Agriculture and Agri-Food Canada    Agriculture et Agroalimentaire Canada

# Aerobiological Surveillance of Wheat Pathogens

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Red Deer, Alberta  January 10, 2018

Canada

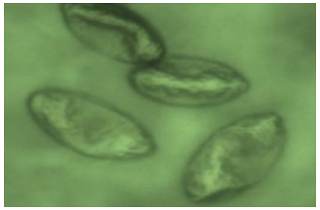
# Presentation Outline

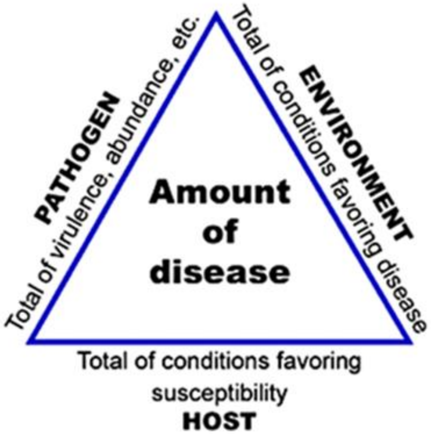
- Wheat pathogen surveillance tools
- Collection of samples
- Detection of samples
- Disease Modeling
- Future prospects

***Puccinia striiformis f.sp. tritici* (*Pst*) causal agent of stripe (yellow) rust**

# Introduction

- **<u>Host-pathogen interaction</u>**
  - Not all interactions are successful
  - Pathogens can cause mild to severe damage
  - Disease Triangle Concept



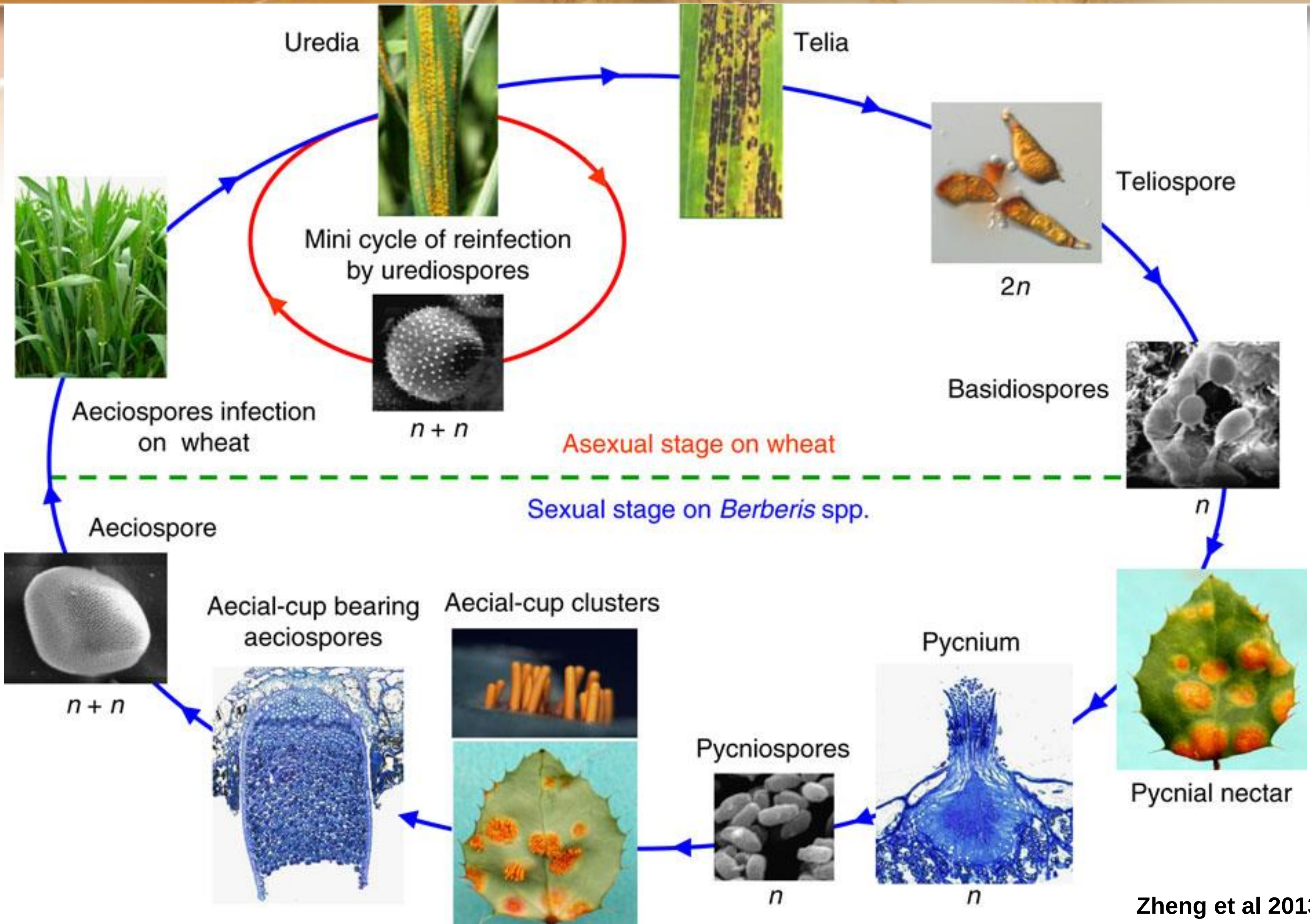



Credits: https://masters.agron.iastate.edu

Credits: http://www.outdoorphotographycanada.com

Credits: http://www.watermarkchurch.hk

# *Puccinia striiformis f.sp. tritici* life cycle




Zheng et al 2013

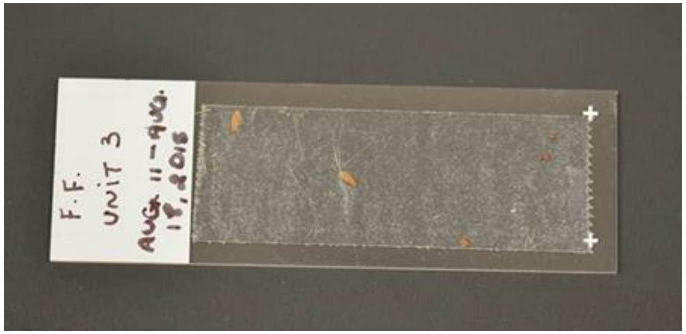
# Wheat pathogen surveillance tools

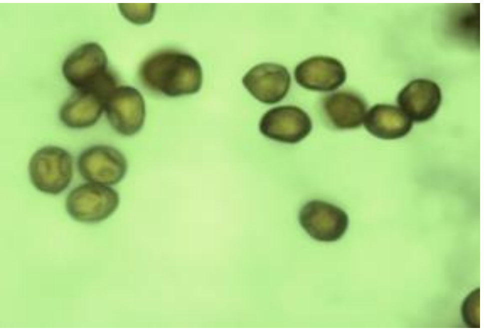
- Currently, we have to wait for symptoms on susceptible lines of wheat

# Wheat pathogen surveillance tools

- We are using sticky slides to detect presence of potential airborne pathogens.

