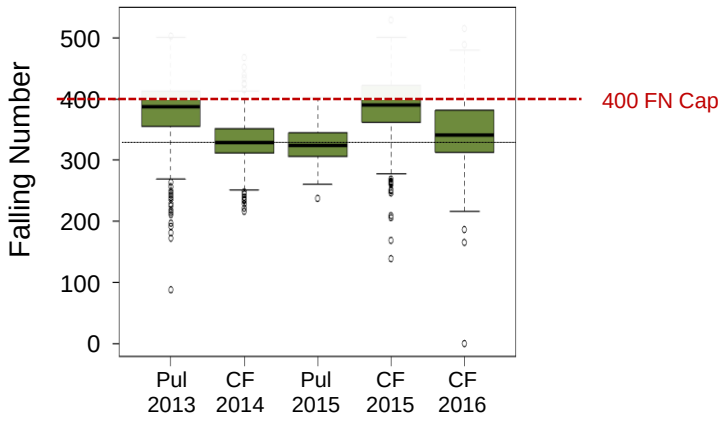
With the two PHS traits, we ran a GWAS using 15,000 90k Illumina markers. We found 9 FN QTL and 34 Visible Sprouting QTL.

Will Only Looking at the Low FN Values Give Different QTN?



Then we looked at the FN data a different way, everything above 400 second, so extremely high FN, was capped at 400 sec. Our focus was to see if the lower FN would show different QTN with those high FN taken out as a large factor.

Genome-wide Association Study of FN and Visible Sprout

15,229 polymorphic markers | 21 chromosomes | 469 accessions

2 *QFN.wsu* 9 *QFN.wsu* 34 *QPHS.wsu*

Germination | Seedling Growth

CF16	Pul15	CF15	Day 3 Day 4	Day 5	Day 6 Day 7	SI
3+2	3	0	12	6	13	3
Pul13		**CF14**				
3		0				

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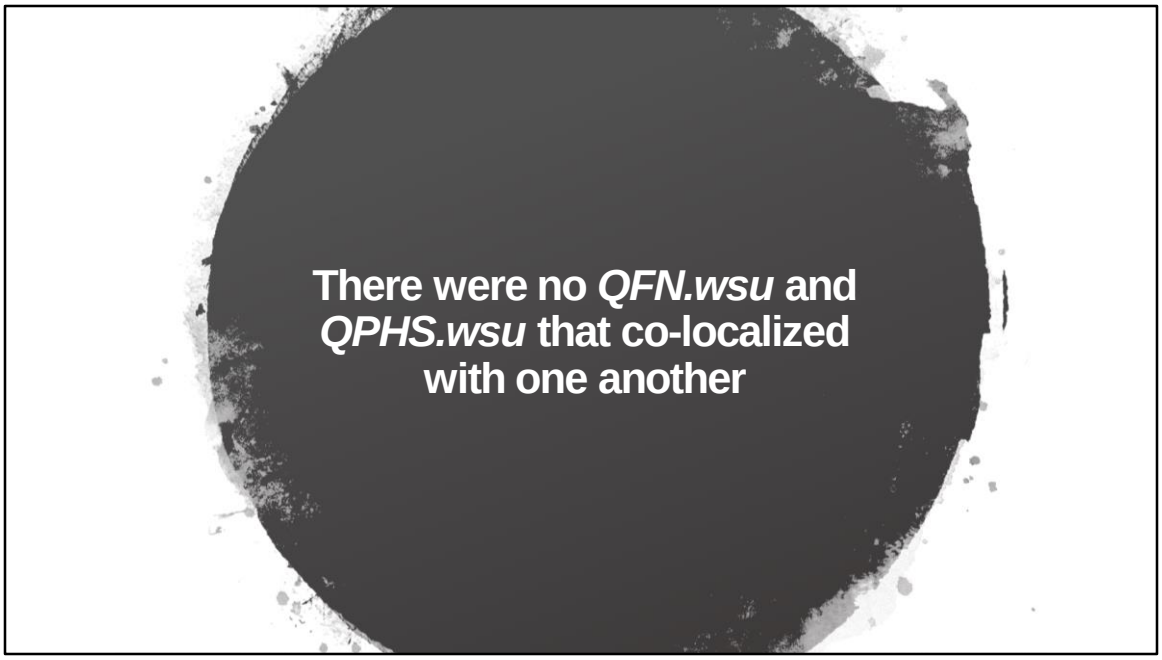
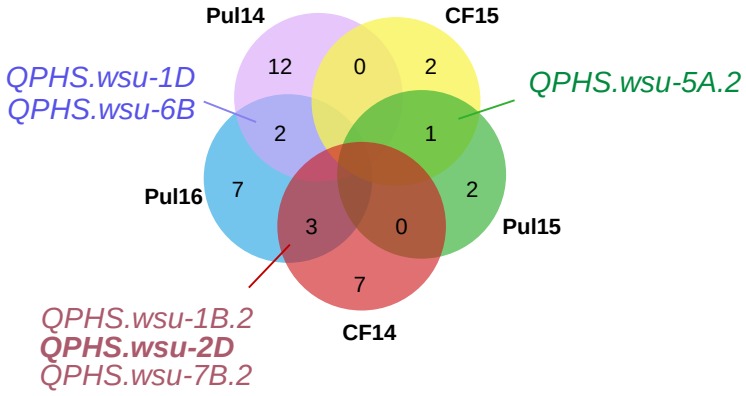
We found 2 more additional FN QTN

