Cereal and Flax Pathology

AgBio Advancements in Agricultural Research Seminar Series

March 14th, 2023

Randy Kutcher, Crop Development Centre

University of Saskatchewan

Overview of program

1) Disease assessment of germplasm

(breeders and exotic material)

Cereal disease nurseries:

- Leaf and stem rust
- Bunt
- Stripe rust
- FHB

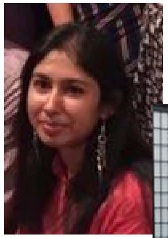
Flax disease nurseries:

- Flax wilt
- Pasmo

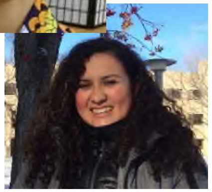
Technicians: **Everett Boots** (senior), **Allison Karstens** and 3 summer students

Overview of program

2) Integrated pest management




Research Assistants: **Trisha Islam, Keiko Nabetani & Alejandra Oviedo**



Research Officers: **Dr. Lipu Wang & Constanza Fleitas**


Postdoctoral fellow: **Dr. Luis Ponce**

Integrated Pest Management

Integrated

- Combine parts into a whole
- Complete something important by addition of parts

Pest

- Annoying person or thing, or
- Competitors for our food and shelter

Management

- skill in dealing with people
- control and organization

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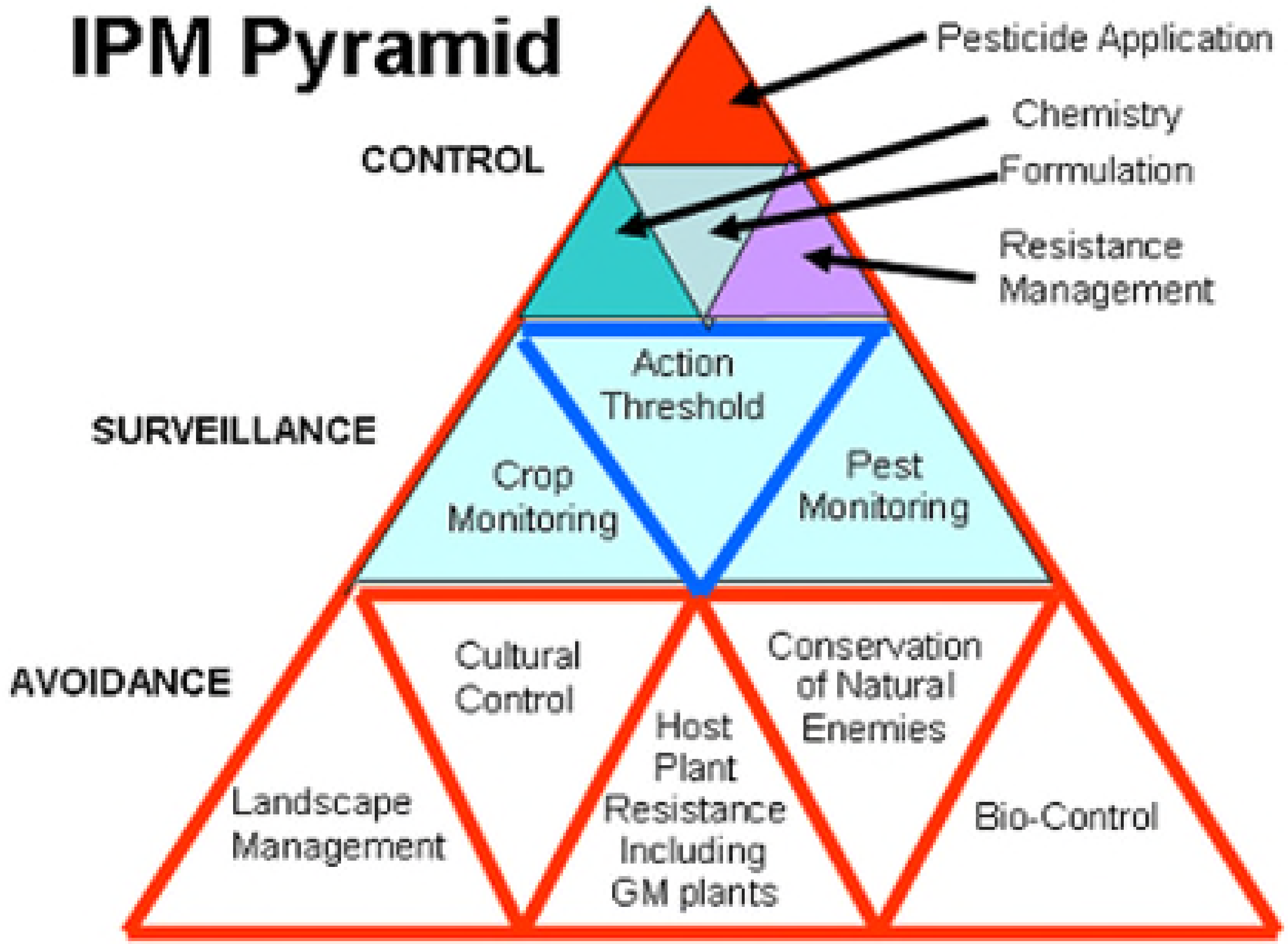
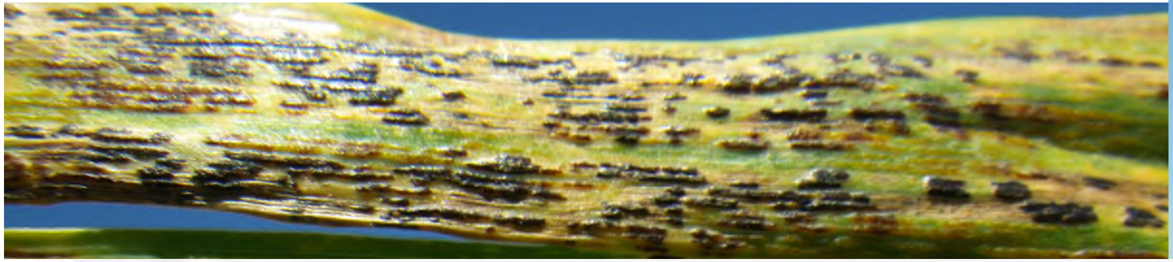
Matthews IPM Pyramid

Graham Matthews, Imperial College, London



Wheat stripe rust

- Worldwide importance
- Caused by obligate biotrophic fungus *Puccinia striiformis* f. sp. *tritici*
- Also known as 'yellow rust'



Photos Credit: Gurcharn Brar

Objective

- characterize race structure of *Pst* in western Canada

  Do this using classical host-pathogen interactions and molecular methods

University of Saskatchewan

International set of near-isogenic wheat genotypes

Differential Name	*Yr-R* gene	Differential Name	*Yr-R* gene
Avocet-S	susceptible check	Avocet-Yr18	*Yr18* (APR*)
Avocet+YrA	*YrA*	Avocet-Yr24	*Yr24*
Avocet-Yr1	*Yr1*	Avocet-Y26	*Yr26*
Avocet-Yr2	*Yr2*	Avocet-Yr27	*Yr27*
Avocet-Yr5	*Yr5*	Avocet-YrSP	*YrSP*
Avocet-Yr6	*Yr6*	Avocet-Yr28	*Yr28*
Avocet-Yr7	*Yr7*	Avocet-Yr29	*Yr29* (APR)
Avocet-Yr8	*Yr8*	Avocet-Yr31	*Yr31*
Avocet-Yr9	*Yr9*	Avocet-Yr32	*Yr32*
Avocet-Yr10	*Yr10*	Yamhill	*Yr2, Yr4a, YrYam*
Avocet-Yr15	*Yr15*	Moro	*Yr10, YrMor*
Avocet-Yr17	*Yr17*	Paha	*YrPa1, YrPa2, YrPa3*

* APR – adult plant resistance

Methodology cont...

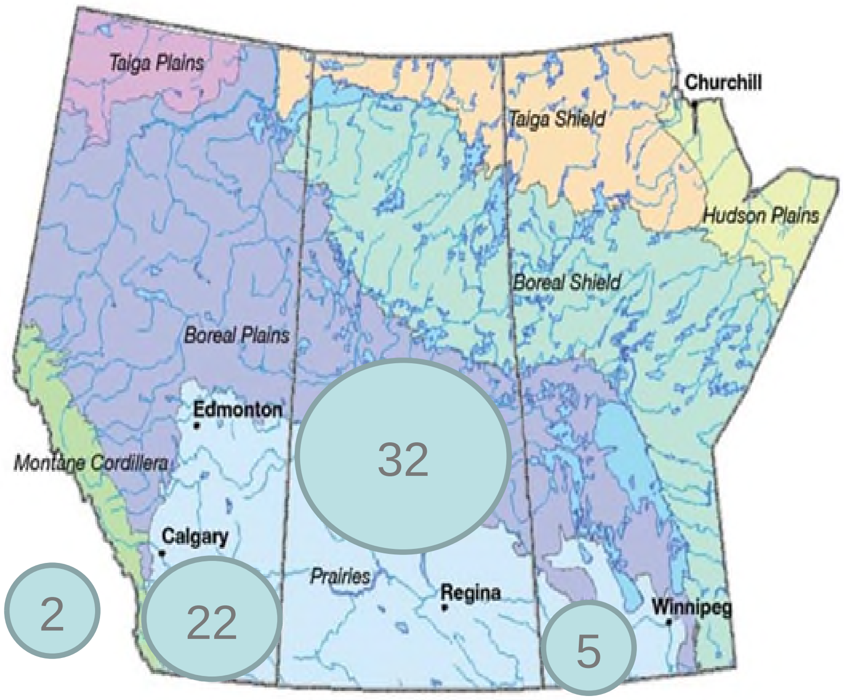
- Planted the differentials in the field at various (8 to 12) locations in Saskatchewan
- Assessed plants for stripe rust at the adult plant growth stage

Photos G. Brar and A.G. Liew

Materials and methods

- 28 near-isogenic wheat genotypes and 4 supplemental

- 61 *Pst* isolates from western Canada



Methodology cont…

- Inoculations at the two-leaf stage

- Disease rating: 14 dpi

Dr. Gurcharn Brar

Effectiveness of *Yr* genes

- Resistant: *Yr5, Yr15, YrSP:* effective against all races of *Pst* in western Canada

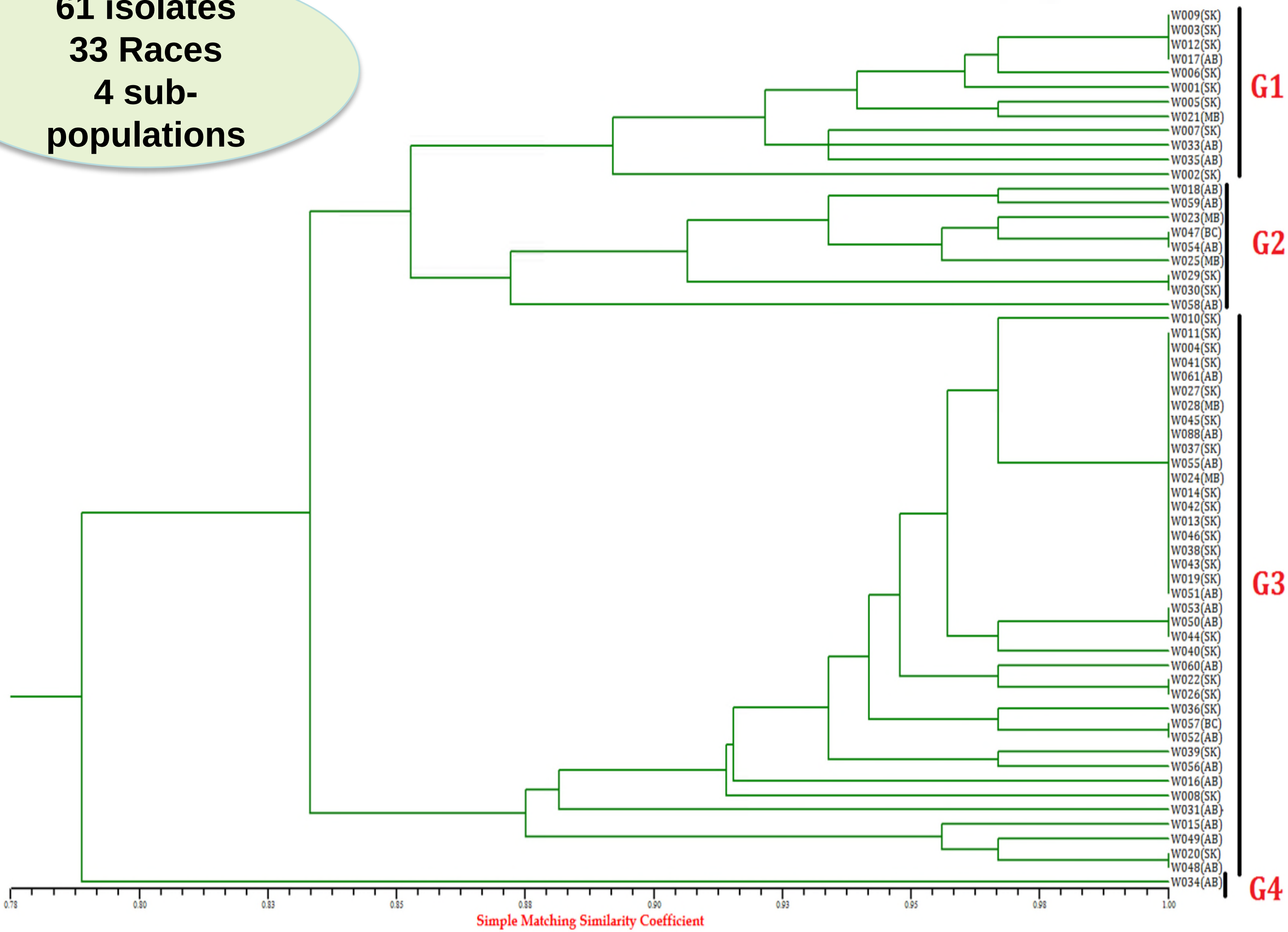
- Susceptible: *Yr6, Yr7, Yr9, Yr17, Yr28, Yr29, Yr31:* defeated by all races in western Canada