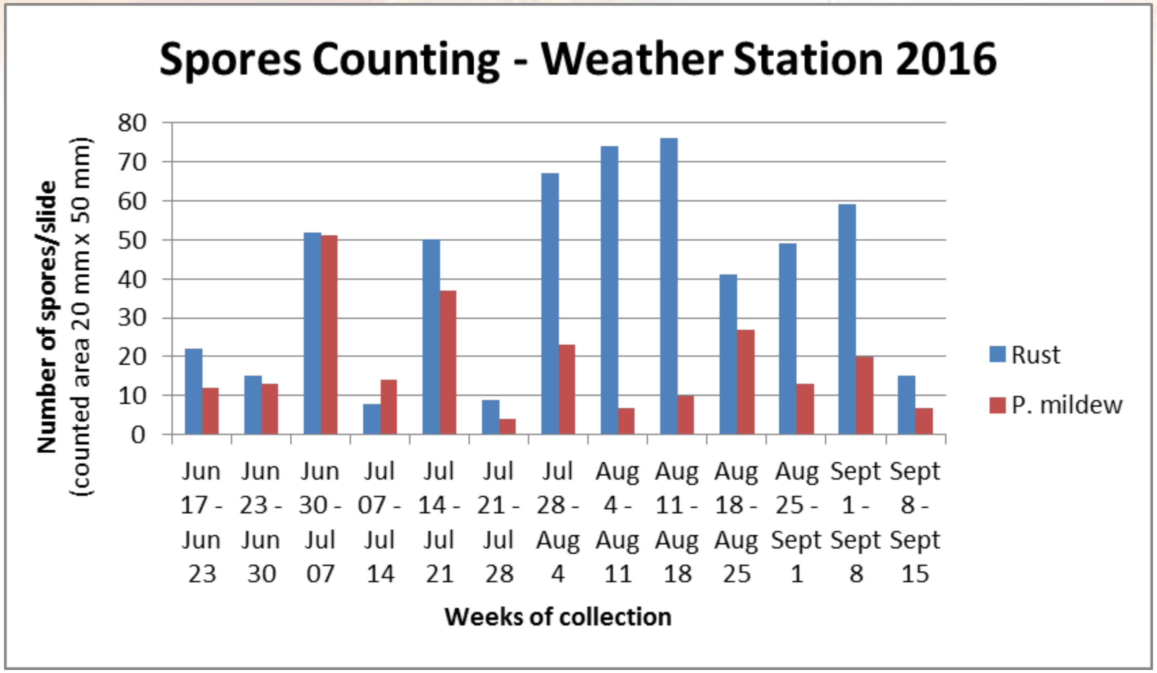
**Adhesive tape**





rust spores – 400X

# Microscopy analyses



# Wheat pathogen surveillance tools

- **We are also using spore collectors to detect presence of potential airborne pathogens.**

