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Multi-omic studies of *Fusarium* species associated with Canadian cereals

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Canada 

Research Questions

(Harris, Overy, Blackwell, Subramaniam)

What mycotoxins are Canadian *Fusarium* species capable of producing?

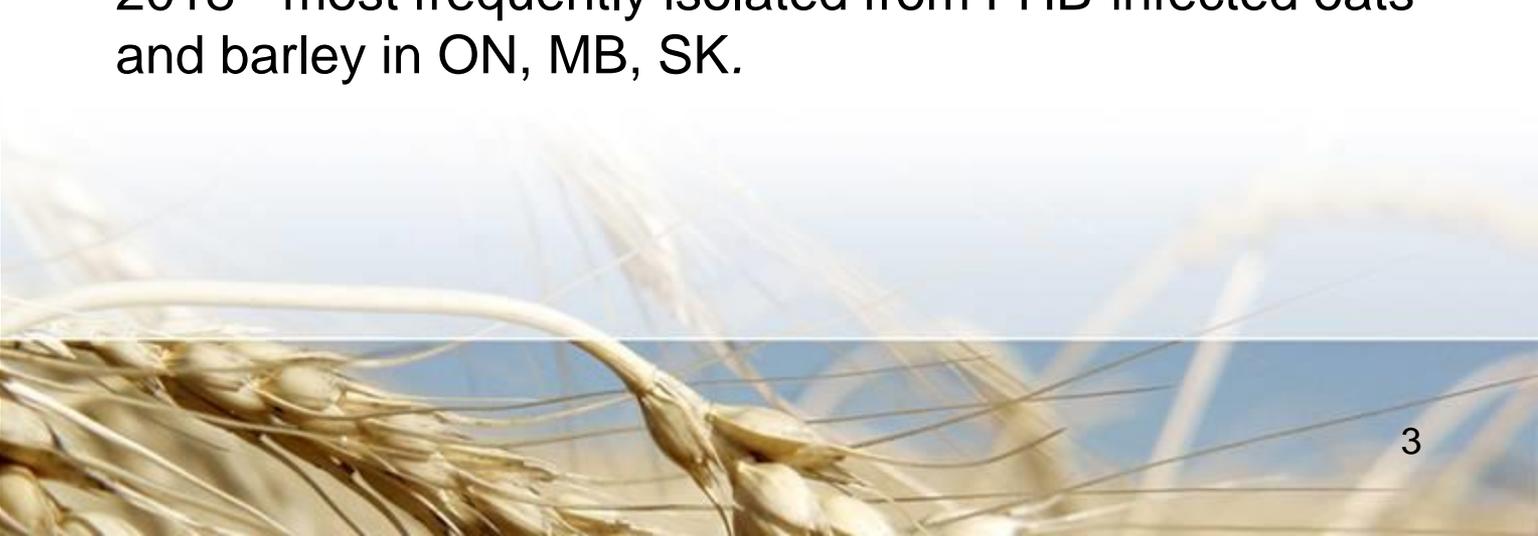
- ❖ genomics, transcriptomics & metabolomics studies of representative *F. poae*, *F. avenaceum*, *F. graminearum* isolates.

Why do these fungi make these mycotoxins (e.g. promote plant infection, compete with other organisms)?

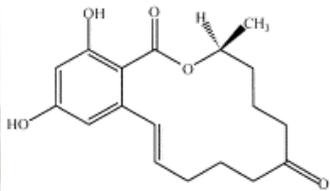
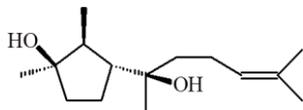
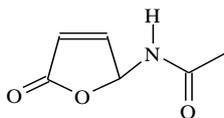
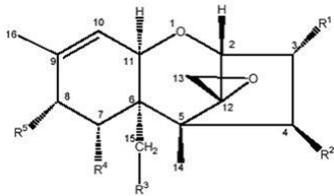
- ❖ gene modification studies, transcriptomic & metabolomic studies of intra- and interspecies competition.