- Variable: *YrA, Yr2, Yr8, Yr10, Yr27, Yr29, Yr32,* Heines VII, Nord Deprez and YrSu

Brar, G.S. and H.R. Kutcher. 2016. Plant Dis. 100(8): 1744-1753. http://dx.doi.org/10.1094/PDIS-12-15-1410-RE
Brar, G.S., R. Graf, R. Knox, H. Campbell and H.R. Kutcher. 2017. Can J Plant Pathol. 39(2): 138-148. https://doi.org/10.1080/07060661.2017.1341433

Stripe rust

61 isolates
33 Races
4 sub-populations



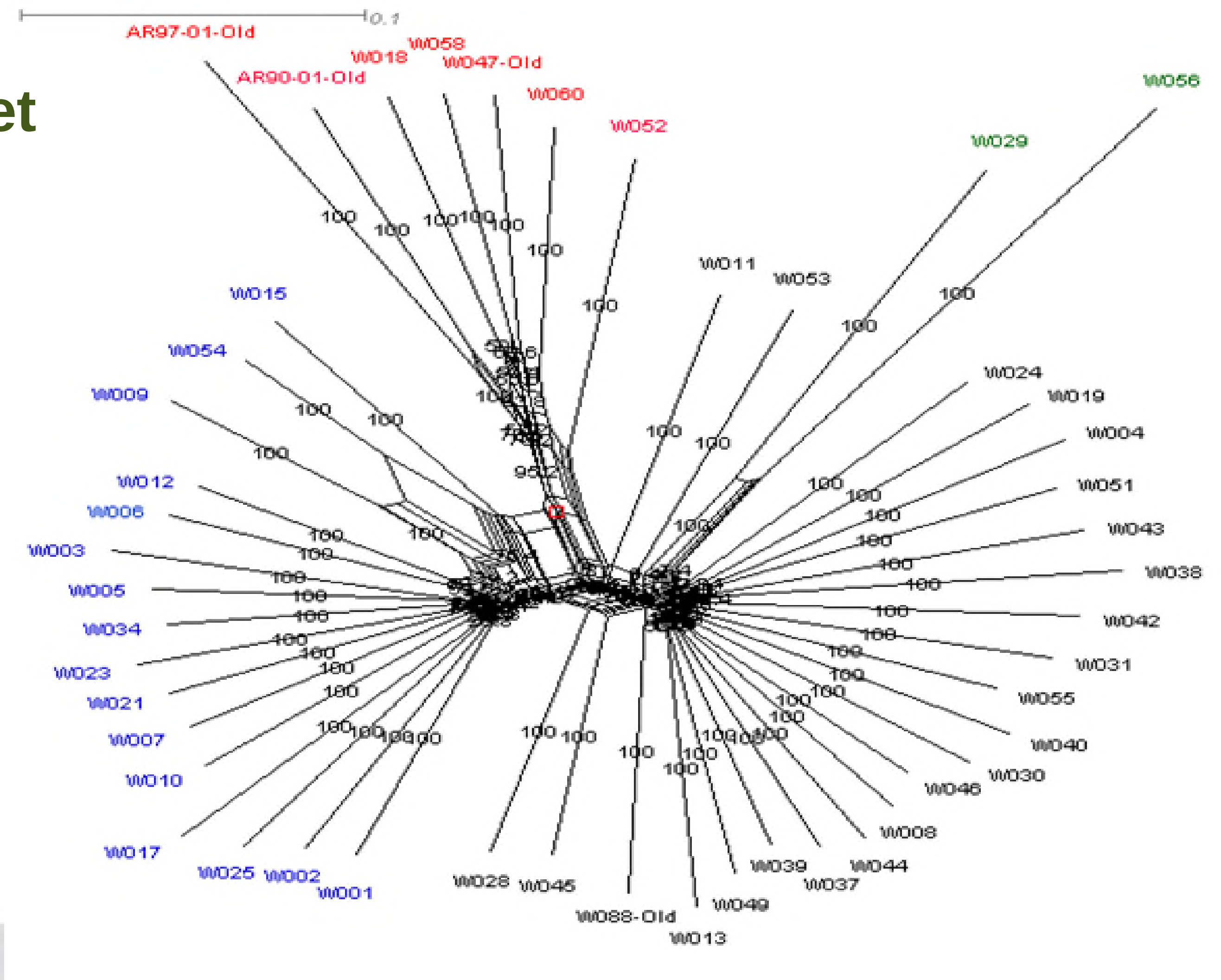
Genotypic (sequence variation)

- Determine genetic diversity of *Pst* in western Canada using genome-wide, single-nucleotide variants (SNVs)

- llumina HiSeq2500 (Illumina®) DNA sequencing
- Variant calling by mapping reads against reference genome 'PST-78'
- Single nucleotide polymorphism (SNP) variants

Diversity analyses

Neighbor-net obtained from SplitsTree4 software



SSR marker analysis

- ***Pst*S0** – GL2, an old lineage previously common in Europe
- ***Pst*S1** – GL1, an invasive, warm-temperature adapted lineage
- ***Pst*S1-related** – GL3, similar to *Pst*S1, but with double the number of recombination events
- ***Pst*Pr** – GL3, a suspected recent foreign incursion

Brar, G.S., Ali, S., Qutob, D., Ambrose, S., Lou, K., Maclachlan, R., Pozniak, C.J., Fu, Y-.B., Sharpe, A.G., and H. R. Kutcher. 2018. Environmental Microbiolology 20: 1498-1515. https://doi.org/10.1111/1462-2920.14067

General conclusions

- The *Pst* population in western Canada has high race and genetic diversity (as detected by traditional and molecular analyses)
- Foreign incursion and recombination events may be the cause of variation in the western Canadian *Pst* population
- We identified single seedling resistance genes, as well as adult plant resistance of benefit to develop resistant wheat varieties

Other studies on stripe rust

- Fungicide x variety control of stripe rust:

  in bread wheat – Tatiana Vera (MSc)

  in winter wheat – Keiko Nabetani (MSc)

Stripe rust studies in Ecuador

Building on past success: stripe rust

- furthering our understanding of wheat stripe rust to mitigate disease and improve resistance
  - Mr. Kun Lou (MSc)

Tan spot of wheat

- Most important disease in the leaf spot complex in western Canada, caused by *Pyrenophora tritici-repentis*
- Yield loss in wheat up to 15% depending on management and environmental factors; losses as high as 50% have been recorded

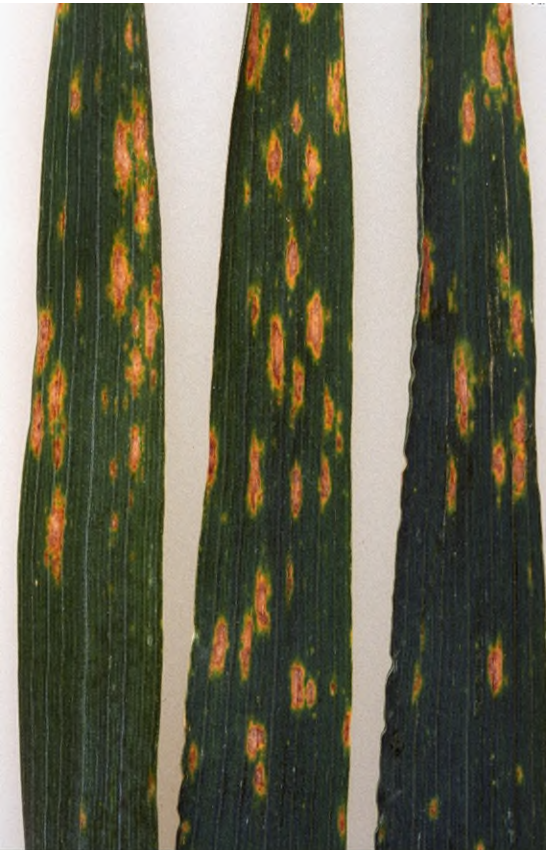
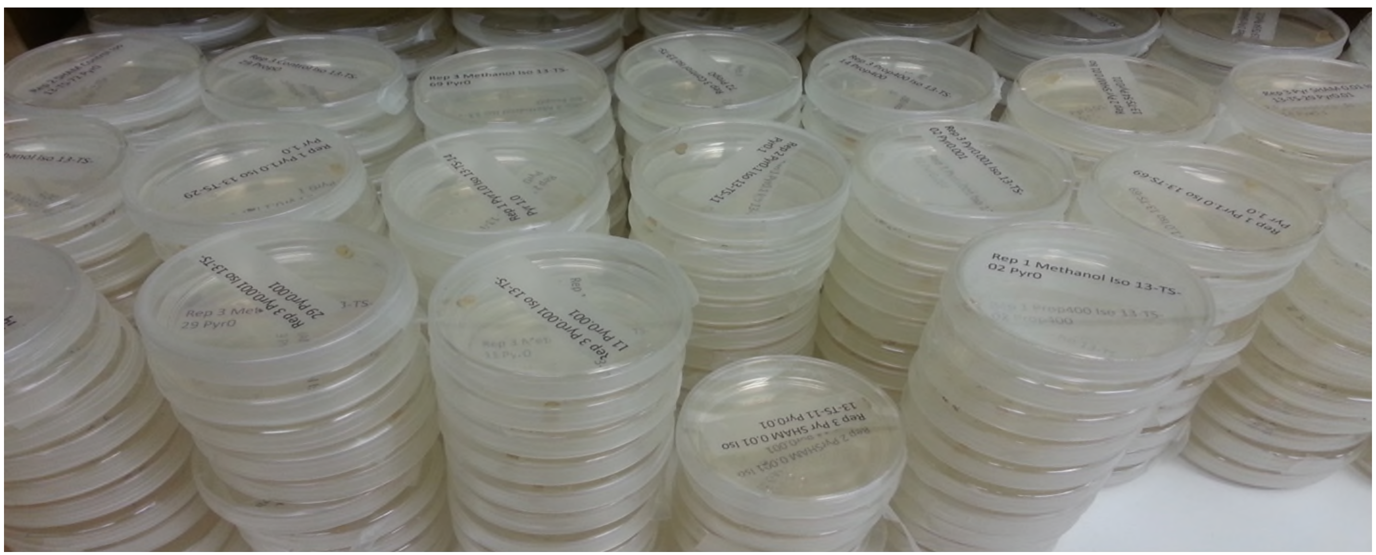


Photo credit: L. Duczek

Objective

- Detect *P. tritici-repentis* fungicide sensitivity in AB and SK by evaluating the effect of fungicides on spore germination and radial growth



D. MacLean

MacLean, D.E., R. Aboukhaddour. V.A. Tran, H. Askarian, S.E. Strelkov, T.K. Turkington, H.R. Kutcher. 2017. Canadian Journal of Plant Pathology 39(4): 433-443. https://doi.org/10.1080/07060661.2017.1387178

