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The population structure of Fusarium pathogens of small grain cereals, their distribution and relationship to mycotoxins

Dilantha Fernando

9th Canadian Barley Symposium, Feb 24, 2020, Winnipeg, Manitoba



UNIVERSITY
OF MANITOBA

➤ **Since, the early 1990s:**

➤ **(1) Losses from FHB in Canada have ranged from \$50 million to \$300 million annually.**

(2) Losses from FHB (Wheat and Barley alone) in the USA is about \$3 billion.

Wheat- *Fusarium graminearum*

Fusarium head blight (FHB)

- Devastating fungal disease
- Major causative agent: *Fusarium graminearum*
- Hosts: Wheat, Barley, Oats and Corn





Fusarium head blight:
Fusarium graminearum
(*Gibberella zeae*)

Why the concern about FHB?





Fusarium in Barley

Affects all of us!!! From Field to Glass



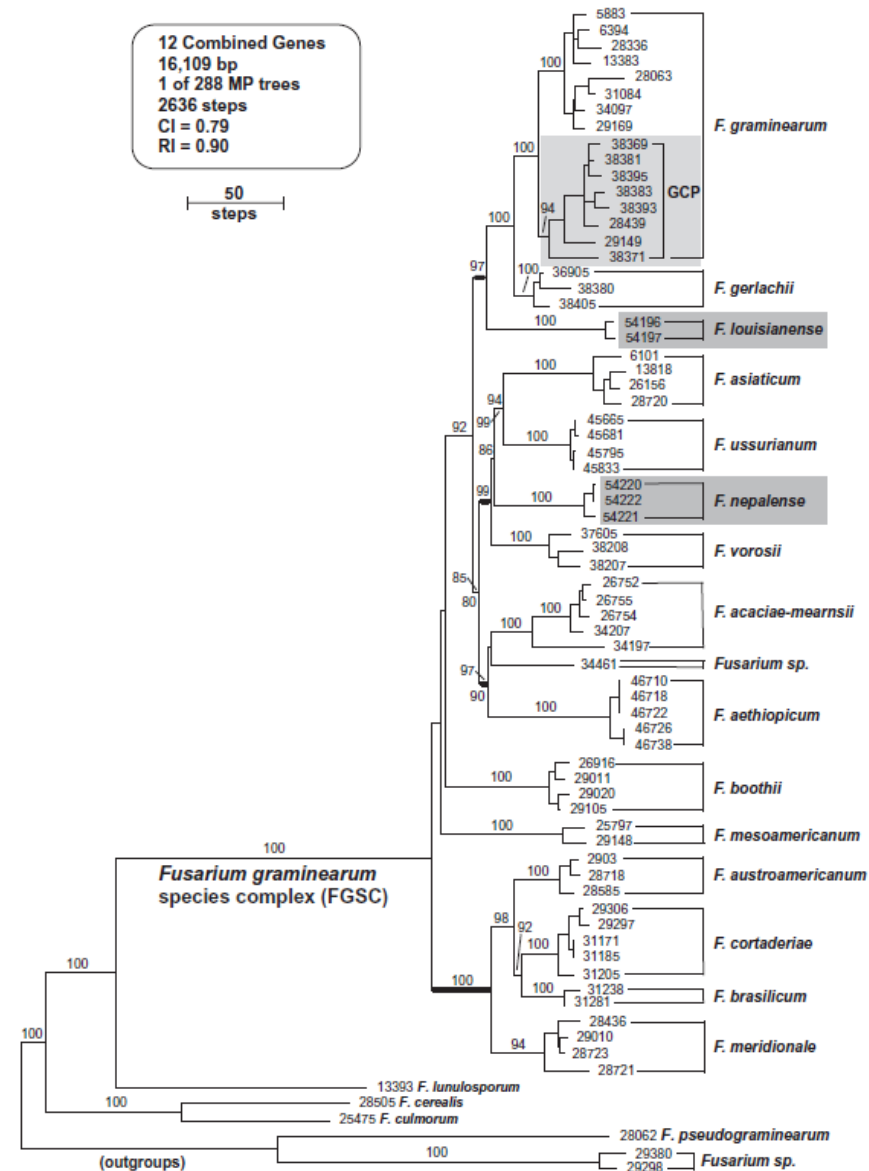
Malting Craft Houses & the Millennials



- FHB is majorly caused by species of the *Fusarium graminearum* species complex (FGSC), comprising 16 species (Sarver et al, 2011. Fung Gen. Biol)

- Other *Fusarium* species implicated in FHB include:

F. culmorum, *F. avenaceum*,
F. verticilloides, *F. oxysporium*,
F. poae, *F. sporotrichiodes*,
F. crookwellense, *F. cerealis*
and *F. equiseti*



Fusarium graminearum species complex (FGSC) -
(Sarver et al, 2011. Fung Gen. Biol)

Effects.....

Mycotoxins.....