**Burkard Cyclone**

# Location of spore collector units in southern Alberta


* 2016 & 2017

¥ 2016

¤ 2017

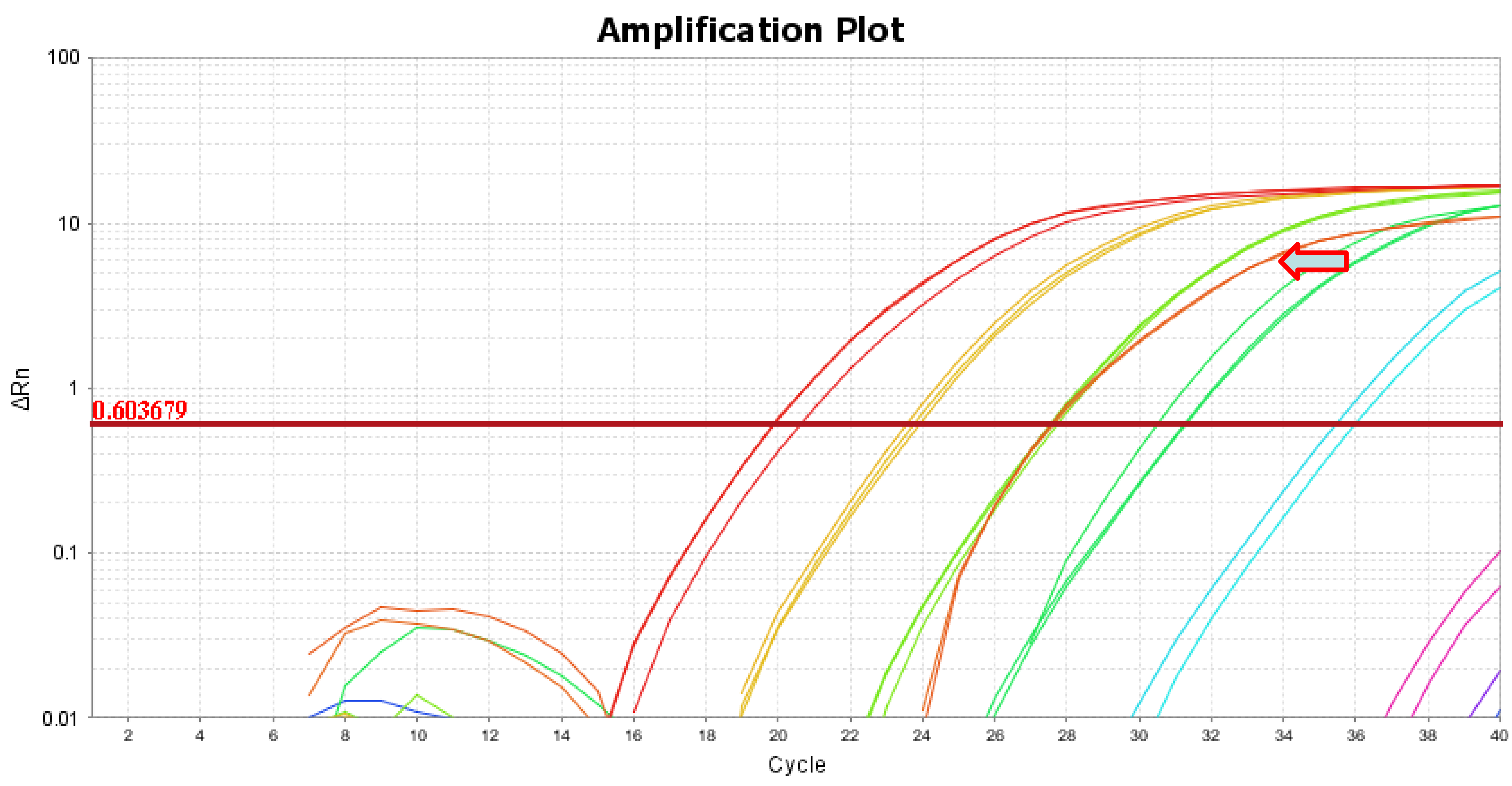
# Detection of samples

- DNA level
  - Different standard approaches enable identification at species level, very sensitive and highly specific
- Immunological level
  - Identification at species level, very sensitive, highly specific and rapid

# Detection of samples

- DNA level
  - Need to break the spore to release the DNA and then detect the presence of a given pathogen based on a unique DNA sequence.
  - DNeasy PowerSoil DNA Isolation Kit (Qiagen) a 2 step protocol that first breaks the spores open and then yield a fraction of DNA that can be used to detect pathogens.
  - Identified and validated unique DNA sequences for each species
  - qPCR to detect and quantify pathogens.

# qPCR results (stripe rust target)



# Pathogen-specific primer sets

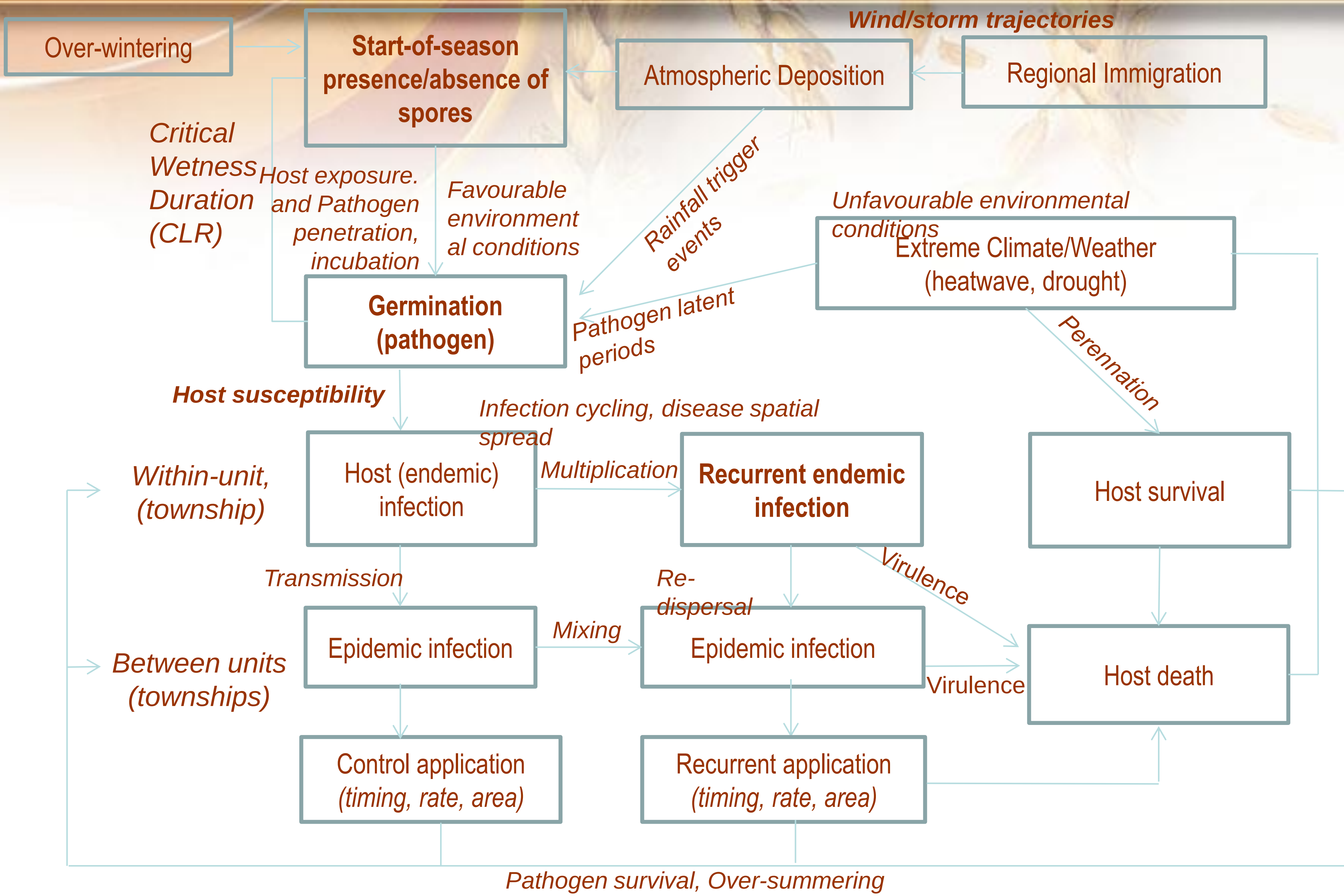
- The seven best PCR primer sets tested

| Primer set (F/R) | Pathogens | Diseases | Detection level |
|---|---|---|---|
| Pg2 | *Puccinia graminis f. sp. tritici* | Stem rust | 0.2 pg - 16 spores |
| PST | *Puccinia striiformis f. sp. tritici* | Stripe rust | 0.3 pg - 25 spores |
| Pt2-032/ Pt1-113#2 | *Puccinia triticina f. sp. tritici* | Leaf rust | 0.5 pg - 20 spores |
| Bgt -6 | *Blumeria graminis f. sp. tritici* | Powdery mildew | 2.0 pg - 307 spores |
| Tox A1/ Tox A2 | *Pyrenophora tritici-repentis* | Tan spot | 0.1 pg - 7 spores |
| Fgram B | *Fusarium graminearum* | Fusarium head blight | 0.2 pg - 8400 spores |
| ITS1/ITS4 | Universal primer | All fungal samples | ------ |