Fusarium species

- *Fusarium graminearum*: Main fungus causing **fusarium head blight (FHB)**: wheat, barley, oats) and **gibberella ear rot** (maize).
- *Fusarium avenaceum*: frequent co-contaminant, especially on durum wheat. Of concern with pulse/wheat rotations.
- *Fusarium poae*: 2001-2017 – most common *Fusaria* isolated from oats, *F. poae* and *F. graminearum* isolated equally from barley.
 - 2018 - most frequently isolated from FHB-infected oats and barley in ON, MB, SK.

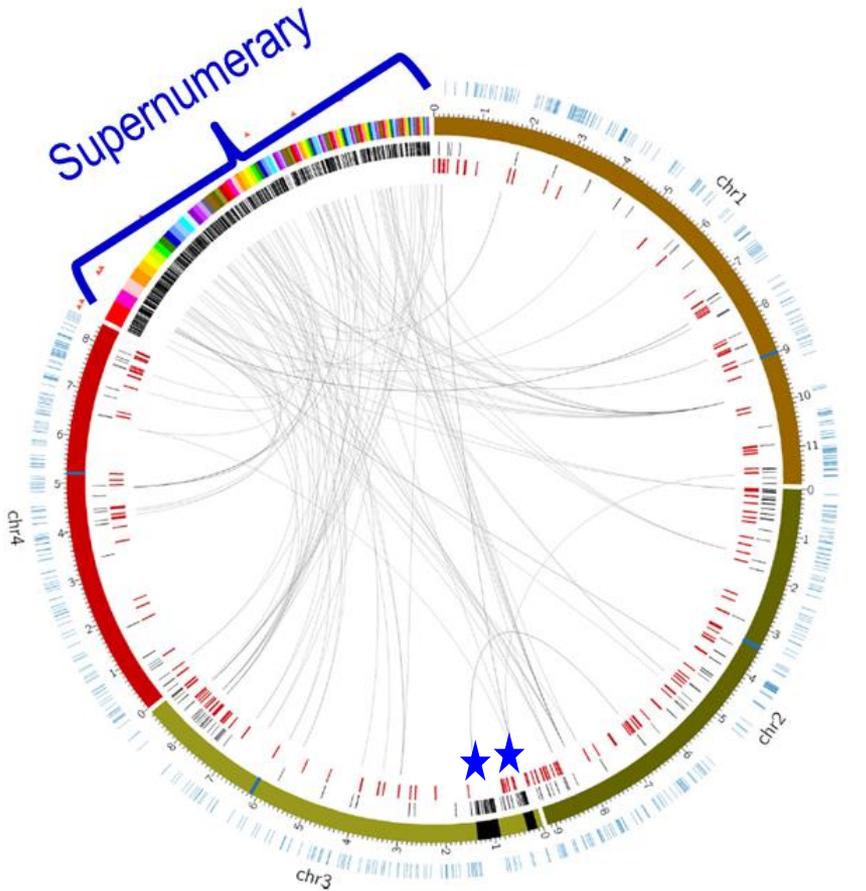


Species-specific cocktails of secondary metabolites



<i>Fusarium graminearum</i>	<i>Fusarium avenaceum</i>	<i>Fusarium poae</i>
Trichothecenes (DON, NX, NIV)	Moniliformin	Trichothecenes (NIV, DAS, NEO,FX)
Zearalenone	Enniatins	Beauvericin
Gramillin A,B	Fuscofusarin	
Culmorin	2-amino-14,16-dimethyloctadecan-3-ol	Culmorin
Cyclonerodiol	Chlamydosporols	Cyclonerodiol
Butenolide	Butenolide	Butenolide
Fusaristatin	Fusaristatin	
Fusaoctaxin	Antibiotic Y	