- **Type B trichothecene mycotoxins:**
 - 1). DON (deoxynivalenol)
 - 15ADON (15-acetyldeoxynivalenol)**
 - 3ADON (3-acetyldeoxynivalenol)**
 - 2). NIV (Nivalenol)
- **Type A trichothecene; NX-2**
- **NIV is less pathogenic to plants, but more toxic to animals than DON**
- **3ADON Fusarium isolates produces more DON and are more aggressive than 15ADON isolates**

Research Focus

Molecular surveillance of Fusarium pathogens in small grain cereals

1. Species of Fusarium pathogens and their potential mycotoxin producing capabilities
2. Population structure of Fusarium pathogens

Species of Fusarium pathogens and their potential mycotoxin producing capabilities

No. of fields

Saskatchewan *

wheat – 56

barley - 7

oats - 11

Corn - 13

Manitoba *

wheat – 60

barley – 10

oats - 8

Alberta

wheat – 7

corn - 19

Ontario

wheat - 41

barley - 41

oats - 33

corn - 54

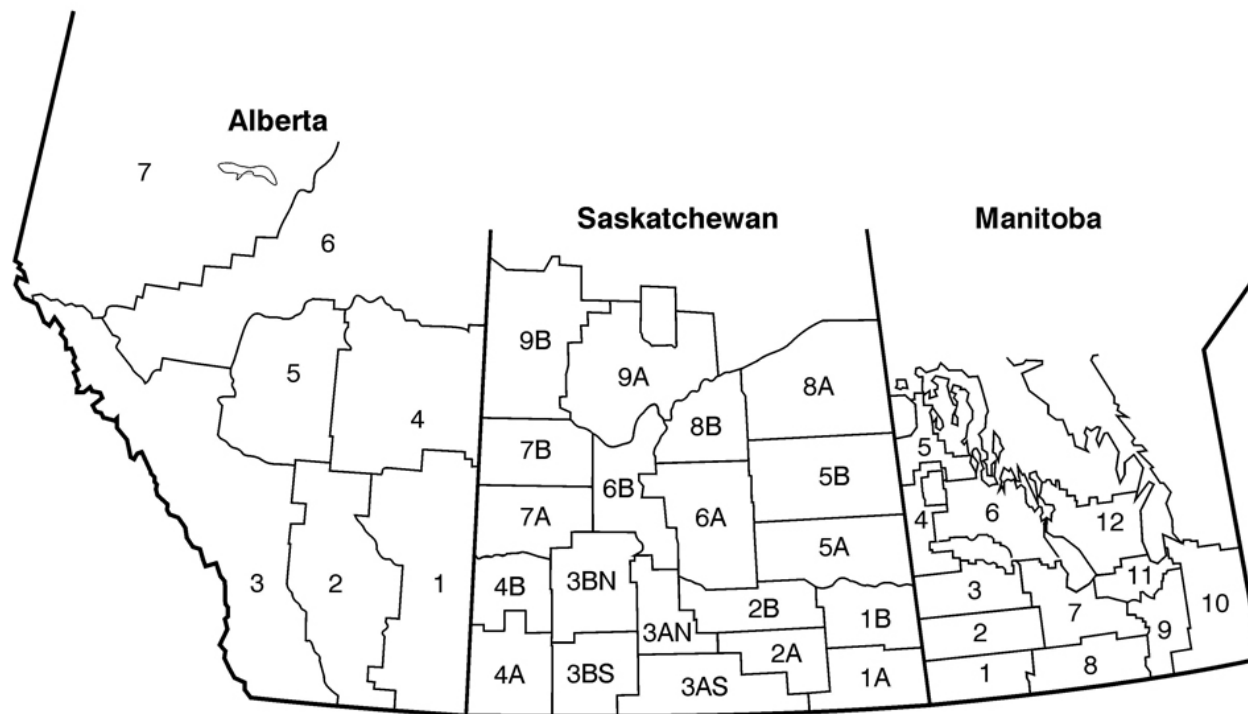
Quebec

wheat - 33

barley - 9

oats - 25

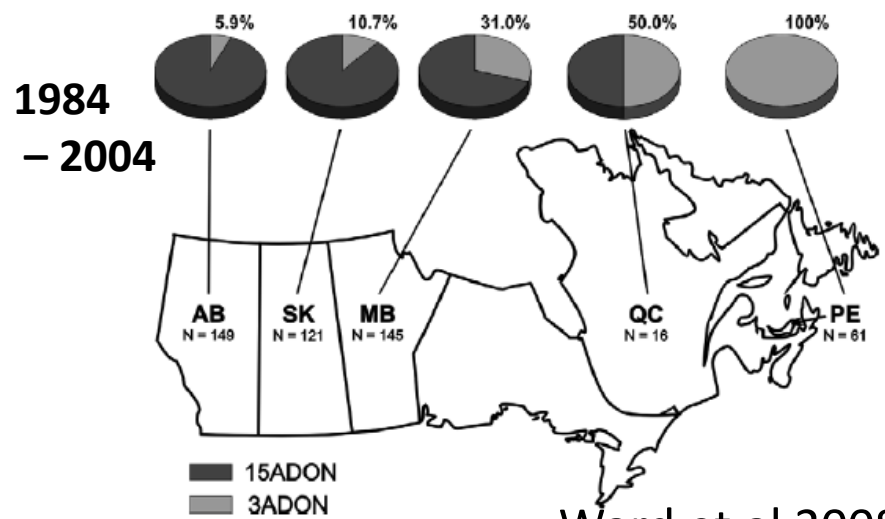
Sampling period – 2018 - 2022



Samples collected from crop districts across the prairies for Fusarium species and chemotype identification