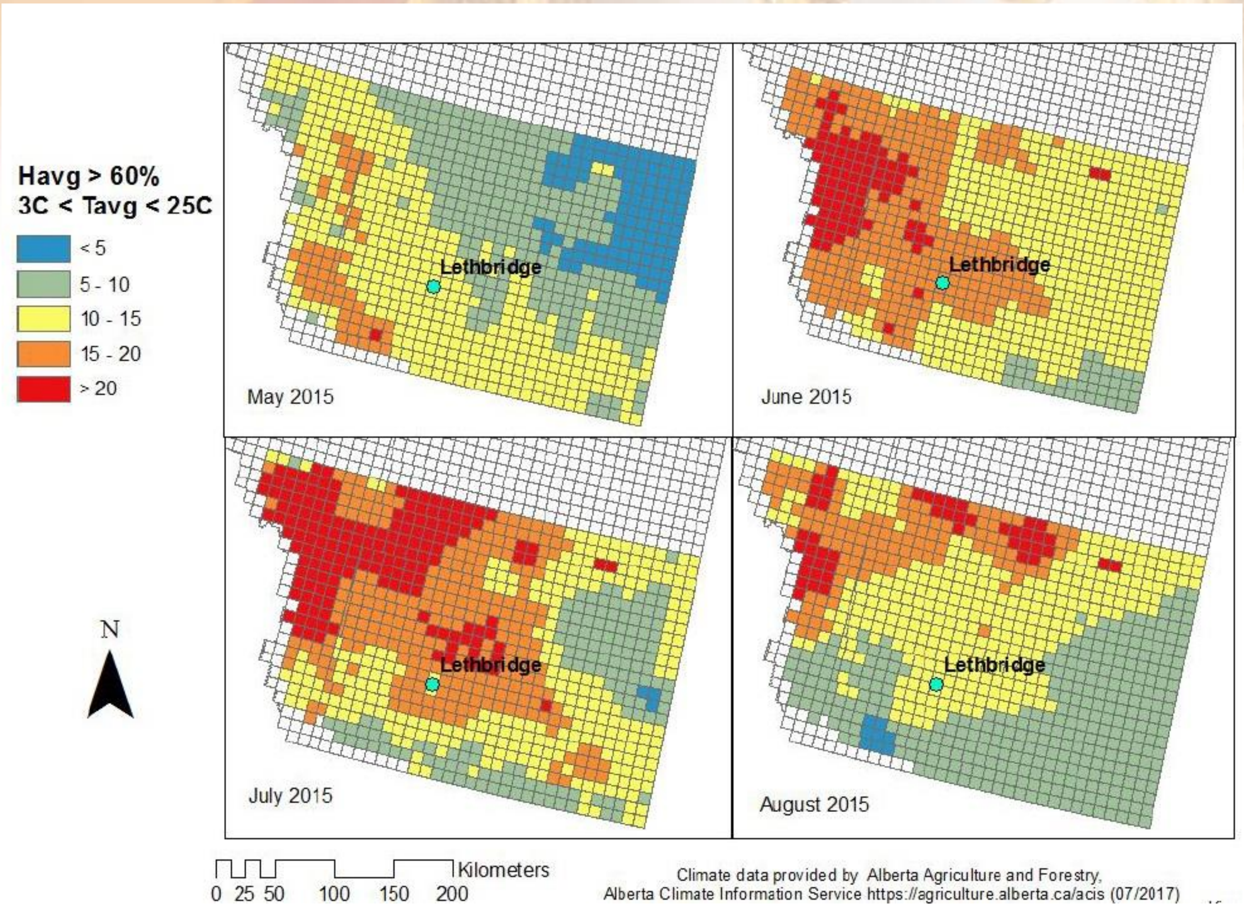
# Summary

- We can reliably detect 6 wheat pathogens at a very low detection level: stripe rust, stem rust, leaf rust, powdery mildew, tan spot and Fusarium head blight
- The assay is rapid and would covers the most prevalent diseases of wheat on the Canadian Prairies
- Detection level well below minimum number of spores needed for successful infection at field level.

# Disease modeling and forecasting framework



# Potential wheat rust disease occurrence based on temperature and humidity empirical thresholds (Alberta municipalities, 2015)




***municipality-interpolated high-resolution climate data from Alberta Climate Information Service (ACIS), https://agriculture.alberta.ca/acis/**

# Spatial endemic epidemic multivariate time-series model (HHH4)

## Assumptions:

- Negative binomial model
- Constant population
- Districts have the same seasonality effect at time t
- Autoregressive and epidemic effects are homogeneous across districts, constant in time
- Spores only spread from the side neighboured districts

Basic model (without covariate spatial random effects):