Fusarium poae genome



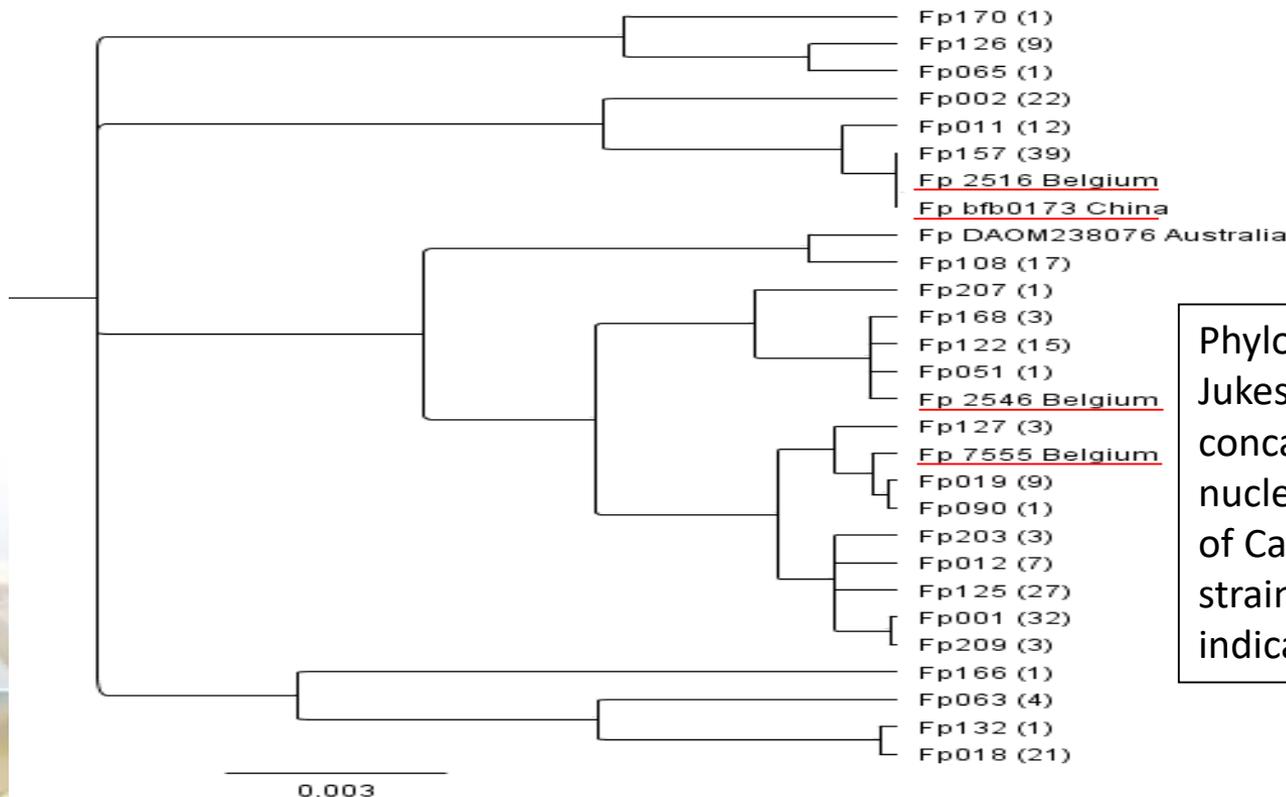
- *F. poae* has 4 core chromosomes (with extensive macrosynteny with the 4 *F. graminearum* chromosomes) and supernumerary chromosomes.
- Supernumerary structures contain many gene duplications and active transposable elements not subjected to RIP and may be evolutionary cradles for pathogenicity.
- Presence of supernumerary chromosomes can influence the expression & diversity of 2nd metabolome.

Vanheule et al. 2016. *BMC Genomics* 7:670

Genetic and mycotoxigenic diversity of Canadian *F. poae*

(with Allen Xue, David Overy, Hai Nguyen, Jeremy Dettman/ORDC)

- 268 *F. poae*: 203 ON, 38 QC, 27 SK oat/barley/wheat samples, 2006-2018
- Sequenced 3 genes to confirm species and explore genetic diversity.
- Genetic analysis → 45 → Nextseq genome seq & LC-HRMS metabolomics.