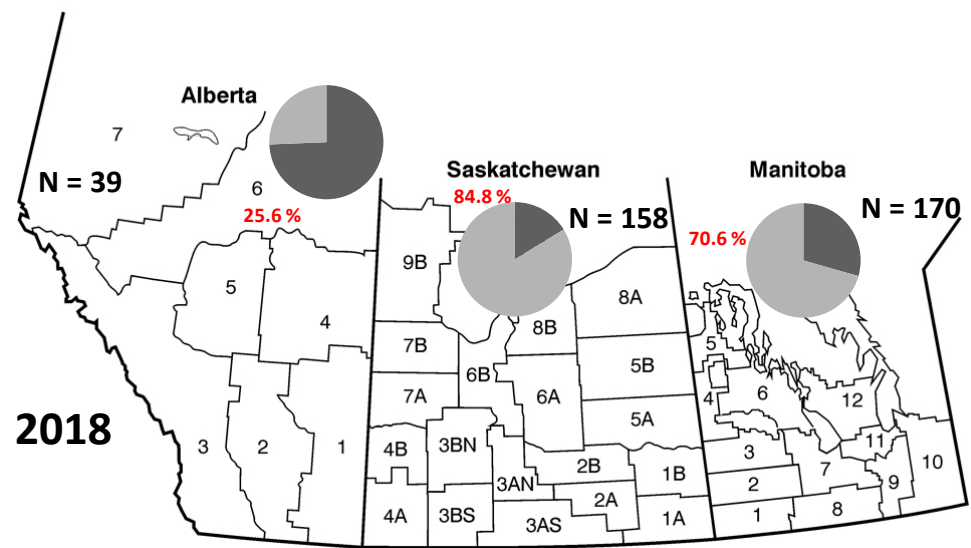
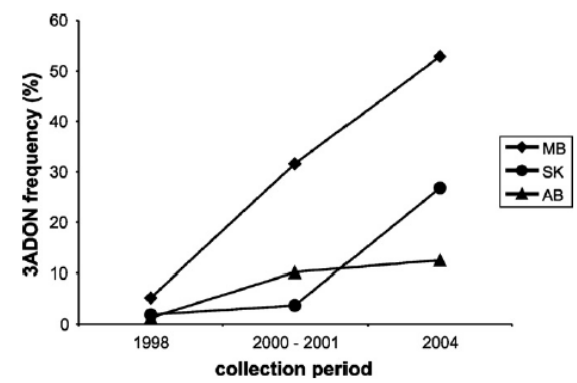
2018, 2019

The story of *F. graminearum* in wheat

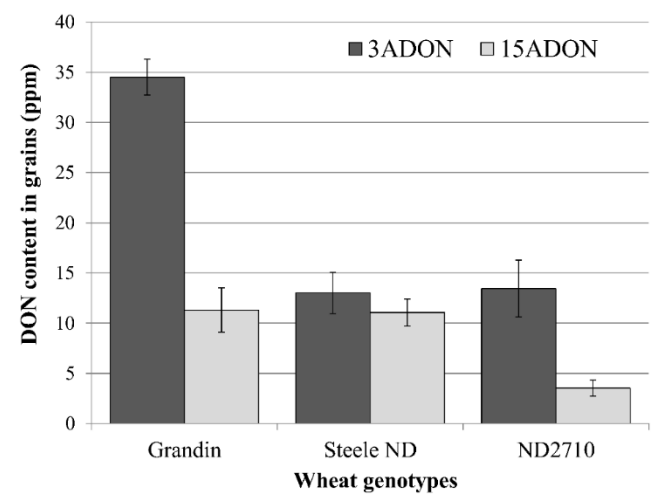


Ward et al 2008. Fung Gen. Biol

Main concern – Change to more aggressive mycotoxin producing isolates!!!