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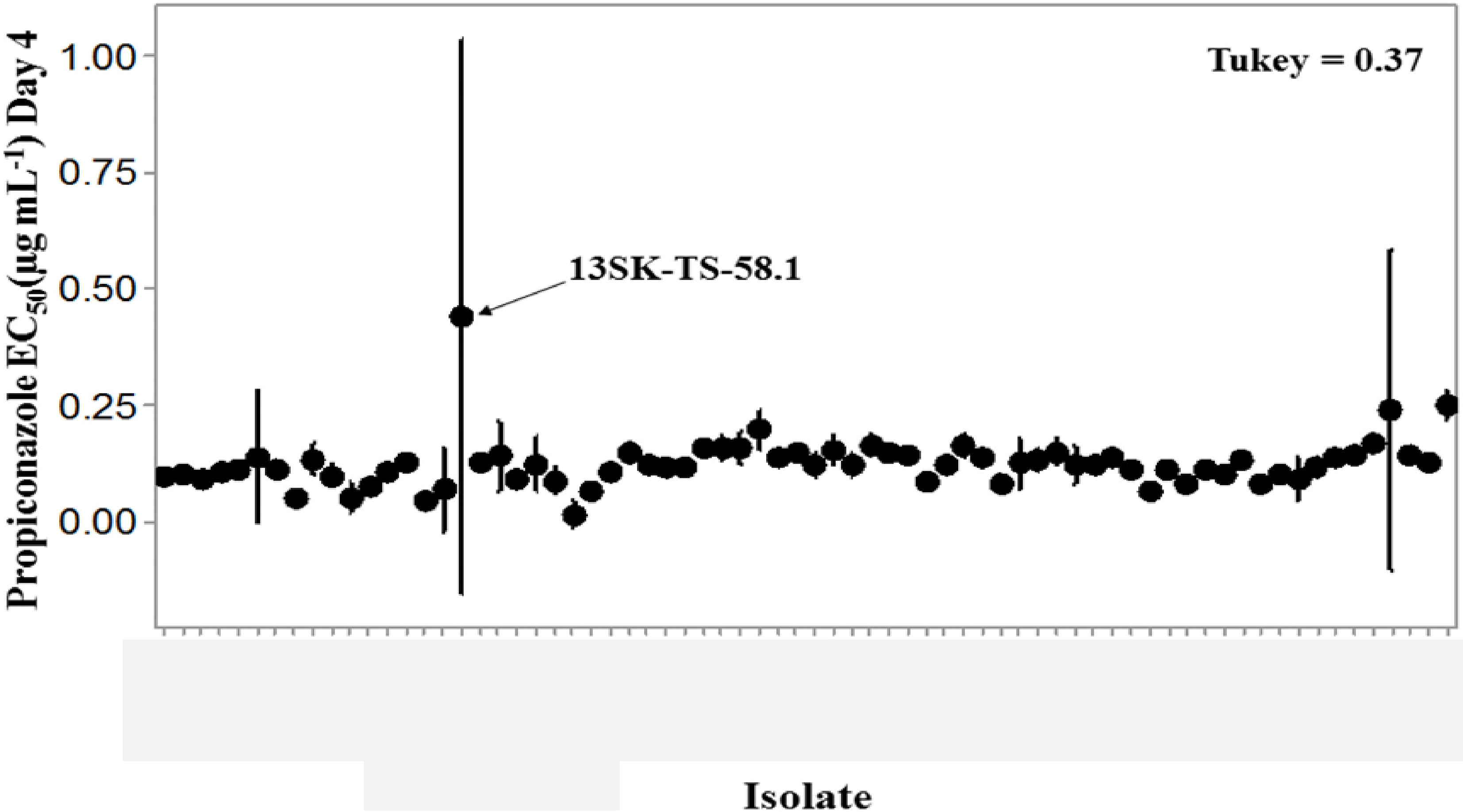
Propiconazole radial growth EC_{50} ($\mu g\ mL^{-1}$) values of 70 isolates of *Pyrenophora tritici-repentis* at Day 4.



Conclusions

- Reduced sensitivity of *P. tritici-repentis* to either azole or strobilurin fungicides has not occurred in Saskatchewan or Alberta.

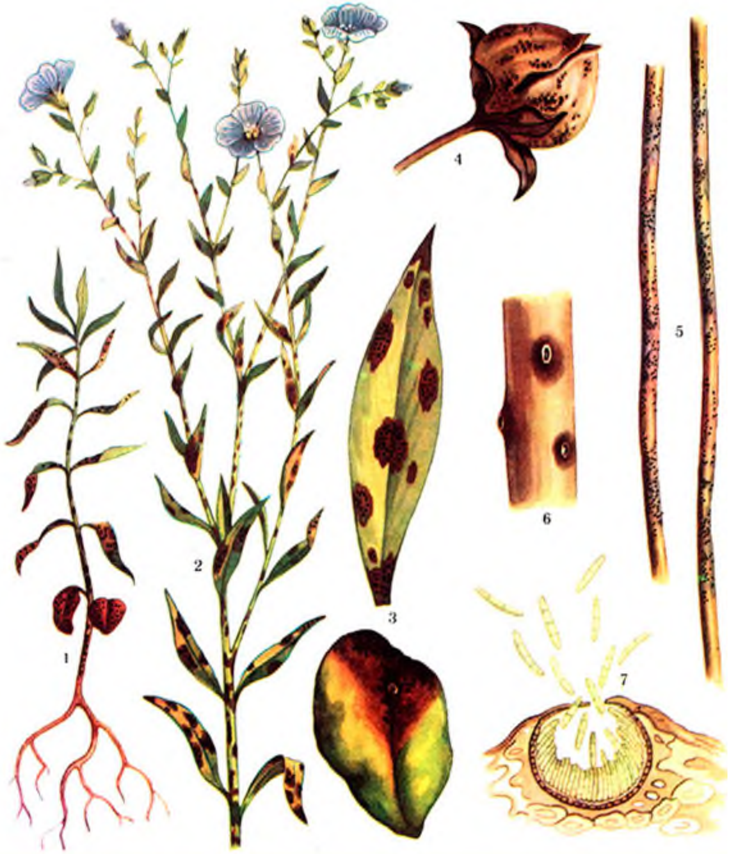
- We have also examined fungicide sensitivity in *Fusarium graminearum* (FHB of wheat) and *Septoria linicola* (cause of pasmo of flax).

Pasmo of flax

- Caused by *Septoria linicola* (Speg.) Garassini

- Warm and humid environment favors disease development (20-21^{o}C)

- May cause 30 - 40% yield loss when the disease is severe



Pasmo of flax

http://www.fitolab.ks.ua/pl.html#!prettyPhoto

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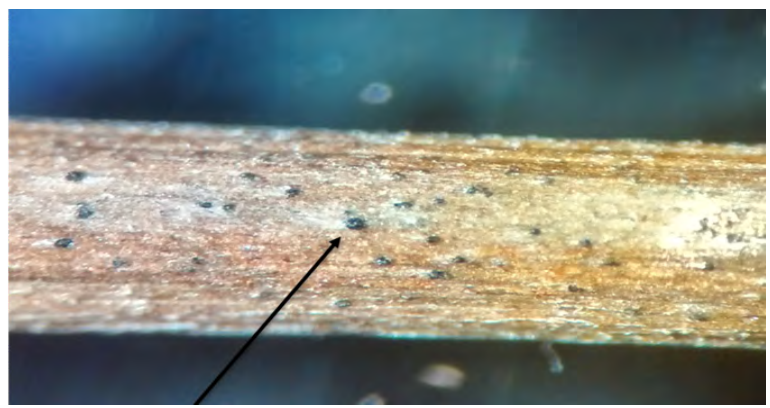
Flax germplasm (*Linum usitatissimum*)

Characterize PGRC flax accessions for resistance to pasmo, Saskatoon, 2014-18

Symptoms

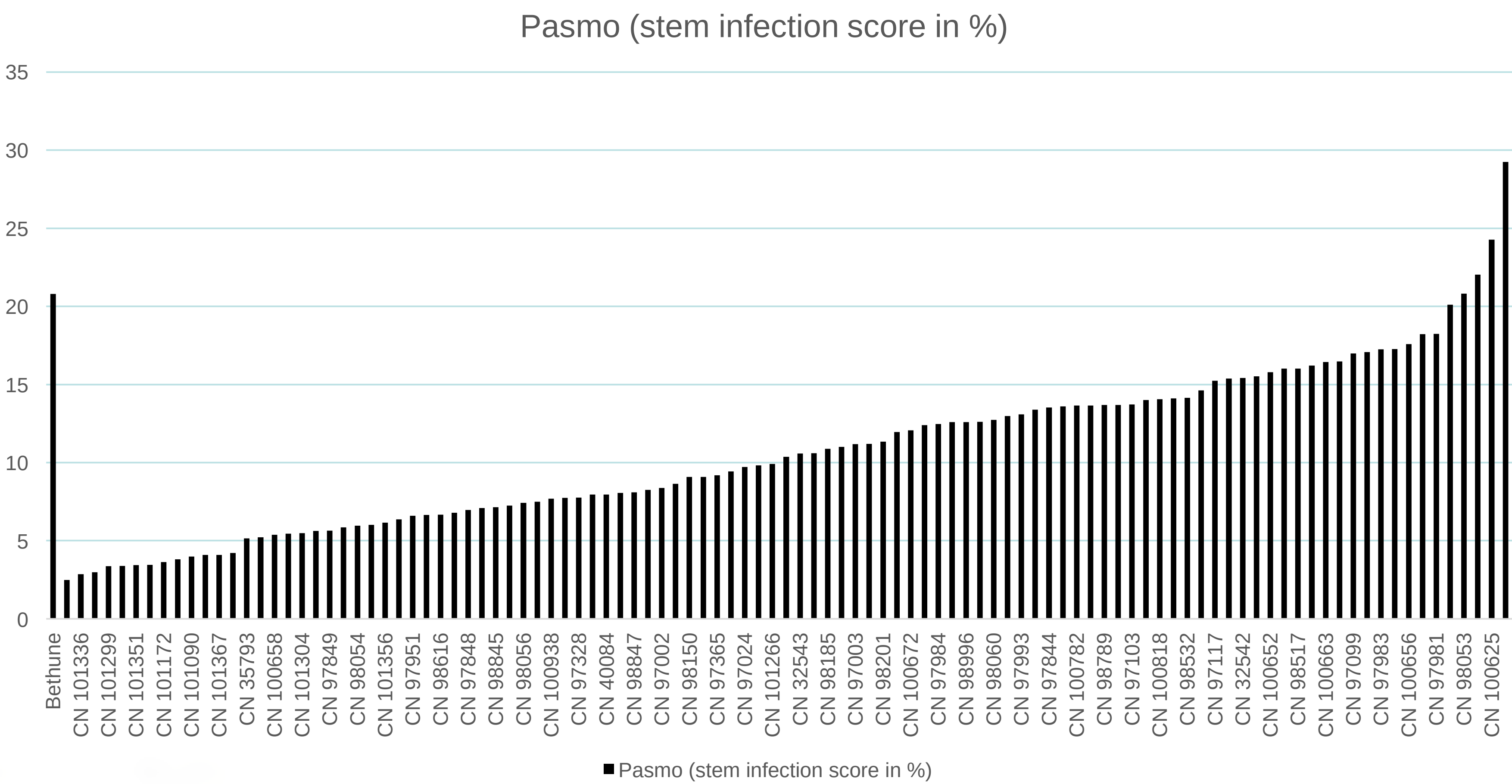
Circular brown lesions on leaves

Green and brown bands on stem



Pycnidia of *S. linicola*

Pasmo reactions of flax lines



n = 40 for Bethune and between 8 and 10 for each accession

Pasmo management with fungicide

- Application timing:

  Early flowering (BBCH-scale 61)

  Mid-flowering (BBCH-scale 65)