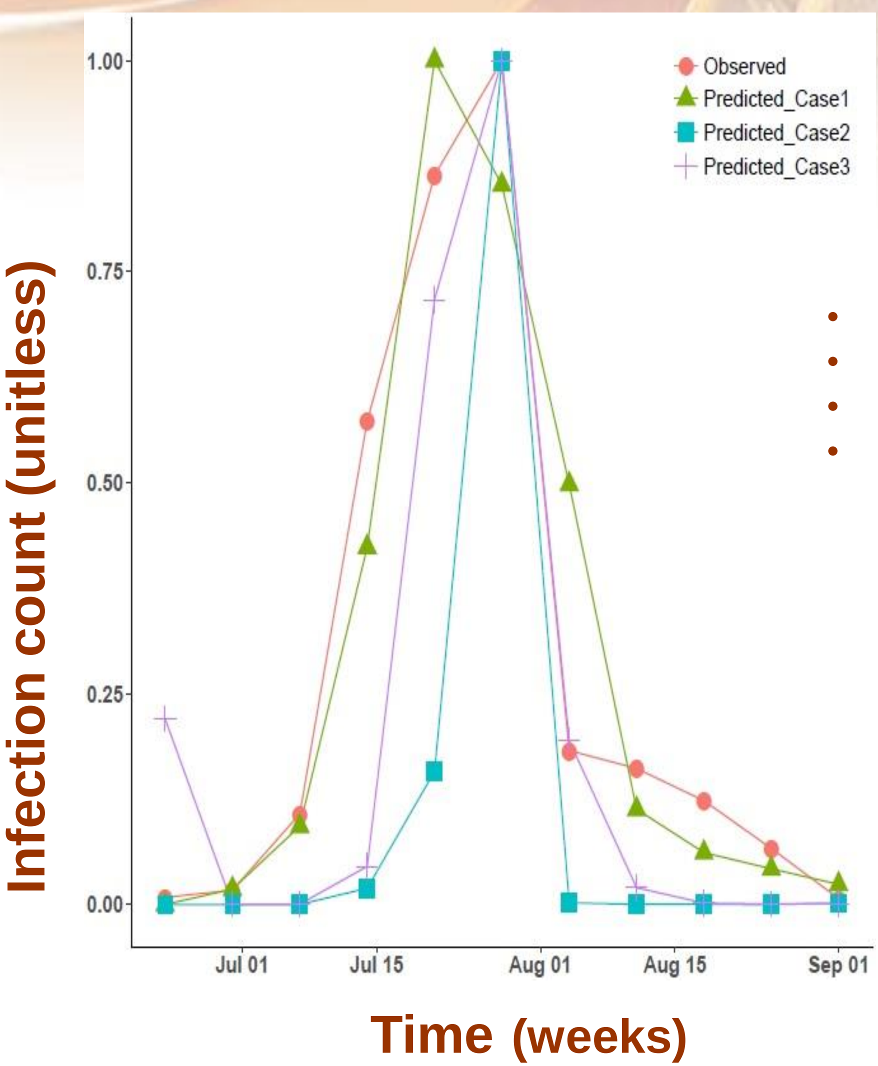
$$\mu_{it} = \underbrace{e_i \nu_t}_{\text{endemic}} + \underbrace{\lambda Y_{i,t-1}}_{\text{autoregressive}} + \underbrace{\phi \sum_{j-\neq i} w_{ji} Y_{j,t-1}}_{\text{epidemic}}$$

$$log(\nu_t) = \underbrace{\alpha^{(\nu)}}_{\text{inital states}} + \underbrace{\beta_t t}_{\text{gradient}} + \underbrace{\gamma sin(\omega t) + \delta cos(\omega t)}_{\text{seasonal trend}}$$

Where

- $e_i$ is the population for district $i$.
- $\nu_t$ is the seasonality effect.
- $\alpha$ and $exp(\beta_t)$ are the **endemic** parameters.
- $\lambda$ is the **autoregressive** parameter, $\lambda = exp(\alpha^{(\lambda)})$.
- $\phi$ is the **epidemic** parameter, $\phi = exp(\alpha^{(\phi)})$.

# Spatial (HHH4) model predictions

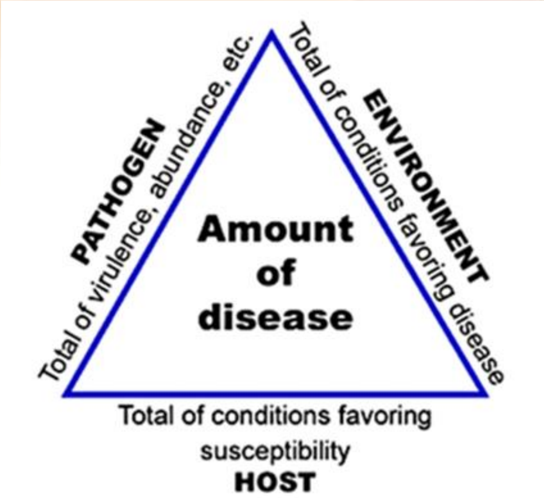



- Observed spore counts (Lethbridge, 2015)
- Case 1 (HHH4 model, observed climate data)
- Case 2 (HHH4 model, ensemble-based reanalysis data)
- Case 3 (HHH4 model, observed and reanalysis data)

# Summary

- Spatial model is able to predict disease occurrence width and peak timing well (Fairfield, Lethbridge validation site)
- Highest model prediction is achieved for the spatial model (HHH4) that assumes disease spread between adjacent districts
- Prediction power increases when combining station measured and ensemble-model reanalysis climate information
- Forecast model development/validation requires larger sample size of disease monitoring data, multiple growing seasons (i.e., larger network of cyclone air samplers needed)

# Future Prospects

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Modelization of infection of stripe rust and other diseases

↓

**Near real-time information to help in decision making toward mitigating impact of pathogens on wheat yield and quality**