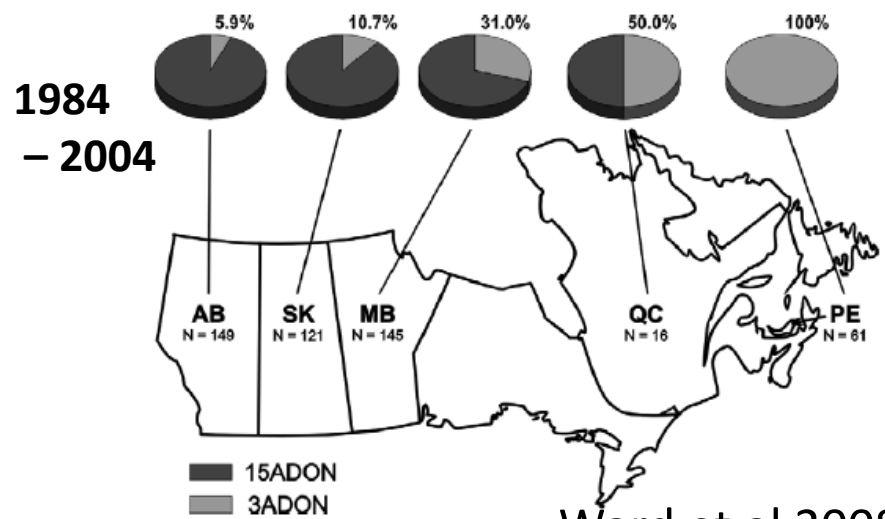


Results from our Lab



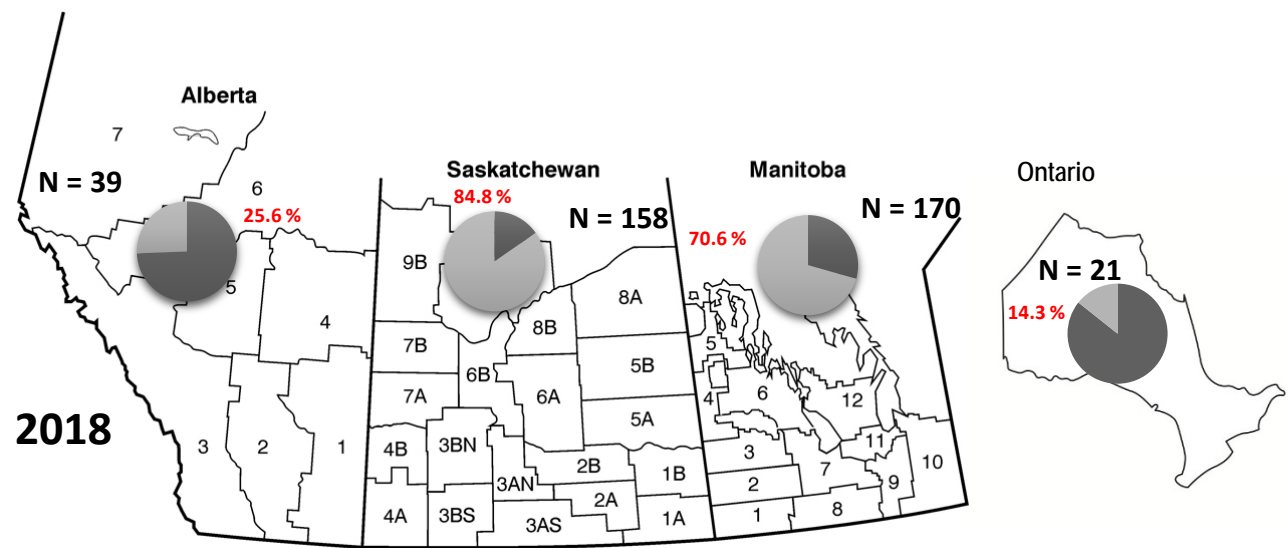
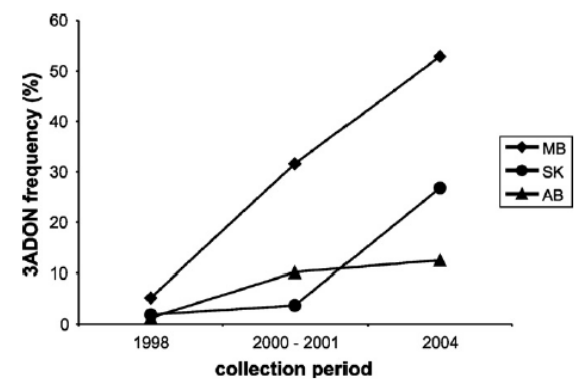
Puri et al 2016. PLOS ONE

The story of *F. graminearum* in wheat

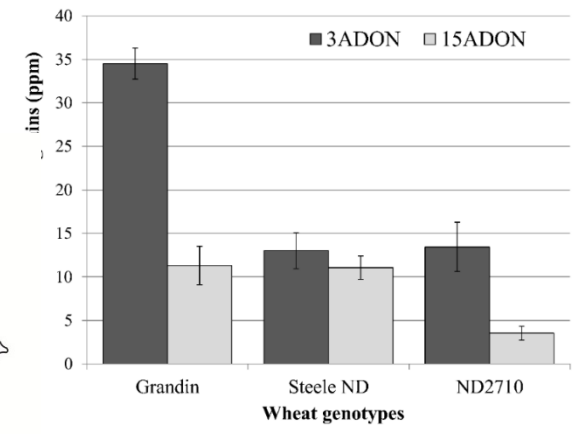


Ward et al 2008. Fung Gen. Biol

Main concern – Change to more aggressive mycotoxin producing isolates!!!



Results from our Lab



Puri et al 2016. PLOS ONE

Species of *Fusarium* and their chemotypes

Percentage of *Fusarium* isolates recovered from **spring wheat** samples collected in 2018 and 2019 in Saskatchewan (SK) and Manitoba (MB)

Location	Year	N. of Sites	<i>F. graminearum</i>		<i>F. culmorum</i>		<i>F. avenaceum</i>		<i>F. poae</i>		<i>F. sporotrichioides</i>		<i>F. equiseti</i>		Total Isolates
			Isolates	%	Isolates	%	Isolates	%	Isolates	%	Isolates	%	Isolates	%	
SK	2018	14	37	69.8	3	5.7	0	0.0	4	7.5	1	1.9	8	15.1	53
	2019	2	9	81.8	1	9.1	0	0.0	1	9.1	0	0.0	0	0.0	11
MB	2018	39	175	86.6	12	5.9	9	4.5	4	2.0	2	1.0	0	0.0	202
	2019	6	12	70.6	3	17.7	0	0.0	1	5.9	1	5.9	0	0.0	17
Totals	2018	53	212	83.1	15	5.9	9	3.5	8	3.1	3	1.2	8	3.1	255
	2019	8	21	70.0	6	20.0	0	0.0	2	6.7	1	3.3	0	0.0	30
	Both	61	233	81.8	21	7.4	9	3.2	10	3.5	4	1.4	8	2.8	285