  Both

- Two fungicide products

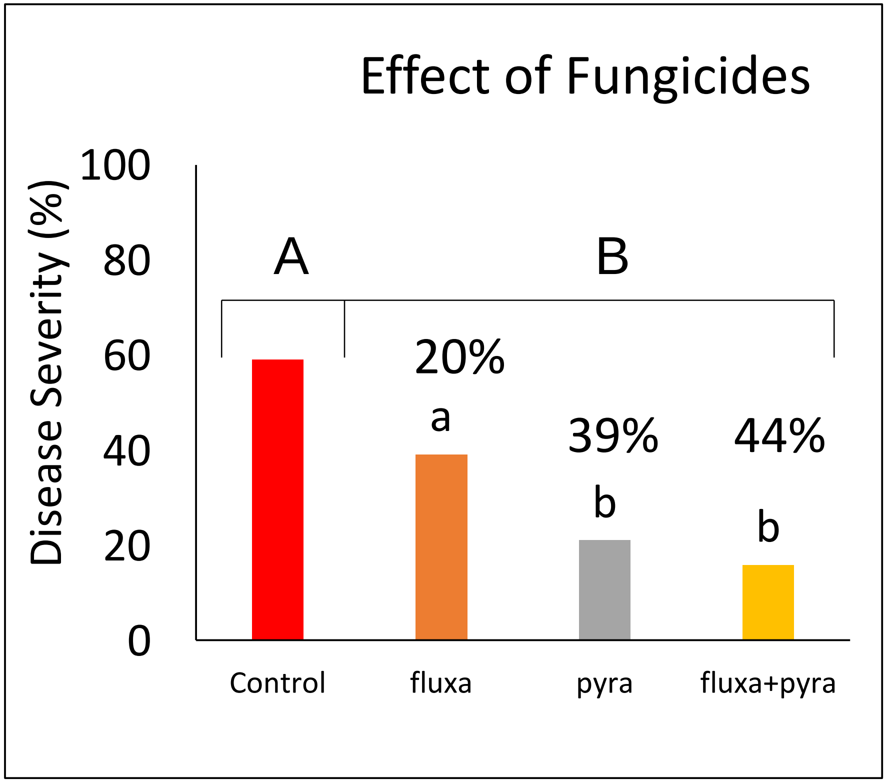
Early flowering stage

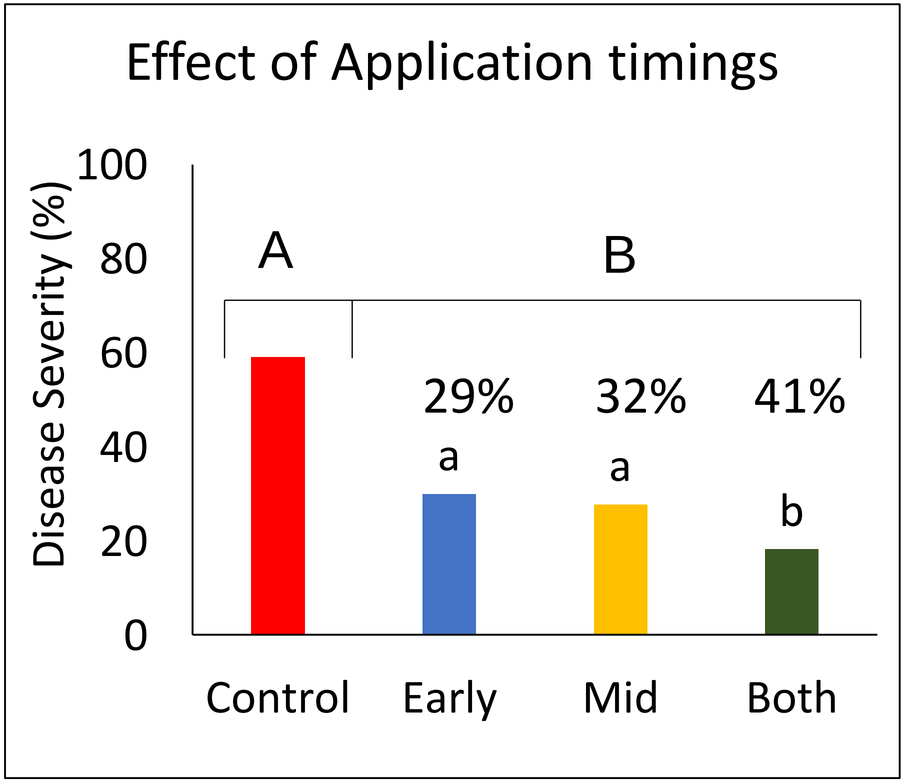
Mid flowering stage

T. Islam

Islam, T., Vera C., Slaski J., Mohr, R., Rashid, K.Y., Booker, H., & Kutcher H.R., 2020. Plant Disease 105(6): 1677-1684 http://doi.org/10.1094/PDIS-06-20-1175-RE

Fungicides and application timing effects on pasmo severity (~60% of site-years)





Upper case letters indicate significant difference between the unsprayed control and the treatments and lower case letters indicate significant differences among the treatments ($P < 0.05$), using Tukey's post hoc test

Conclusions

- Fungicide application decreased disease severity and increased seed yield over the control at ~60% of the site-years.

- Pyraclostrobin (Headline®) and pyra + fluxapyroxad (Priaxor®) fungicides effectively controlled pasmo and improved yield; however, when fungicide resistance is considered, application of pyra + fluxa would be the best option

- Little difference between fungicide application timings

DISEASE MANAGEMENT

Fungicide timing for wheat leaf disease and FHB

A single fungicide application at the anthesis stage may produce the best results

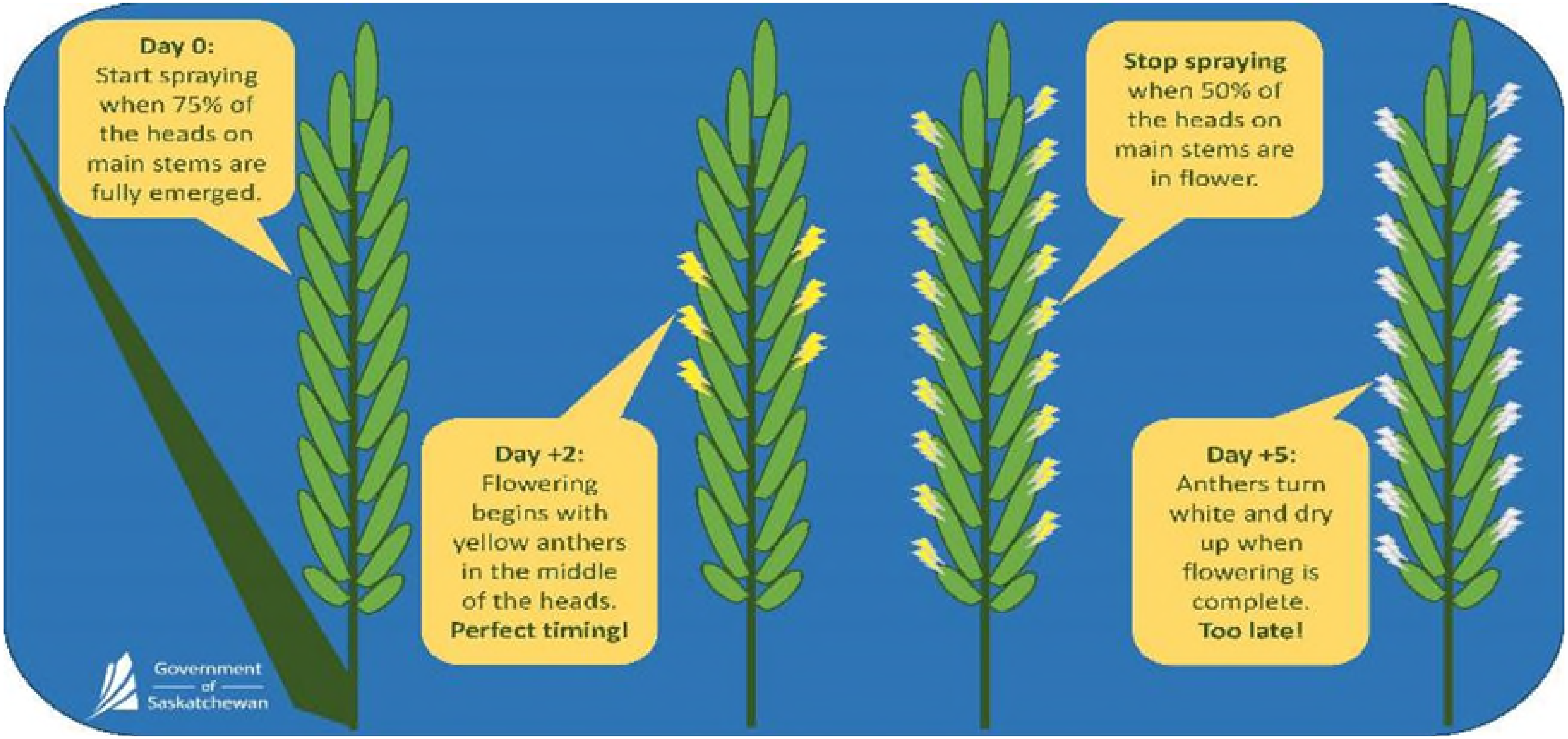
By Mark Halsall

Prairie grain producers always keep close tabs on the weather during the growing season to see if conditions turn favourable for disease development in their crops.

If that happens, there may not be a simple answer to the question of whether to spray or not. And much of that has to do with timing.

"The first question growers usually ask is should I spray?" says Randy Kutcher, a plant scientist at the University of Saskatchewan. "The second question is, 'I've decided to spray — what's the best timing?' Those are the two main questions you get with any fungicide application in field crops."

Application timing: anthesis for FHB



BBCH 59 **BBCH 61** **BBCH 65** **BBCH 69**

BBCH growth stage scale; Lancashire et al. 1991, Ann. Appl. Biol. 119: 561-601.

http://www.saskwheatcommission.com/producer-info/fusarium-risk-assessment-map/

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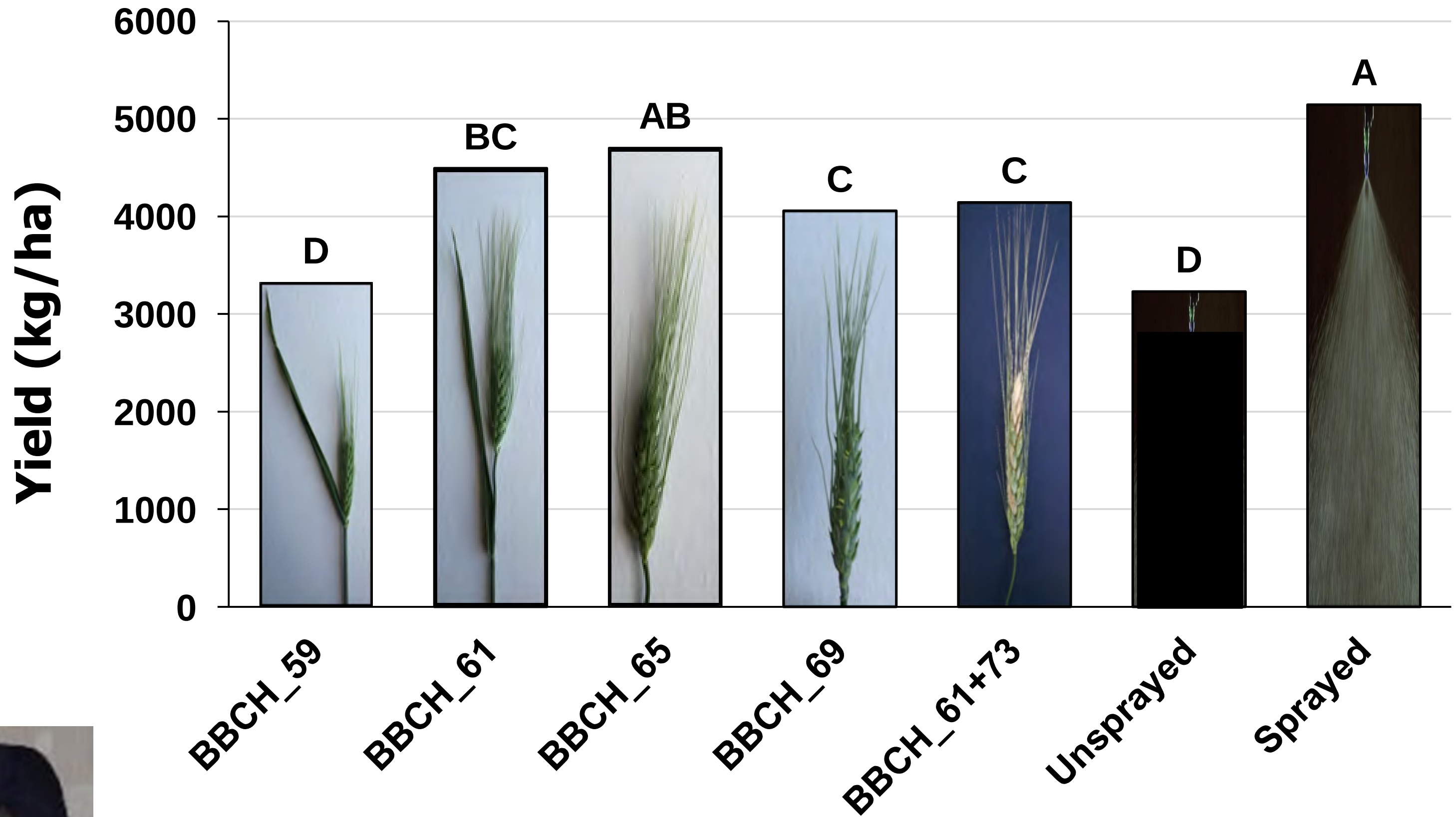
Fungicide timing effect on yield