Credits: https://masters.agron.iastate.edu

Credits: http://www.watermarkchurch.hk

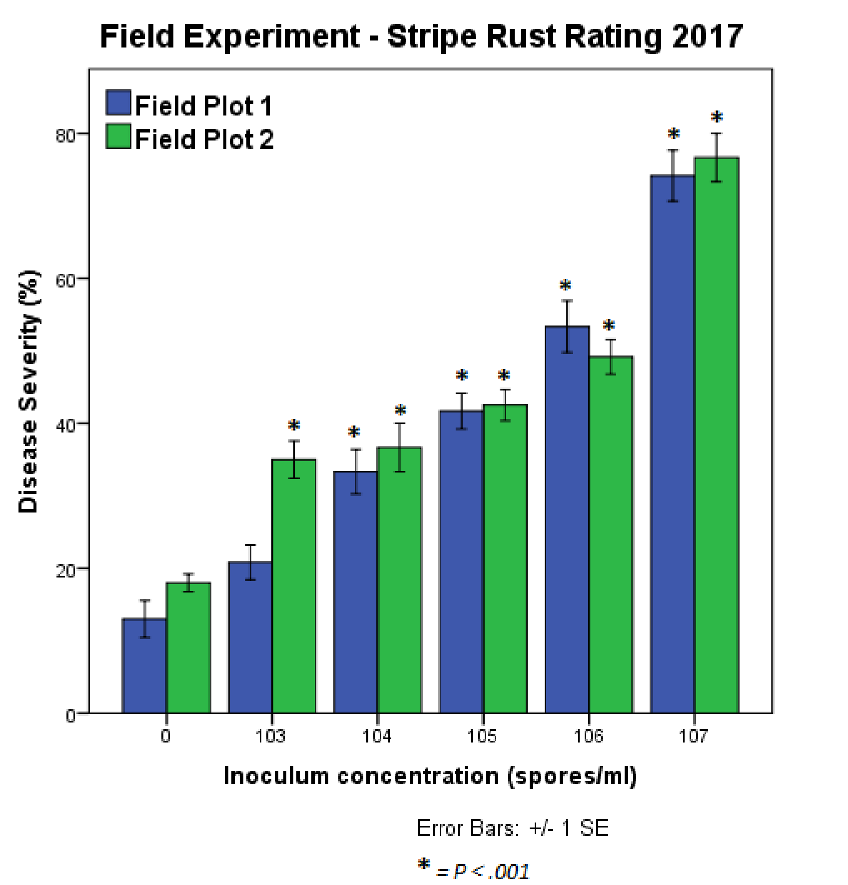
# Acknowledgements

**AAFC Peer Reviewed Projects & GRDI**

?

# Previous experiment

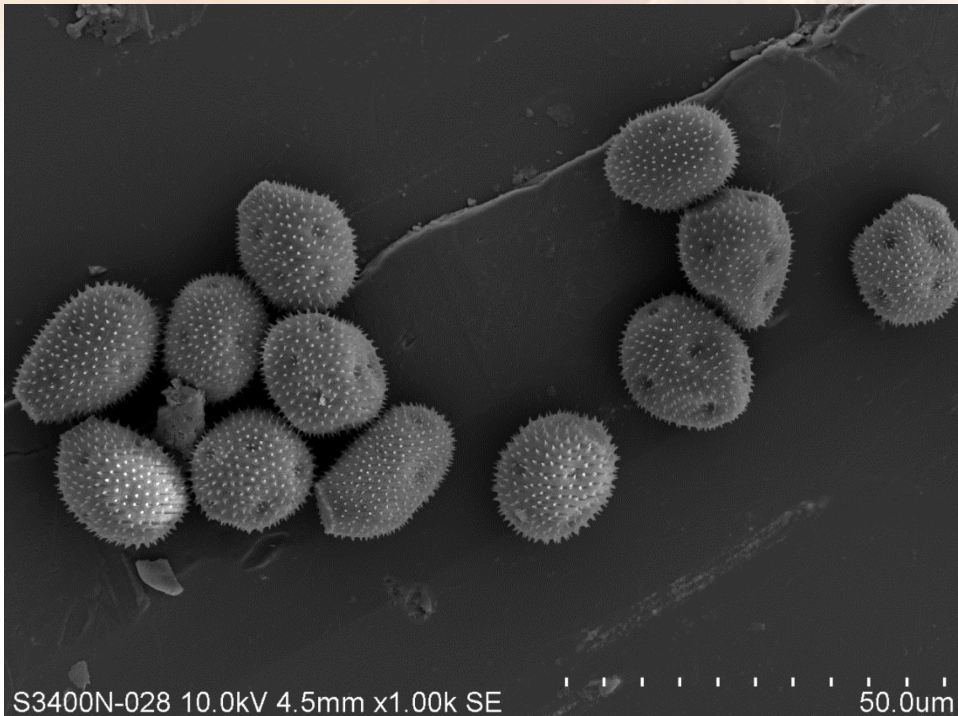
- **Field experiment – stripe rust**

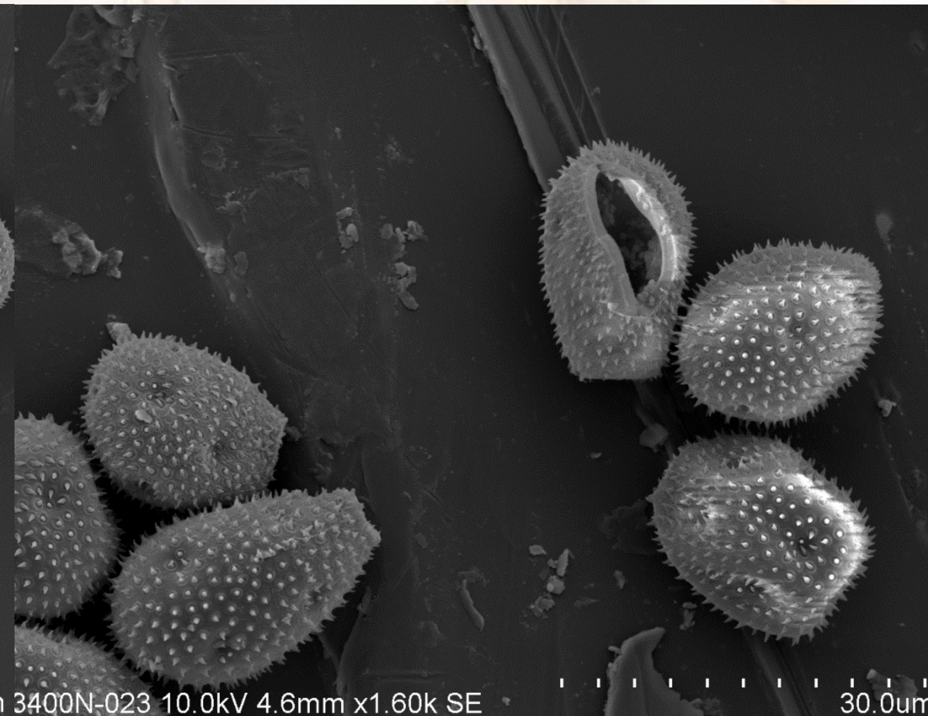


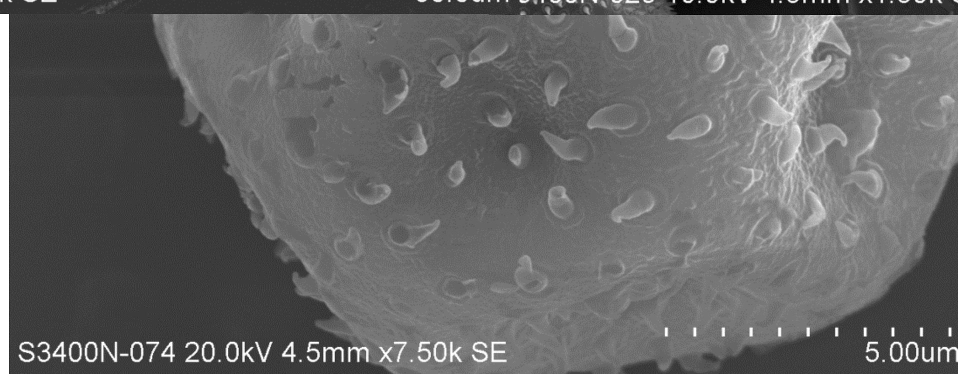
# Detection of samples

- Light Microscopy
  - Limited resolution and best approximation at genus level
- Electron Microscopy
  - Additional information but slow process