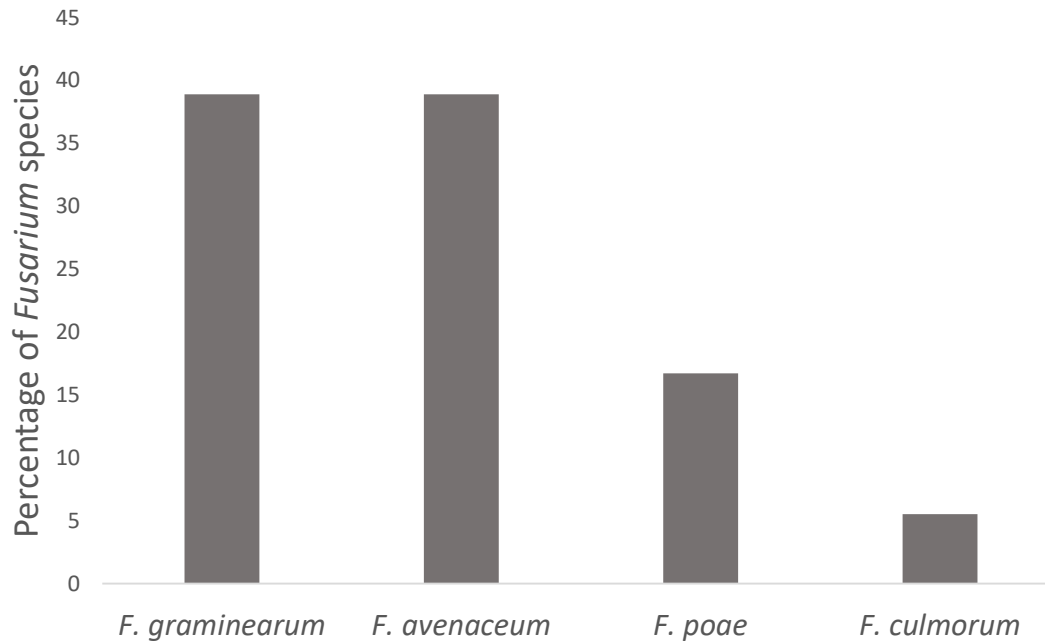
Chemotype (3ADON and 15ADON) percentages of *Fusarium graminearum* isolates recovered from samples

		SK (%)	MB (%)
2018	3ADON	84.8	70.6
	15ADON	15.2	29.4

		SK (%)	MB (%)
2019	3ADON	79.3	83.3
	15ADON	20.7	16.7

Species of *Fusarium* and their chemotypes

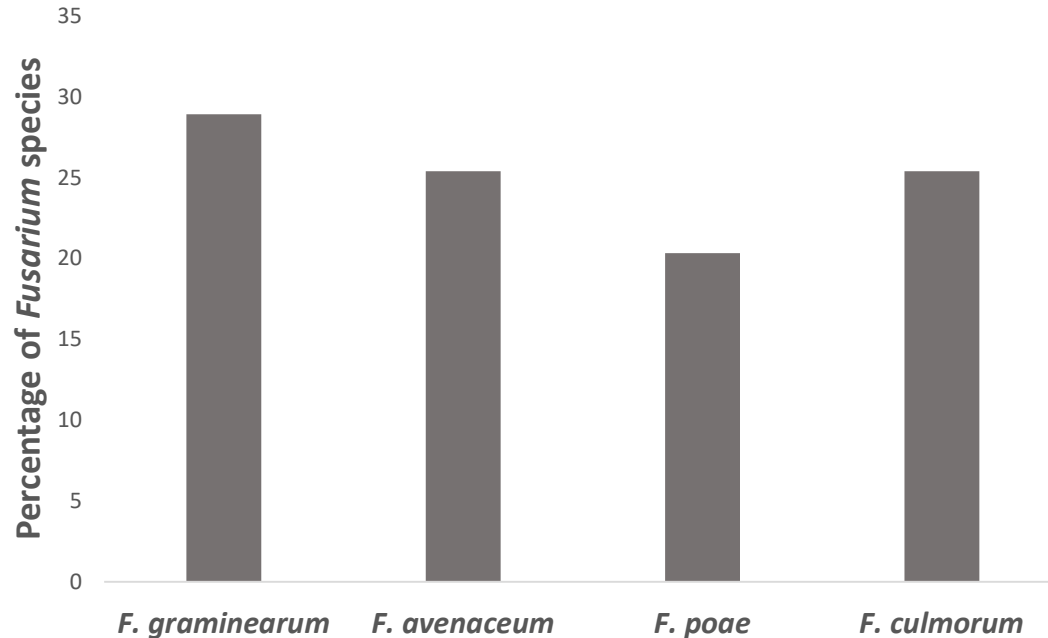
Percentage of *Fusarium* isolates recovered from **Barley** samples collected in 2019 in 3 sites in Saskatchewan



	(%)
3ADON	87.5
15ADON	12.5

Species of *Fusarium* and their chemotypes

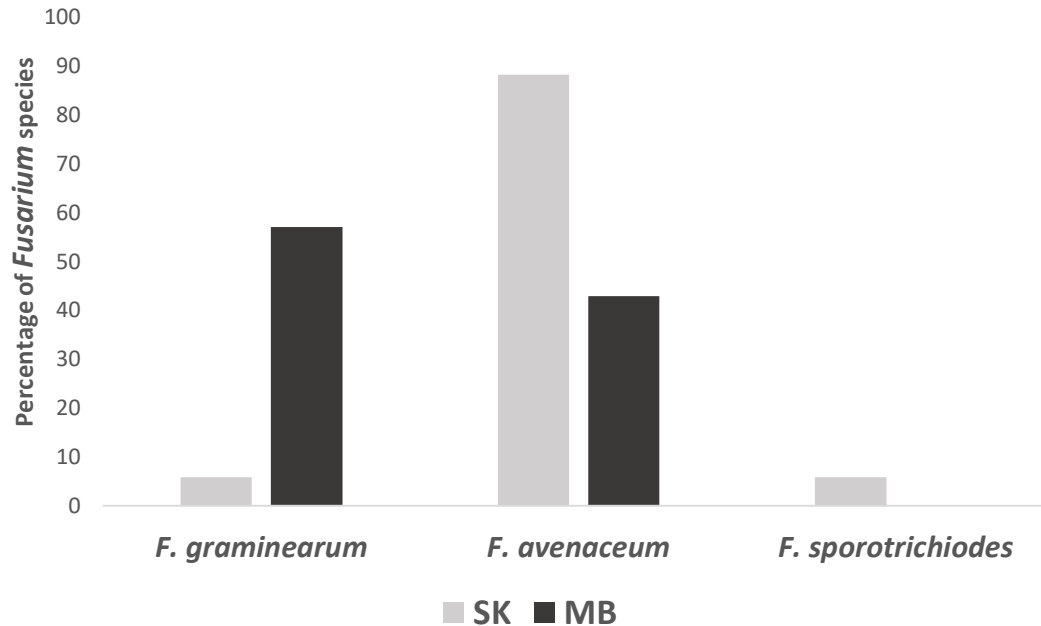
Percentage of *Fusarium* isolates recovered from **Oat** samples collected in 2019 in 3 sites in Saskatchewan