LSD$_{(0.05)}$

Saskatoon & Outlook 2016

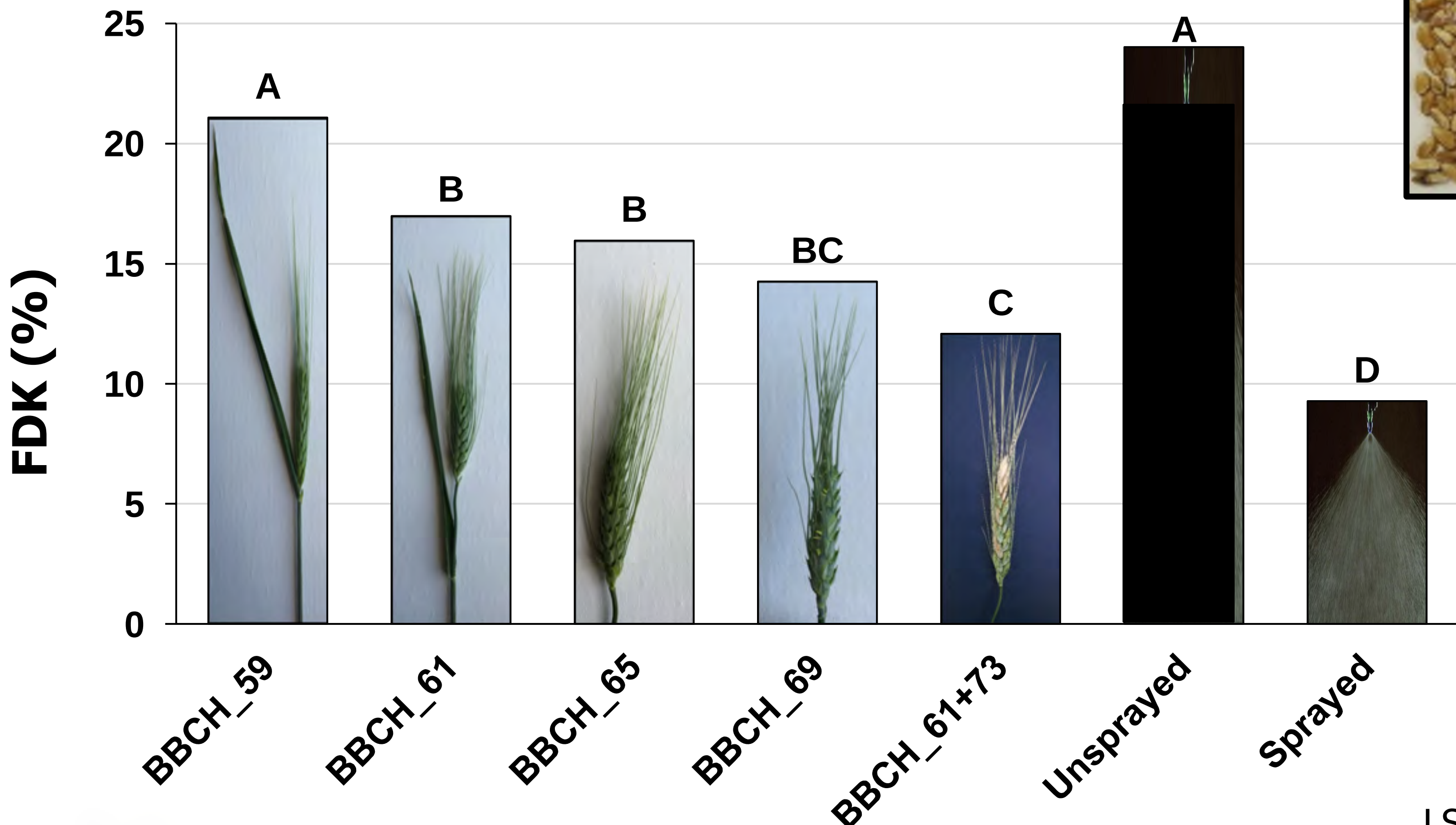
Gursahib Singh

Photo credits: G. Singh and Sprayers 101

www.usask.ca

Singh, G., Hnatowich G., Peng G., & Kutcher H.R., 2021.
Plant Disease 105(11):3481-3489. https://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS-02-21-0335-RE

Fungicide timing effect on FDK

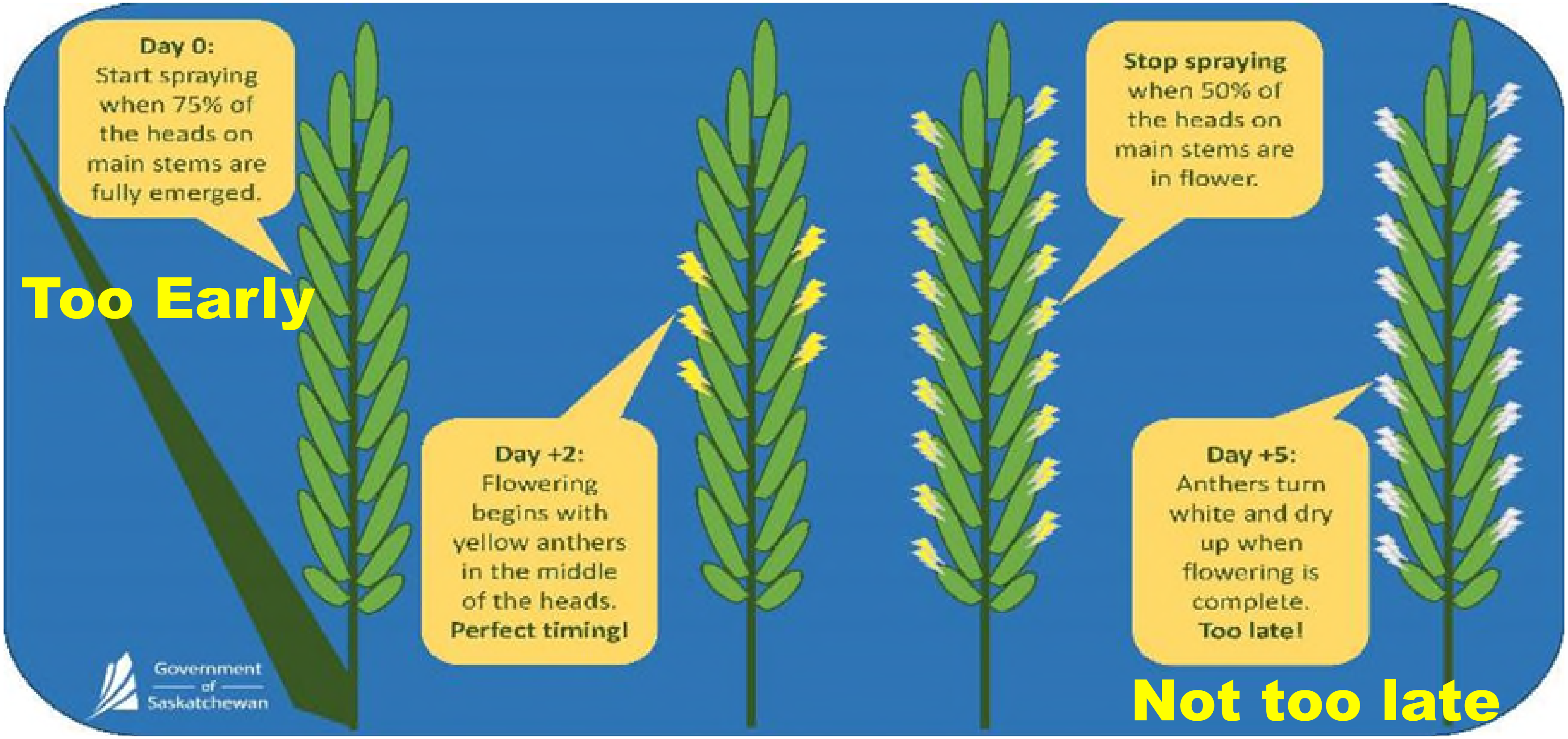


$LSD_{(0.05)}$

Saskatoon & Outlook 2016

Photo credits: G. Singh and Sprayers 101

Application timing: anthesis for FHB



BBCH 59 | BBCH 61 | BBCH 65 | BBCH 69

BBCH growth stage scale; Lancashire et al. 1991, Ann. Appl. Biol. 119: 561-601.

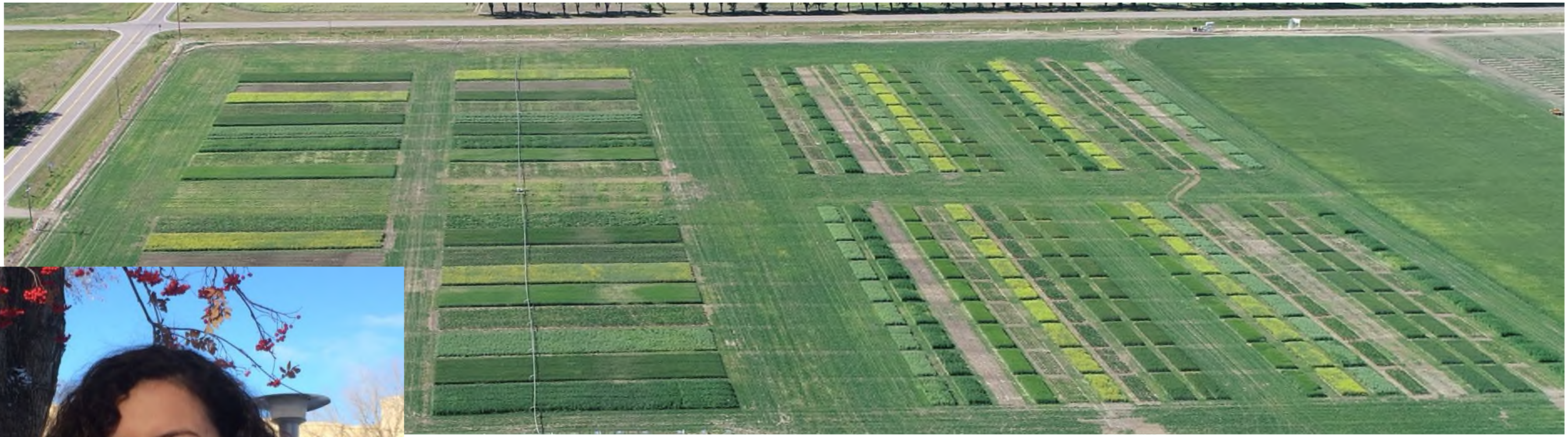
http://www.saskwheatcommission.com/producer-info/fusarium-risk-assessment-map/

FHB fungicide timing

- Fungicide timing to mitigate FHB in cereal crops and temperature effects on chemotypes – Anya Illingworth (current MSc student)

Crop sequence study – cultural FHB control

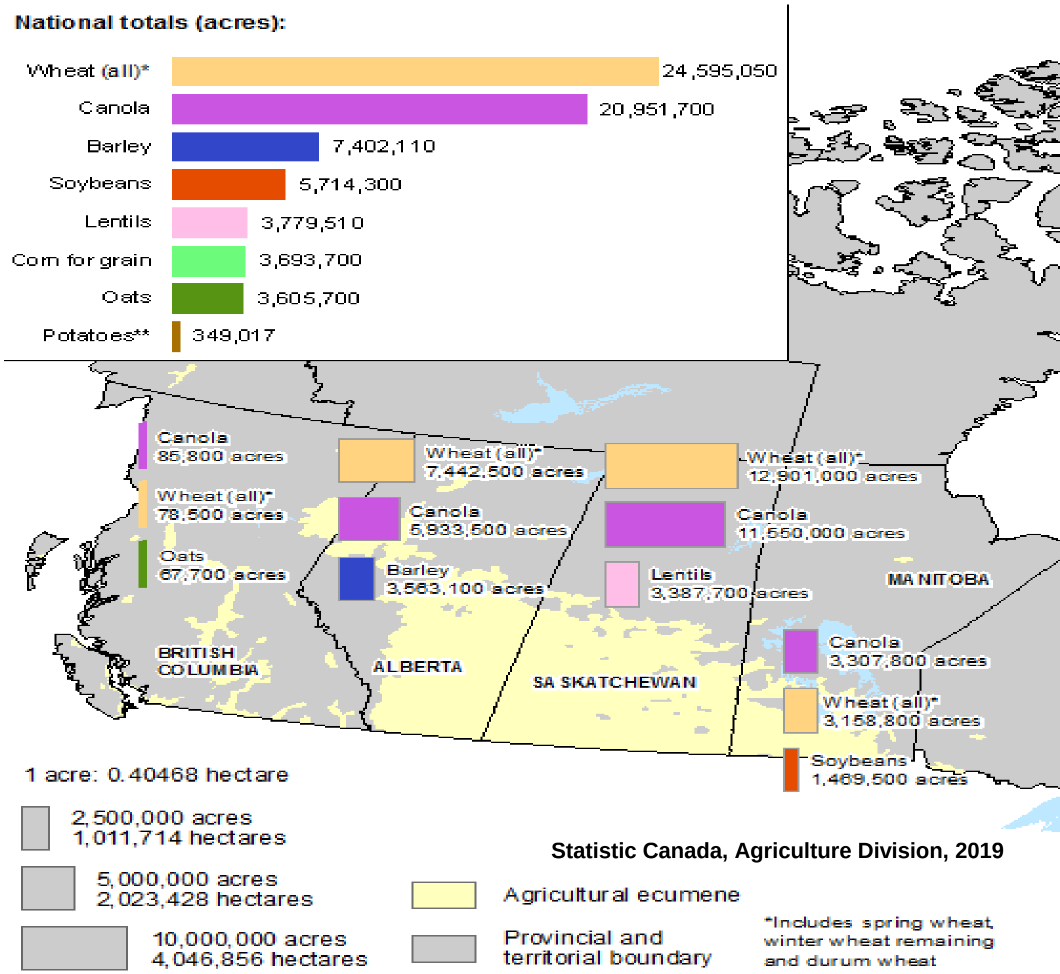
- study focused on FHB management in wheat and barley; five collaborators across MB, SK and AB – Alejandra Oviedo (MSc & now RA)



Alejandra Oviedo Ludena

How diverse are crop rotations in western Canada?