# *Pst* spores under scanning electron microscope



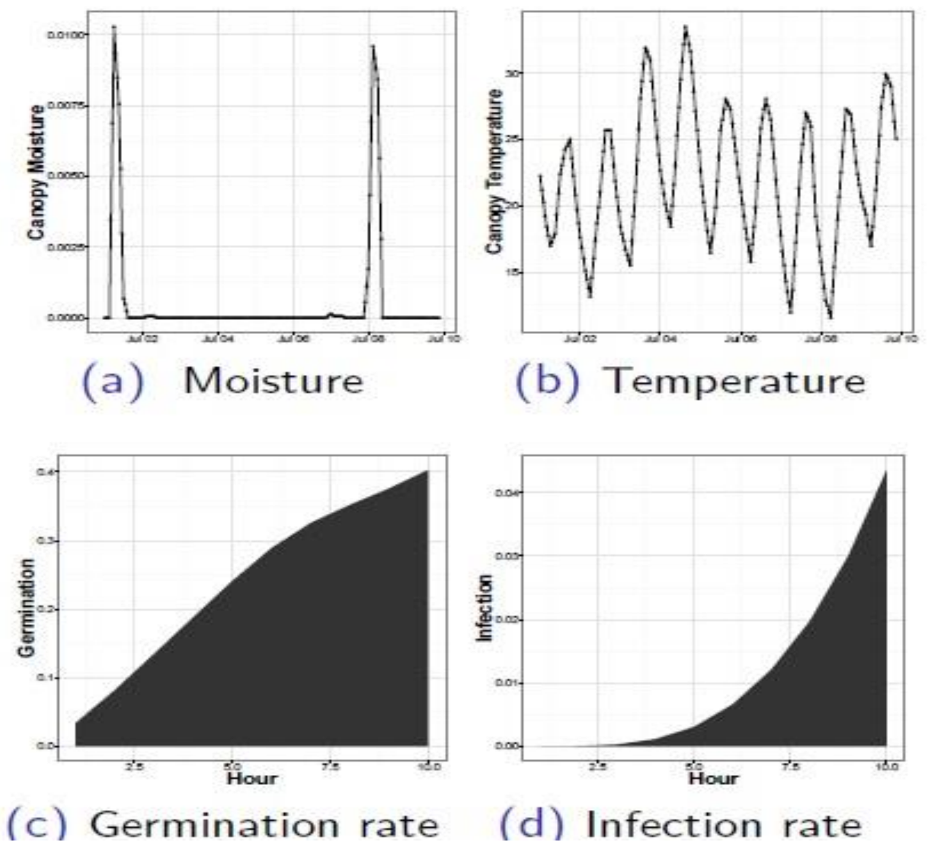

**Courtesy C. Sheedy LRDC**

# Wheat stripe rust - Modeled germination and infection rate
## (Lethbridge, 2015 growing season)

## Infection profile (site-specific distribution) (CLR model)

## Assumptions:

- Temperature is constant over each hourly interval
- Equal spore cohorts germinating at the start of each wet hour
- No spore germination process during the dry periods
- No neighbouring infections
- Spore germination, infection are only affected by canopy temperature and moisture
- The process of spore germination and infection is Weibull-distributed