	(%)
3ADON	82.3
15ADON	17.7

Species of *Fusarium* and their chemotypes

Percentage of *Fusarium* isolates recovered from **Corn** samples collected in 2018 in 3 sites in Manitoba and 10 sites in Saskatchewan



Chemotype (3ADON and 15ADON) percentages of *Fusarium graminearum* isolates recovered from samples

	(%)
3ADON	55.6
15ADON	44.4

Population structure of *Fusarium* pathogens

Why study population structure???

- There is an arms race (evolution) between pathogens and hosts
- One strategic goal to manage plant health and improve food security is to understand the population biology of plant pathogens.

Population structure of *Fusarium* pathogens

Genetic structure – amount and distribution of genetic variation within and between populations.

Application of this knowledge

1. Amount of genetic variation maintained in a population indicates how rapidly a pathogen can evolve; information that can be used to predict how long a control measure would be effective.
2. Fungal populations with high levels of genetic variation are likely to adapt more rapidly to fungicides or resistant hosts.

Population structure study of *Fusarium* pathogens

Variable number of tandem repeats (VNTR)

Molecular Ecology Notes (2004) 4, 468–470

doi: 10.1111/j.1471-8286.2004.00703.x

PRIMER NOTE

Development of VNTR markers for two *Fusarium graminearum* clade species

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^{*}Life Science Research Center, Gifu University, Gifu 501–1193 Japan, ⁺Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA, [‡]USDA-ARS, Cereal Disease Laboratory, 1551 Lindig Street, St. Paul, MN 55108, USA

Abstract

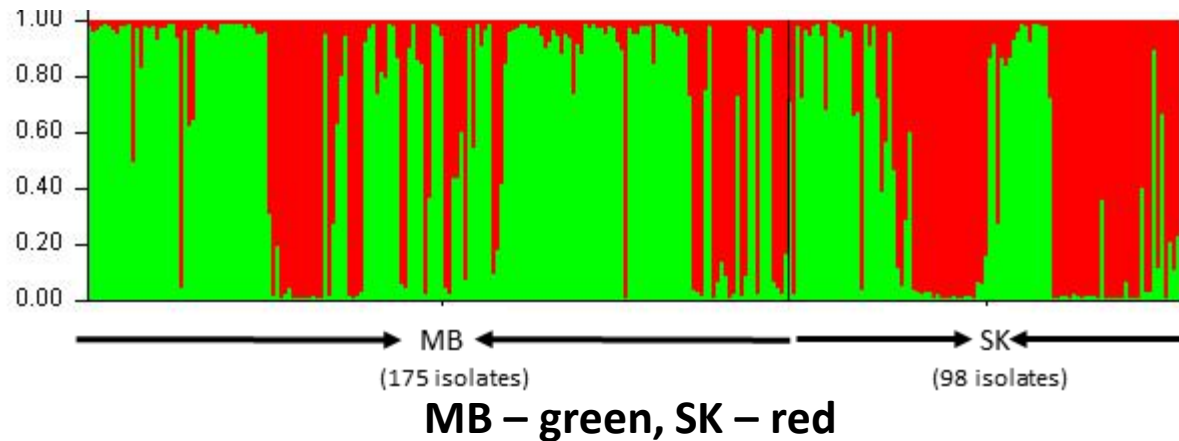
Using a bioinformatics approach, we developed 10 variable number of tandem repeats (VNTR) markers for *Fusarium graminearum* and *Fusarium asiaticum* useful for population genetic studies. Repeat sequences in the genome sequence of *F. graminearum* were identified by a tandem repeat finding program. Length polymorphisms at 54 loci were identified in five strains each from the United States, Italy and China. From these 54 loci, 10 were selected based on polymorphisms detected across species, ease of scoring, and their dispersed location in the genome.

Keywords: bioinformatics, *Fusarium*, population genetics, VNTR

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Go to PC settings to activate Windows.