- Main field crops in (western) Canada:
- Rotations tend to be intensive, e.g. wheat-canola, durum-lentil
- low crop diversity increases cereal disease risks



Statistic Canada, Agriculture Division, 2019

University of Saskatchewan

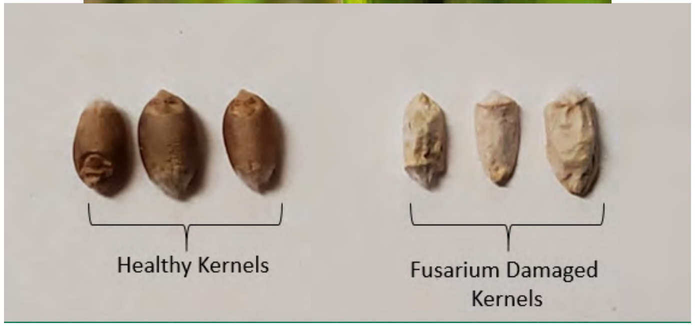
To best manage FHB in durum follow an integrated pest management program

- An integrated approach is paramount to manage FHB; economically beneficial and stable across environments,
- Follow a diverse crop rotation (minimum of 3 crops) and use resistant cultivars (when available),
- Timely fungicide application with increased seeding rate improves FHB suppression and facilitates crop staging.
- **Combining a diverse rotation with resistant cultivars and fungicide (when warranted) is more efficacious than any single approach.**

Fusarium head blight

- Varietal resistance is a key component of IPM

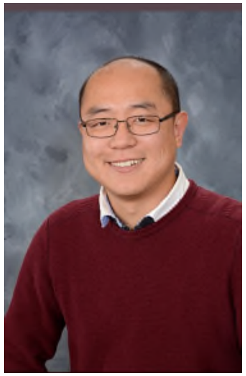
- Sources of resistance are needed to keep varieties high yielding and resistant



FHB evaluation of PGRC accessions

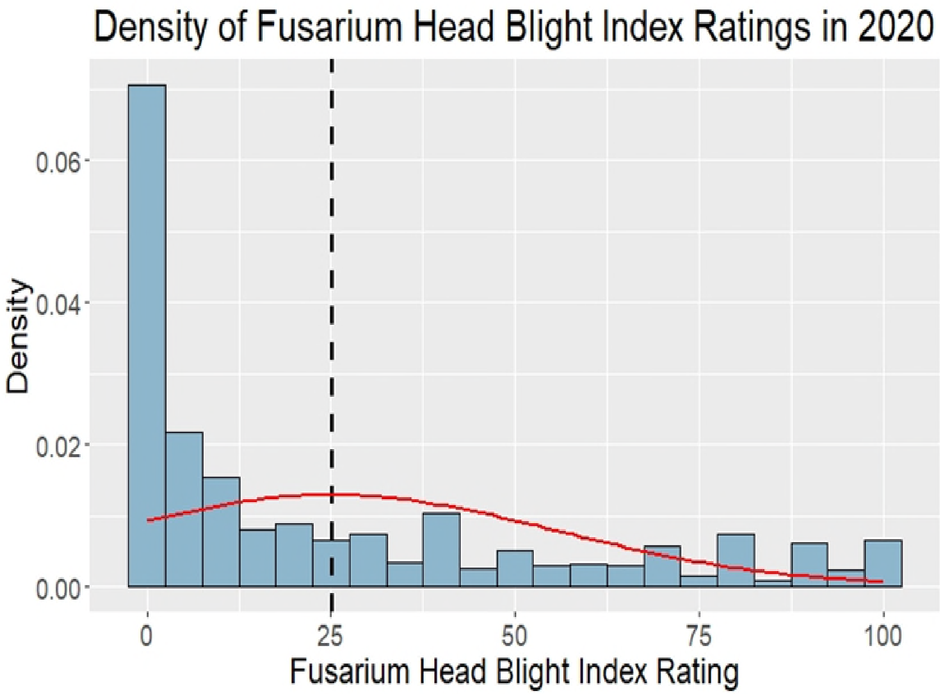
>10,000 PGRC bread wheat accessions screened → **4,000 accessions evaluated in the field for 5 year** → **400 accessions with high resistance selected for genetic evaluation** → **200 PGRC accessions chosen for greenhouse screening**

Mackenzie Hladun

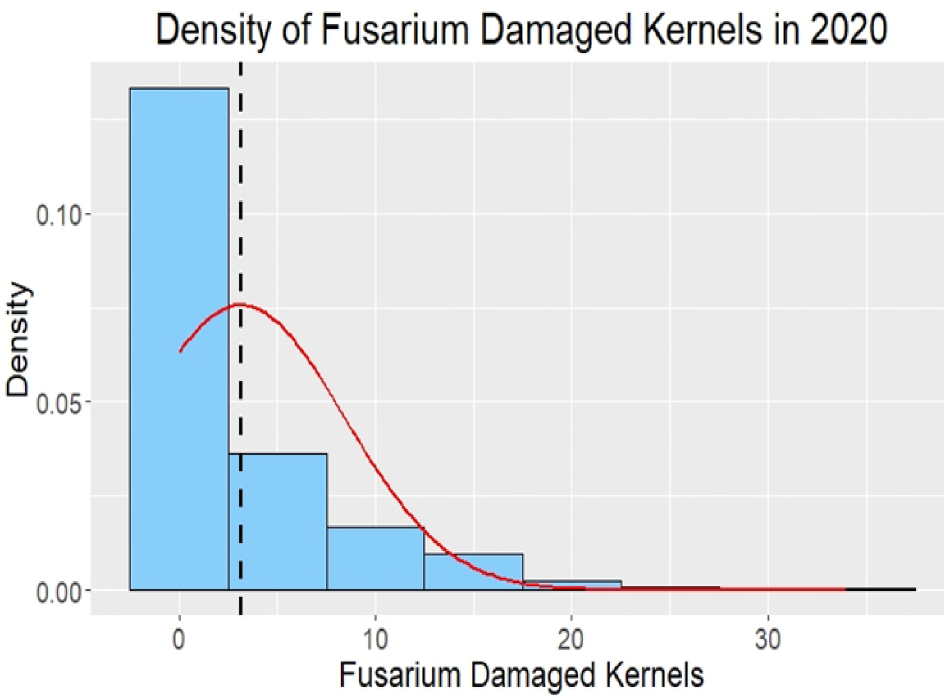


Dr. Lipu Wang

Dr. Luis Ponce



Density of Fusarium Head Blight Index Ratings in 2020
Density
0.06
0.04
0.02
0.00
0
25
50
75
100
Fusarium Head Blight Index Rating



Density of Fusarium Damaged Kernels in 2020
Density
0.10
0.05
0.00
0
10
20
30
Fusarium Damaged Kernels

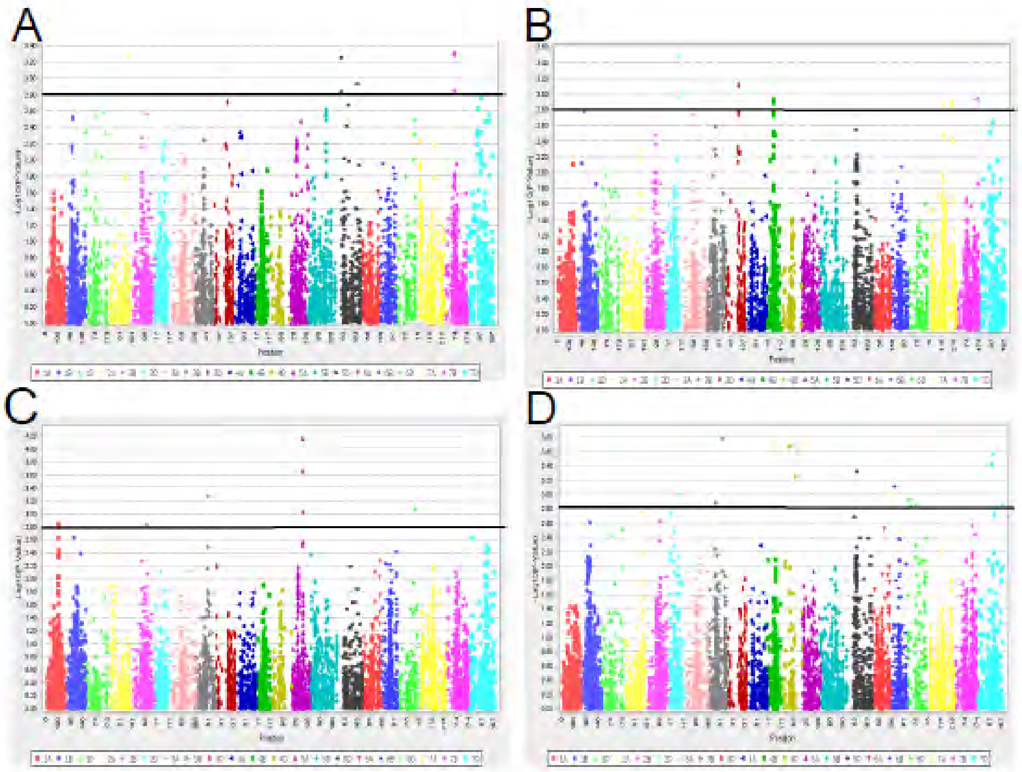
Physical Data

- **FHB Index**
- **FDK**

GWAS Pipeline

Genetic Data

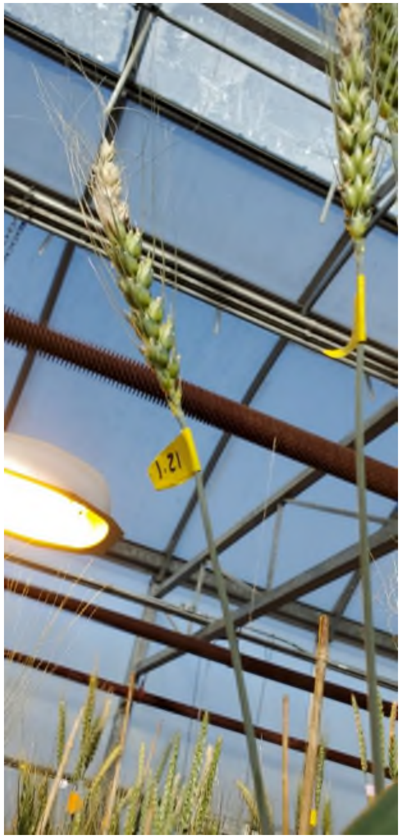
- **90K Infinium SNPs**



Wang, 2019 Soils and Crops Conference Poster

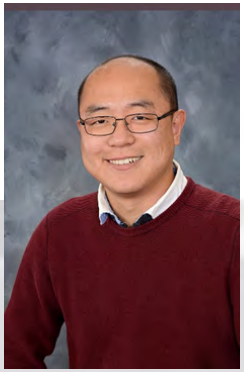
Searching the gene bank

Four PGRC accessions identified as potential sources of FHB resistance out of 400 accessions evaluated



Fusarium spp. produce mycotoxins

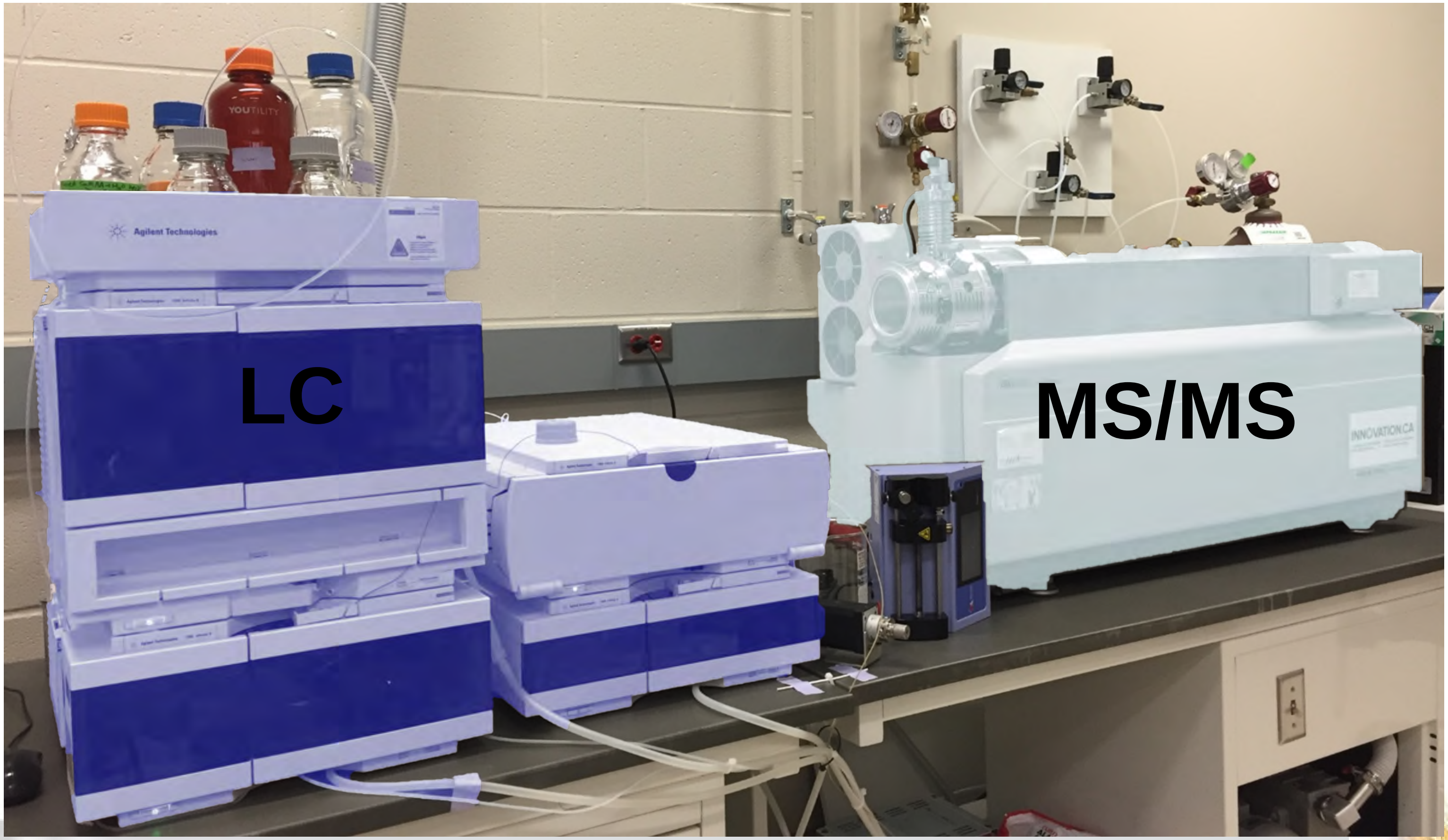
- Harmful to humans and livestock, reduced grain quality and price, poor-quality food products (e.g., bread and beer)
- Regulations on the maximum acceptable mycotoxin level
- Development of a mycotoxin quantification platform to support FHB research and breeding programs