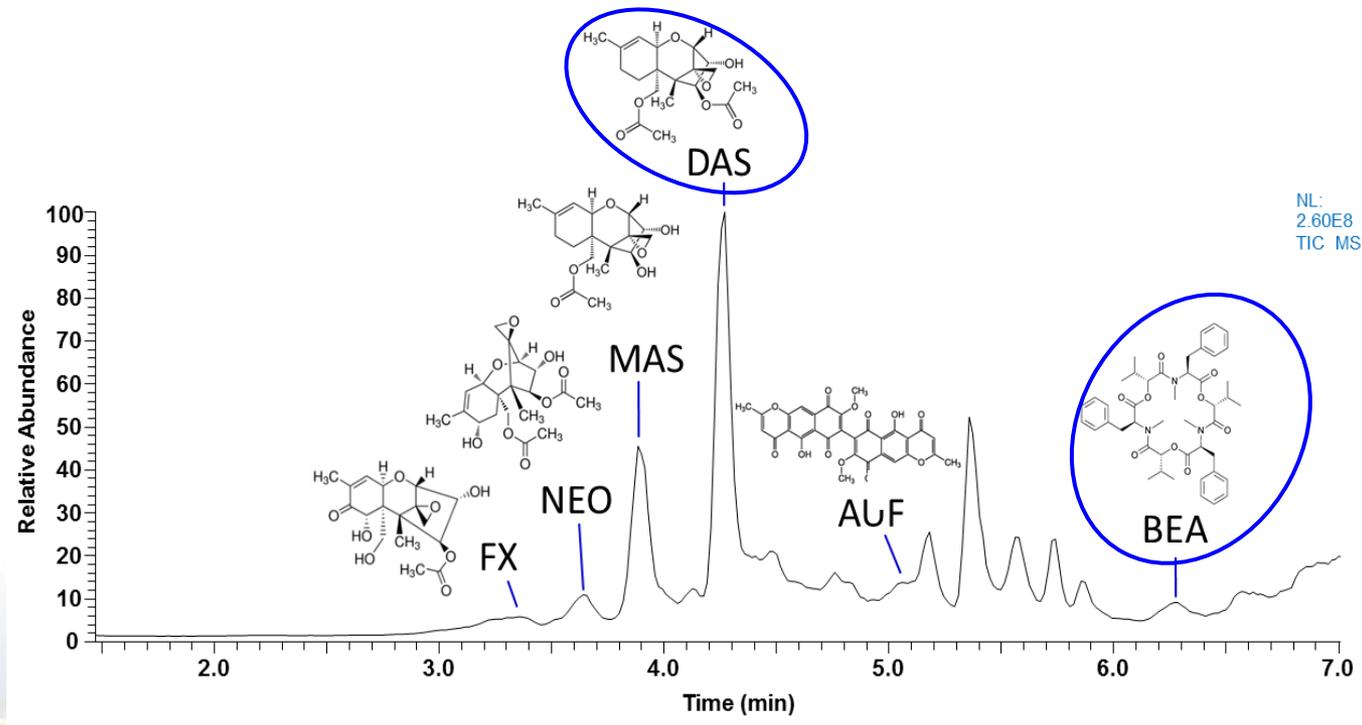


Genome sequenced
Vanheule et al 2016

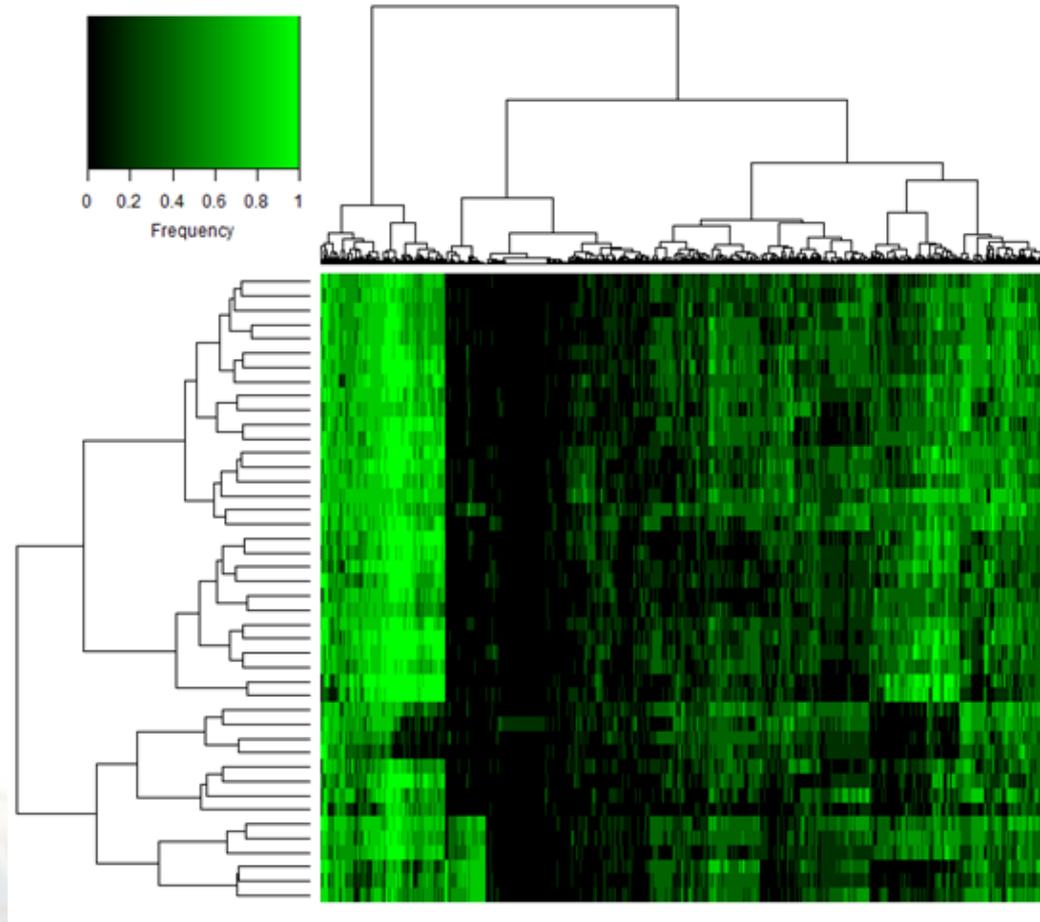
Phylogenetic tree (UPGMA, Jukes-Cantor) based on concatenated *tef1a-TRI1-TRI8* nucleotide