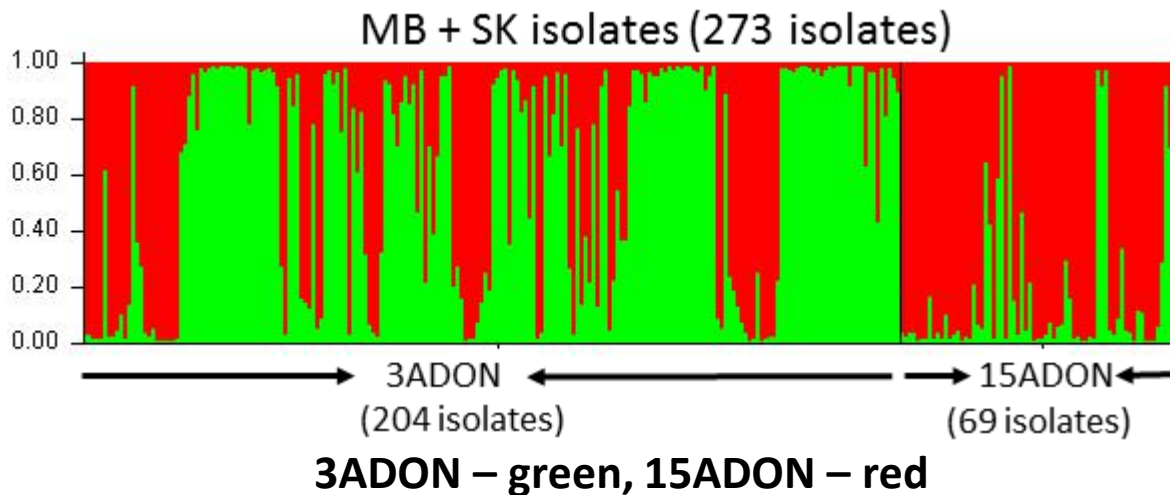
Advantage of VNTR over AFLP and RFLP – Collection of accurate polymorphic data.

Comparative population genetic analysis of 273 isolates of *Fusarium graminearum* collected in Manitoba (MB) and Saskatchewan (SK) in 2018



Low genetic differentiation

Clustering by province
($F_{ST} < 0.0208$, $P < 0.001$)



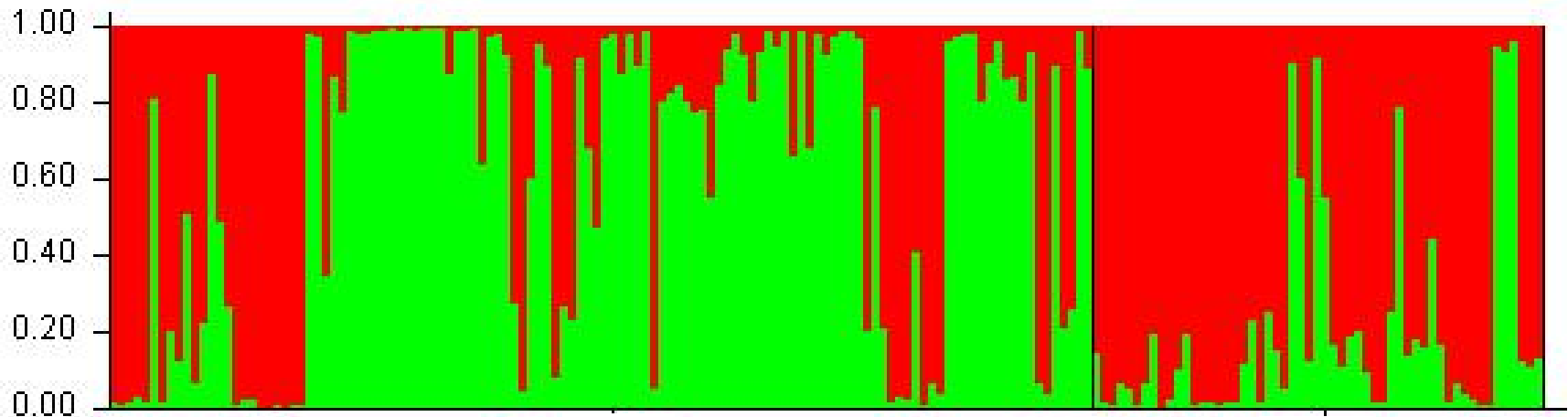
Significant genetic differentiation

Clustering by chemotype
($F_{ST} < 0.1073$, $P < 0.001$)

Horizontal axis – represents isolates each by a vertical line
Vertical axis – estimated membership coefficient in each cluster

Comparative population genetic analysis of 273 isolates of *Fusarium graminearum* collected in Manitoba (MB) and Saskatchewan (SK) in 2018

MB isolates (175 isolates)



← 3ADON (120 isolates) ← 15ADON (55 isolates) ←

3ADON – green, 15ADON – red

Significant genetic differentiation

Clustering by chemotype
($F_{ST} < 0.0544$, $P < 0.001$)