Dr. Lipu Wang

Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS)

The Core Mass Spectrometry Facility, College of Pharmacy and Nutrition, USask



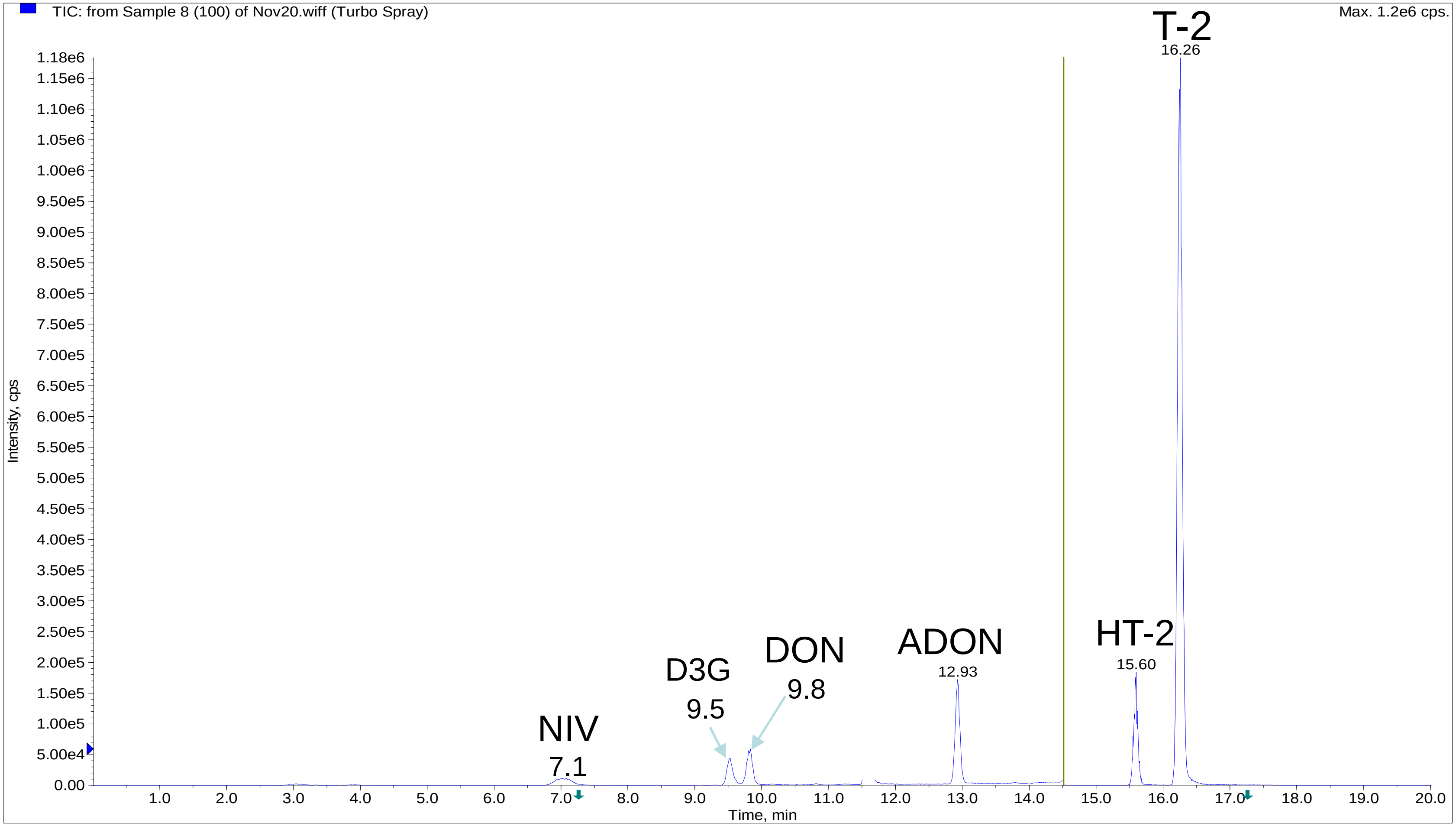
High-throughput DON phenotyping assay

small amount of sample request	100 mg of any tissue from plant
Simple extraction	One-step extraction
Fast test	Shorten the analysis time from 20 min to 2 min per sample
High throughput	96 samples per run and 4 runs per day (24 hours)
High precision and accuracy	The CV of all QCs < 12% and the accuracy > 86%
High sensitivity	The Lowest Limit of Quantification at 3.12 ppb
High extraction recovery **Less matrix Effect**	ER of QCs > 85% and ME of QCs >96%
Low cost	Competitive price than ELISA

Validated to US Food and Drug Administration (FDA) Guidelines for Bioanalytical Methods

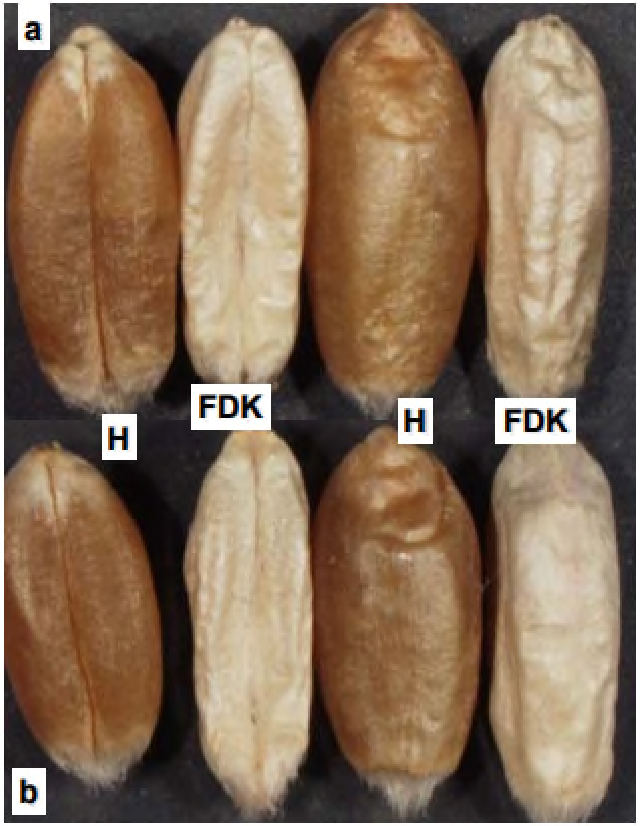
Wang, L., Michel D., Zhang W., El-Aneed A., Fobert P.R., Ruan Y., Berraies S., Cuthbert R., & Kutcher H.R. 2022. Phytofrontiers Online: https://doi.org/10.1094/PHYTOFR-03-22-0024-TA

Multiple mycotoxins quantification assay

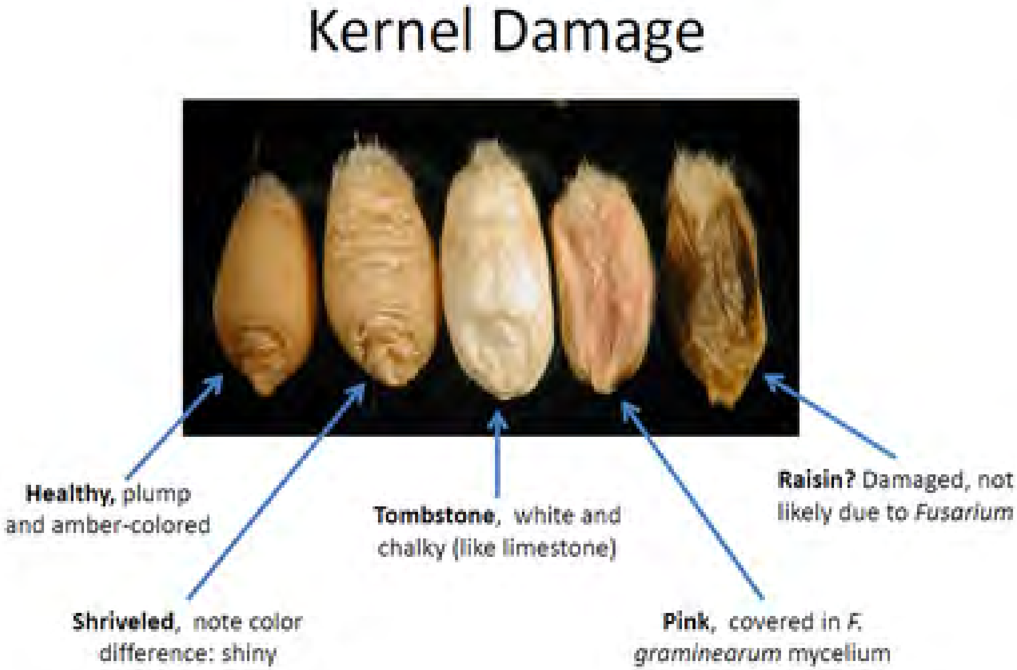


LC-ESI-MS/MS TIC of a wheat sample spiked with 7 mycotoxins at a concentration level of 100 ng/mL.

FDK an important characteristic of FHB



Canadian Grain Comission

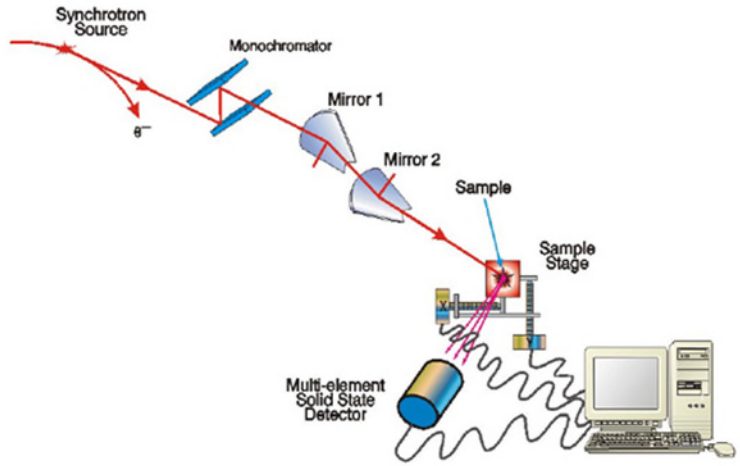


Objective

- New techniques for FDK assessment would be of benefit to wheat breeders and the industry in general.
- FHB resistance is a polygenic trait; breeding requires analysis of large numbers of samples (phenotyping)
- Automatic methods based on computer vision techniques and high-throughput phenotyping provide a promising path to overcome this challenge

Two imaging technologies

1. Synchrotron X-ray image



https://www.lightsource.ca/

2. Portable RGB imaging system (BELT)

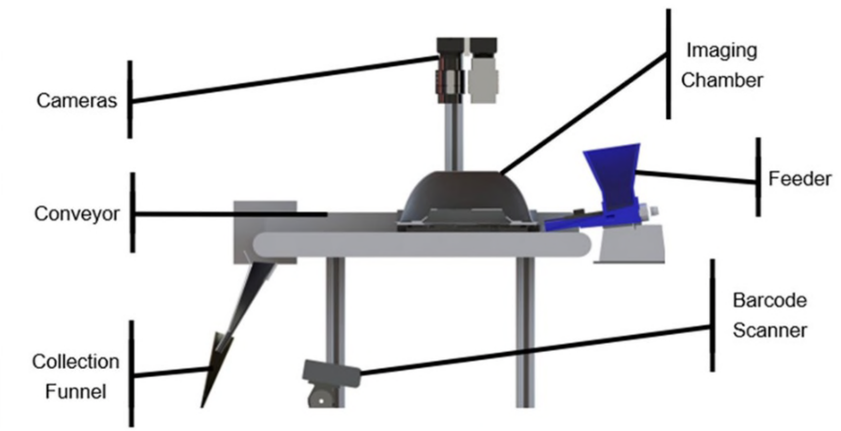
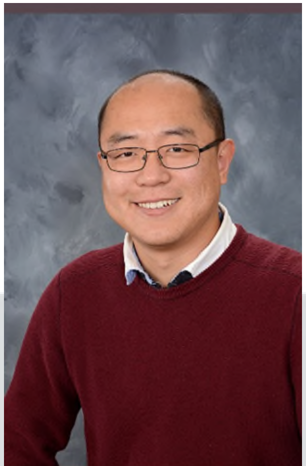