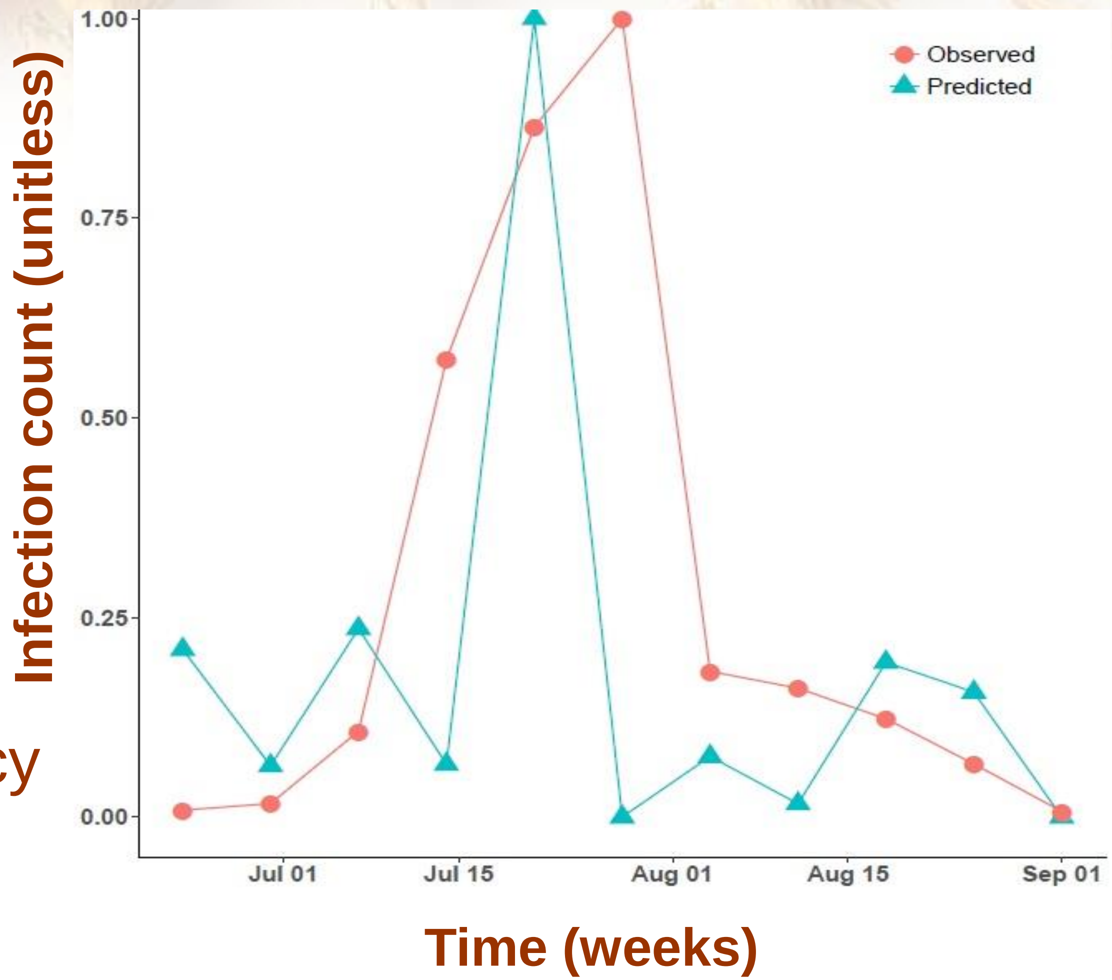


# CLR model predictions (normalized) (2015 growing season, Lethbridge spore profile)

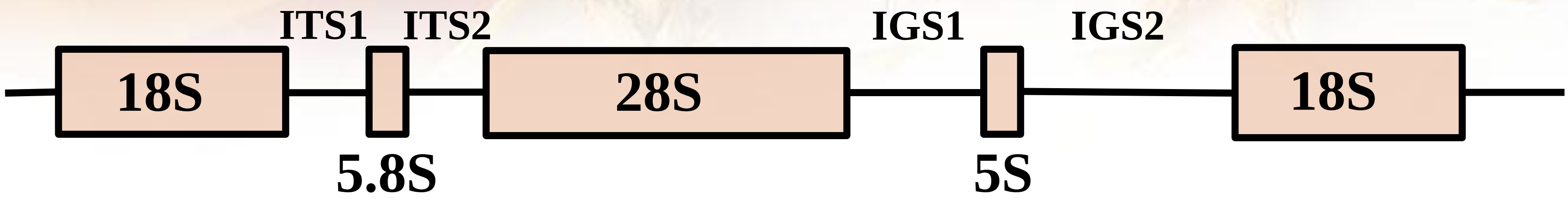
- higher variability in prediction error during early/late season

- observed infection rate close to predicted

- timing of infection shows largest discrepancy



# Schematic of rDNA locus



# Fungi placement

| | Eukaryota | |
|---|---|---|
| Kingdom: | Fungi | Fungi |
| Phylum: | Basidiomycota | Ascomycota |
| Class: | Agaricomycetes | Sordariomycetes |
| Subclass: | Agaricomycetidae | Xylariomycetidae |
| Order: | Atheliales | Xylariales |
| Family: | Atheliaceae | Microdochiaceae |
| Genus: | *Athelia* | *Microdochium* |
| Species: | *A. arachnoidea* | *M. nivale* |

# Wheat rusts

stripe rust

stem rust

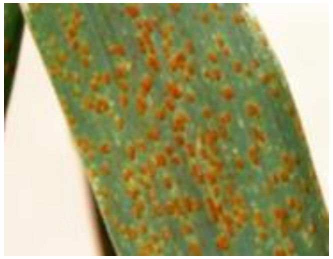
leaf rust

www.agric.wa.gov.aus

# Common diseases of wheat in Western Canada

- Stripe rust (*Puccinia striiformis f. sp. tritici)*
- Leaf rust (*Puccinia triticina* )
- Stem rust (*Puccinia graminis f. sp. tritici* )
- Powdery mildew (*Blumeria graminis f. sp. tritici)*
- Tan spot (*Pyrenophora tritici-repentis )*
- Fusarium head blight (*Fusarium graminearum)*

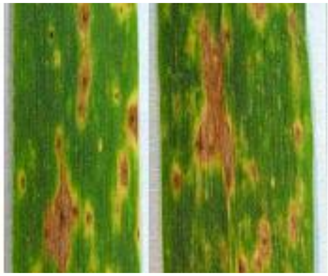

http://msue.anr.msu.edu


http://theconversation.com


http://agric.wa.gov.au


https://www.ag.ndsu.edu


http://agric.wa.gov.au


http://www.grdc.com.au

# Wheat rusts

- Rusts are obligate biotrophs
- *Puccinia striiformis f.sp. tritici* (*Pst*) is causal agent of stripe rust 117 Mb
- *Puccinia triticina* (*Pt*) is causal agent of leaf rust 135 Mb
- *Puccinia graminis f.sp. tritici* (*Pgt*) is causal agent of stem rust 89 Mb
- Around the world, wheat rusts can cause yield reduction from 20% to 90%

# Genome size of different organisms and number of genes

| | Size (Mb) | Genes (1000) |
|---|---|---|
| *Escherichia coli* (bacteria) | 4.7 | 3 |
| *Saccharomyces cerevisiae* (yeast) | 15 | 6 |
| *Tilletia carries* (bunt) | 23 | |
| *Arabidopsis thaliana* (mouse cress) | 70 | 23 |
| *Puccinia striiformis* (stripe rust) | 117 | |
| *Drosophila melanogaster* (fruit fly) | 140 | 15 |
| *Oryza sativa* (rice) | 580 | 27 |
| *Brassica napus* (rapeseed) | 1,200 | |
| *Medicago sativa* (alfalfa) | 1,600 | |
| *Zea mays* (maize) | 2,400 | |
| *Homo sapiens* (man) | 3,300 | 30 |
| *Hordeum vulgare* (barley) | 5,100 | |
| *Thinopyrum elongatum* (2x) | 6,000-8,000? | |
| *Secale cereale* (rye) | 8,300 | |
| *Avena sativa* (oat) | 11,300 | |
| *Triticum aestivum* (wheat) | 16,000 | 57 |
| *Fritillaria assyriaca* (lily) | 123,000 | |

Arumuganathan and Earle, 1991; Ausubel et al. 1995; Brown 1991; Dean and Schmidt, 1995; Mills 1996.

# Acknowledgements


## Associates and collaborators at LeRDC:

Michele Frick
Alejandro Peña
Briana Smith
Eric Amundsen

Denis Gaudet
Harpinder Randhawa
Robert Graf
Jamie Larsen

Ottawa RDC:
Scott Redhead
Hélène Labbé

AAFC Peer Reviewed Projects

Alberta Crop Industry Development Fund