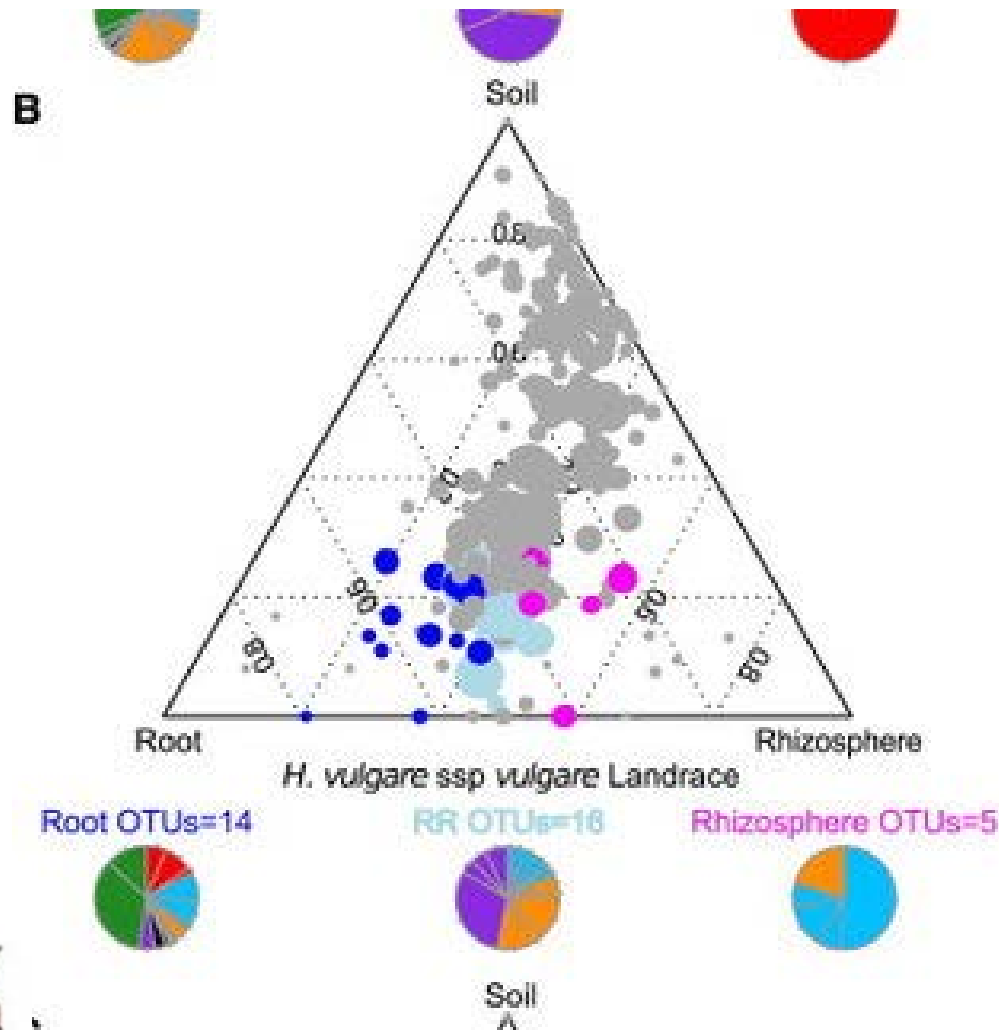
Horizontal axis – represents isolates each by a vertical line
Vertical axis – estimated membership coefficient in each cluster

Take home messages

- Chemotype shift from 15ADON to 3ADON is on going.
- *Fusarium graminearum* is still the most prevalent species in wheat.
- *Fusarium avenaceum* is rapidly becoming very relevant as a causative agent in FHB in barley, oat and corn.
- Population genetic analyses of *F. graminearum* isolates in Manitoba and Saskatchewan show that;
 1. Isolates of different trichothecene chemotype represented distinct populations, but show no genetic differentiation with regards to provinces.
 2. There was more gene-flow from 3ADON isolates to 15ADON than from 15ADON to 3ADON.



Newly funded research with Matt Bakker, James Tucker and others





Linking malt quality defects with traits of *Fusarium* species and with malt microbiomes

A new collaborative project with:

University of Manitoba
Agriculture & Agri-Food Canada
Canadian Grain Commission
CMBTC



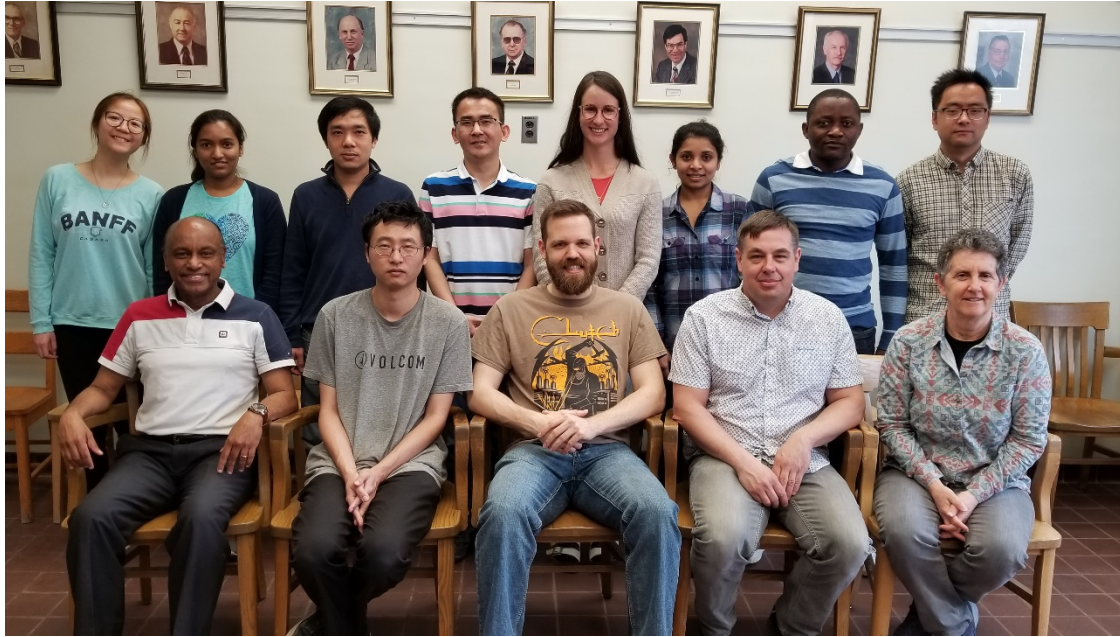
- Barley infested in the field with individual pathogen isolates
- Malted
- Microbiomes profiled
- Malt quality assessed
- Link malt characteristics to strain-level features of the pathogen



University
of Manitoba



Acknowledgements



Fernando Lab

Abbot Oghenekaro – UM Post doc who did the work

Randy Kutcher – University of Saskatchewan

James Tucker – AAFC, Brandon, Manitoba

Xiben Wang - AAFC, Morden, Manitoba

Allen Xue – AAFC, Ottawa, Ontario

Michael Harding - Crop Diversification Centre South, Alberta