Fig. 1 BELT Cross section render of the physical BELT system

Halcro et al., 2020

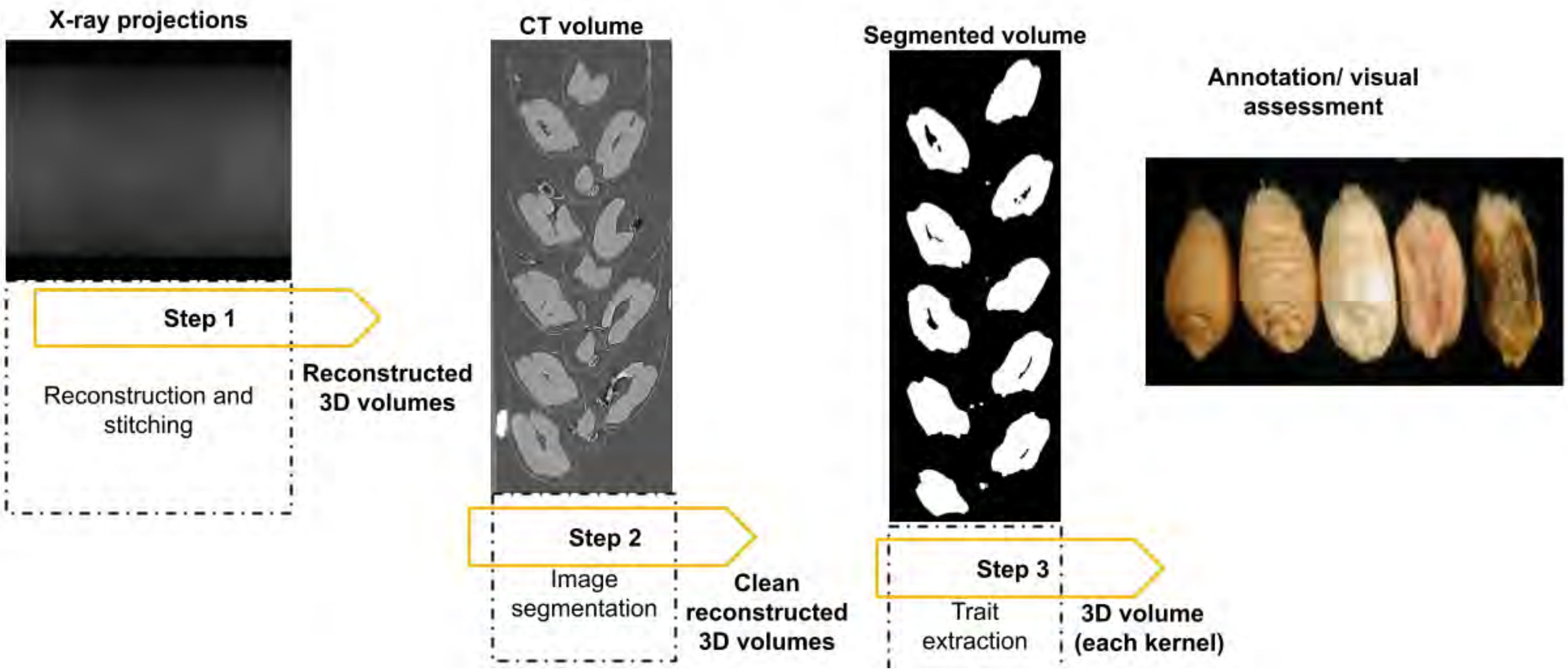
Sheila Andrade



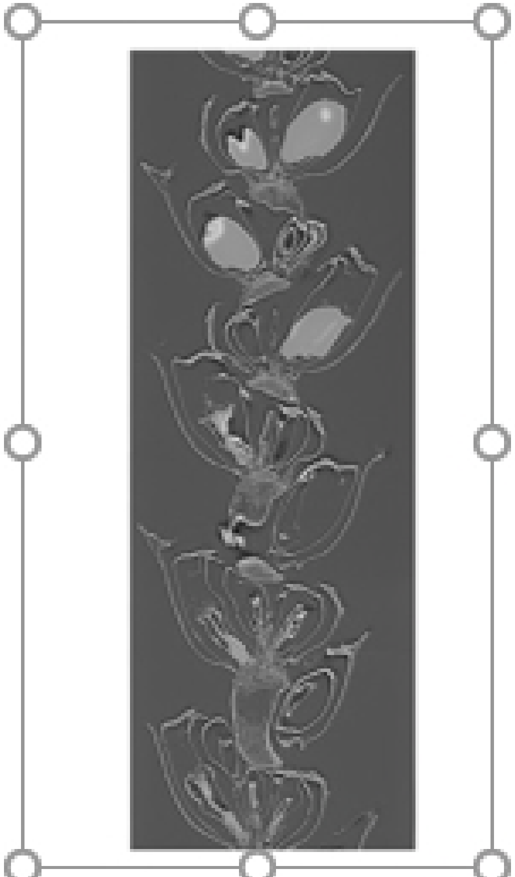
Dr. Lipu Wang

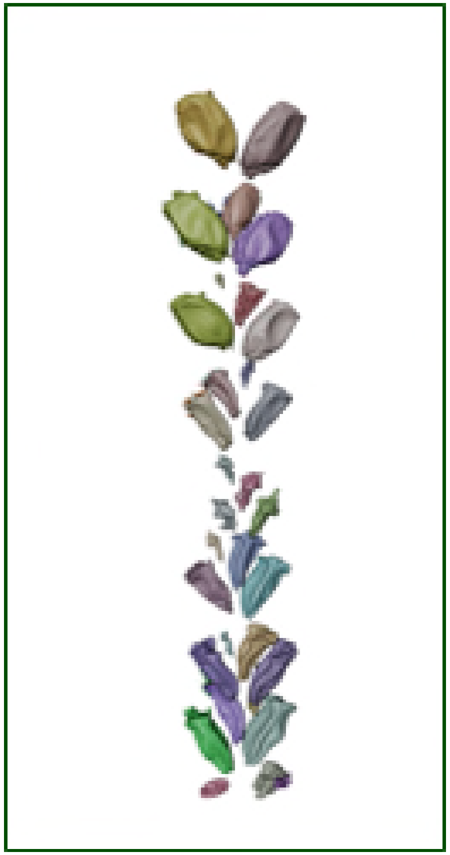
Logan Orenchuk

Sheila's PhD research

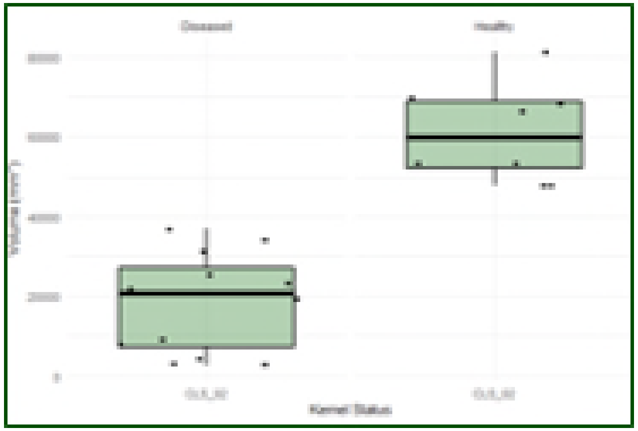


Sample 02- Healthy and Diseased kernels





Mean (mm^3)	
Healthy	61067.65
Diseased	18324.45



Bacterial leaf streak

- Caused by *Xanthomonas translucens*
- Rod-shaped, gram-negative bacteria
- Different pathovars affect cereal hosts
  - *X. translucens* pv. *undulosa*
  - *X. translucens* pv. *translucens*
- Bacterial leaf streak when on the leaves
- Black chaff when on the glumes

Courtesy Dr. Michael Harding

Photo: Tyler Mays, Texas AgrilLife

BLS is not a new disease to the prairies

Ibra Lockwood Conners (1894–1989)

Survey of the

REVALENCE OF COMMON PLANT DISEASES

in the

DOMINION OF CANADA

1920

First Annual Report

Division of Botany
Experimental Farms Branch
Department of Agriculture.

Minor diseases of Barley

"Bacterial blight (Ps.avenae) and Helminthosporium were common north of Sudbury, Ontario. Considerable loss in some fields where infection ran from 5. to 25%." – Faull.

"Bacterium translucens was collected at Winnipeg; but search at other points of the province did not reveal any of the disease". Connors.

BLS in wheat

- Phenotypic screening methods and a diagnostic seed test to assess bacterial leaf streak in Canadian wheat germplasm – Constanza Fleitas, Research Officer, and new MSc student, Valentina Anastasini

Valentina Anastasini

Constanza Fleitas

Other crops and diseases

- Canaryseed and leaf mottle, Fusarium seed infection – Paulina Cholango Martinez (MSc)

- Oat and crown rust
- Biocontrol products for oat diseases, FHB in wheat
- Intercropping flax and chickpea; flax wilt – Pree Edirisinghe (MSc)

Teaching and tech transfer

- PLSC 335 Field Crop Disease Management
- Active tech transfer program:
  e.g. Ministry Diagnostic School;
  SaskWheat – 'Think Wheat' grower meetings

  Media articles: Grainews, Top Crop Manager, Canadian Agronomist, Germination Magazine, GrainsWest, SaskBarley

UNIVERSITY OF SASKATCHEWAN

Current graduate students

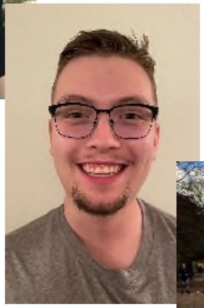
Kun Lou – MSc, stripe rust of wheat

Mackenzie Hladun – MSc, FHB of wheat

Anya Illingworth – MSc, FHB of multiple cereals

Sheila Andrade – PhD, FHB of wheat

Logan Orenchuk – MSc, FHB of wheat



Valentina Anastasini – MSc, BLS of wheat

Completed graduate students
(2015 – present)

Gurcharn Brar (MSc and PhD)
Pree Edirisinghe (MSc)
Wali Soomro (MSc)
Paulina Cholango Martinez (MSc)
Dustin MacLean (MSc)
Trisha Islam (MSc)
Keiko Nabetani (MSc)
Gursahib Singh (PhD)
Tatiana Vera (MSc)
Alejandra Oviedo Ludena (MSc)









Acknowledgements

Saskatchewan Ministry of Agriculture

University of Saskatchewan

Agriculture and Agri-Food Canada

Western Grains Research Foundation

Sask Wheat Development Commission

Canadian Agricultural Partnership
Innovate. Grow. Prosper.

SaskBarley Development Commission

Alberta Wheat Commission

NSERC CRSNG

SaskFlax
Saskatchewan Flax Development Commission

Manitoba Crop Alliance

Canary Seed Development Commission of Saskatchewan

Brewing and Malting Barley Research Institute

Growing Forward 2
A federal-provincial-territorial initiative

30 Goals for 2030

- 1.4 million people living in Saskatchewan.
- 100,000 new jobs.
- Grow private capital investment in Saskatchewan to $16 billion annually.
- Increase the value of exports by 50 per cent.
- Grow the number of international markets to which Saskatchewan exports more than $1 billion.
- Grow Saskatchewan's agri-food exports to $20 billion.
- Increase crop production to 45 million metric tonnes and livestock cash receipts to $3 billion.
- Expand irrigation in Saskatchewan.
- Increase agriculture value-added revenue to $10 billion.
- Crush 75 per cent of the canola Saskatchewan produces in Saskatchewan.
- Process 50 per cent of the pulse crops Saskatchewan produces in Saskatchewan.
- Double meat processing and animal feed value-added revenue to more than $1 billion.
- Increase oil production by 25 per cent to 600,000 barrels per day.
- Increase the annual value of uranium sales to $2 billion.
- Increase the annual value of potash sales to $9 billion.
- Double the growth of Saskatchewan's forestry sector.
- Grow Indigenous participation in Saskatchewan's natural resource industries.
- Triple the growth of Saskatchewan's technology sector.
- Increase the value of Saskatchewan manufacturing exports by 50 per cent.
- Increase tourist expenditures in Saskatchewan by 50 per cent.
- Enhance oil recovery, carbon capture utilization and storage and position Saskatchewan as the best place in North America to test, commercialize and scale new oil and gas technologies.
- Invest $30 billion in infrastructure over the next decade.
- Build and upgrade 10,000 kilometres of highways.
- Expand Saskatchewan's export infrastructure.
- Keep the budget balanced.
- Keep Saskatchewan's debt-to-economic growth (GDP) ratio within the top three in Canada.
- Deliver on Saskatchewan's climate change strategy, *Prairie Resilience*.
- Advance development of zero-emission small modular reactor technology.
- Support communities through $2.5 billion in revenue sharing.
- Reduce surgical wait times to a three-month target.

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