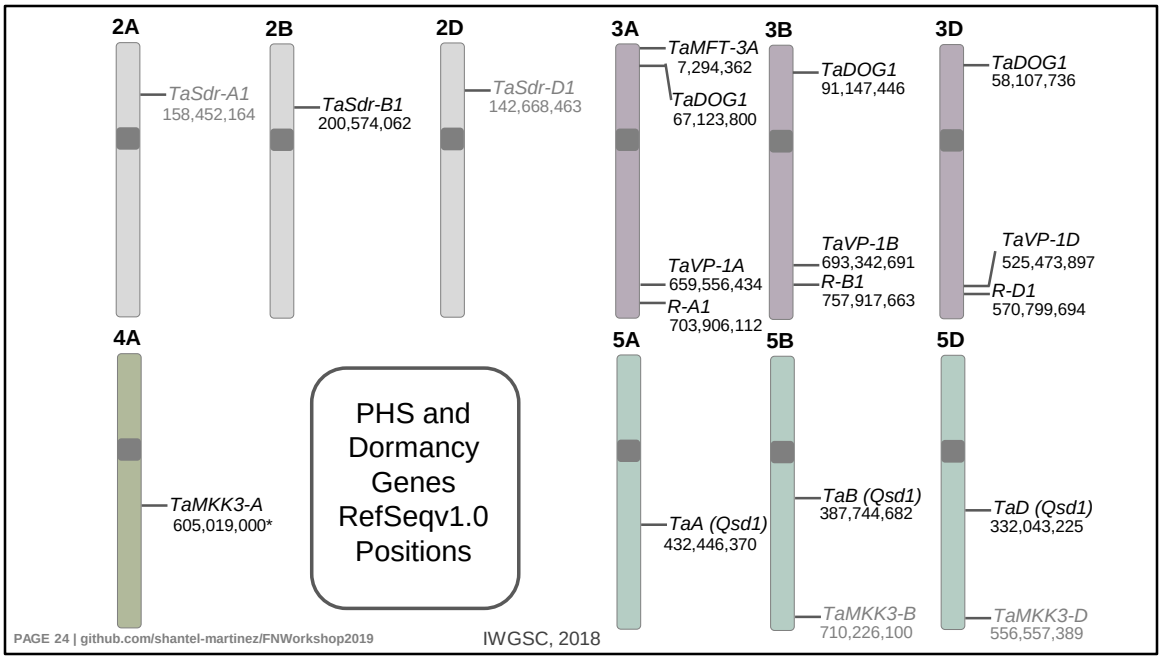


So we may not be able to rely **entirely** on SWT to select for High FN, or FN to entirely select for low visible sprouting, especially in situation were it's a very mild case of sprouting were you cant see it. As we have heard over and over, the FN is to measurement for millers/bakers to make the product with what they have, and the spike wetting test is used to tell you whether or not you will actually be able to see sprouting.

Visible Sprouting QTL *QPHS.wsu* Across Environments



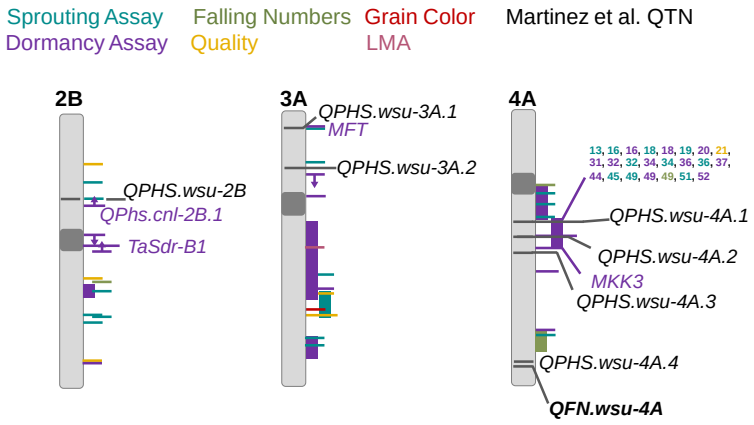
Going more deeply in the QTL we did find, we got a really large number (34) of visible sprout QTL and only 6 showed up across multiple environments. These are the QTL you would want to emphasis across a breeding program because they are **more likely** to be effective in different environments rather than one environment. If I wanted to increased PHS in my breeding program in the PNW, I would start with these 6 QTL. Ongoing work right now by Stephanie Sjoberg is looking to see of whether or not these 6 show up in an independent PNW mapping panel.



We should also be paying attention to the published PHS genes cloned and characterized.

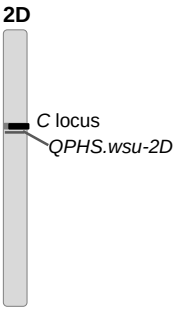
The advantage to these PHS tolerance genes will need to be weighed with the disadvantage of bringing in outside germplasm, which means breeders need time to integrate these genes in addition to selecting for high yield, disease resistance, and quality for our region.

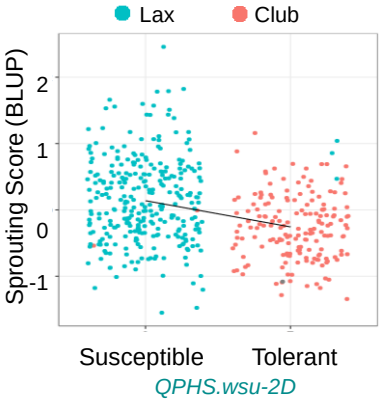
Visible Sprouting QTL Located Near Known PHS Genes



Tests have not confirmed that the QTL we identified are due to the genes nearby, but based on proximity, it appear we may already have germplasm with dormancy genes such as MFT on 3A and MKK3 on 4A. This in addition to the QTL contributed by that Clark's Cream on Chrm 2B.

Strongest PHS QTL, *QPHS.wsu-2D*, Close to the *Compactum* (*C*) Locus





Photos by T. DeMacon | Johnson et al., 2008

Interestingly, the QTL that showed up in all days of spike-wetting test misting and in "multiple" environments (2) was a 2D QTL.
There are many theories as to why the club wheat seems to be linked to PHS tolerance, it could be historical breeding effort from Dr. Bob, it could be the number of tolerance QTN from other chromosomes,
Currently research has the c locus spanning the centromere, Kim Garland-Campbell may have a more up to date location unpublished, so further research into the co-localization of the c locus and this strong PHS QTL should be teased apart.

2 of the 11 *QFN.wsu* appear to be unique

10 of the 34 *QPHS.wsu* appear to be unique

The others were found near other known PHS-related loci

Breeding for PHS Conclusion

GWAS for FN and visible sprout detected different QTN, although both co-localized with known PHS-related loci

FN is a measure of a-amylase activity. The lack of correlation between FN and visible sprout may mean that a-amylase is regulated differently with respect to the timing of germination in different varieties.

The club *C* locus was linked to the strongest *QPHS.wsu-2D* QTL

We suspect that the majority of our germplasm does not have a bad FN problem and most of the lines fall well above 325, therefore the significant markers we see in our FN AM is due to other factors contributing to FN rather than a-amylase.
We have to question whether or not the spike wetting test is the ideal method for selecting PHS tol in a breeding program. IF the breeding objective is to maintain high FN.
Also, the time we scored germination in the SWT may not be correlating with alpha amylase activity

github.com/shantel-martinez/FNWorkshop2019

Panel Topic:

How do we make these markers/QTL useful in breeding programs

How do we turn this into a tool the breeders can use now