sequence alignment of Canadian and international strains. Numbers in brackets indicate # strains represented.

F. poae and Emerging Mycotoxins

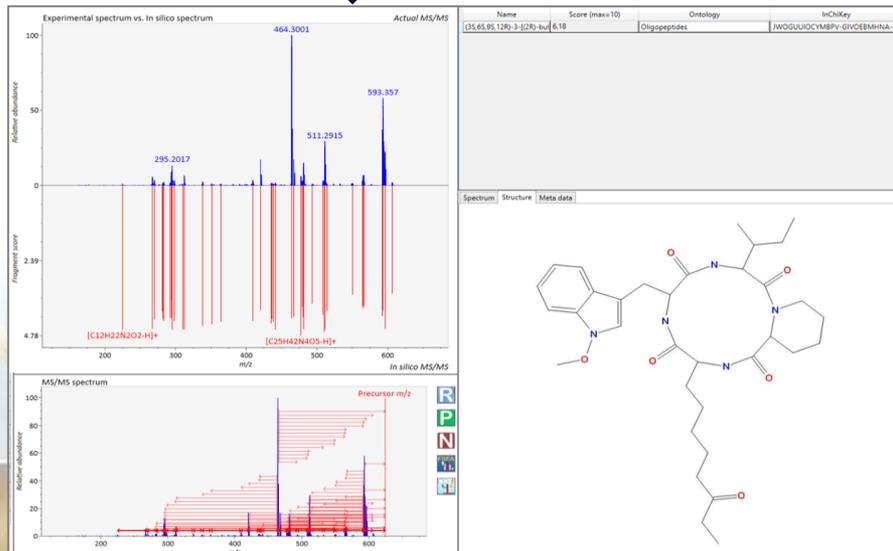
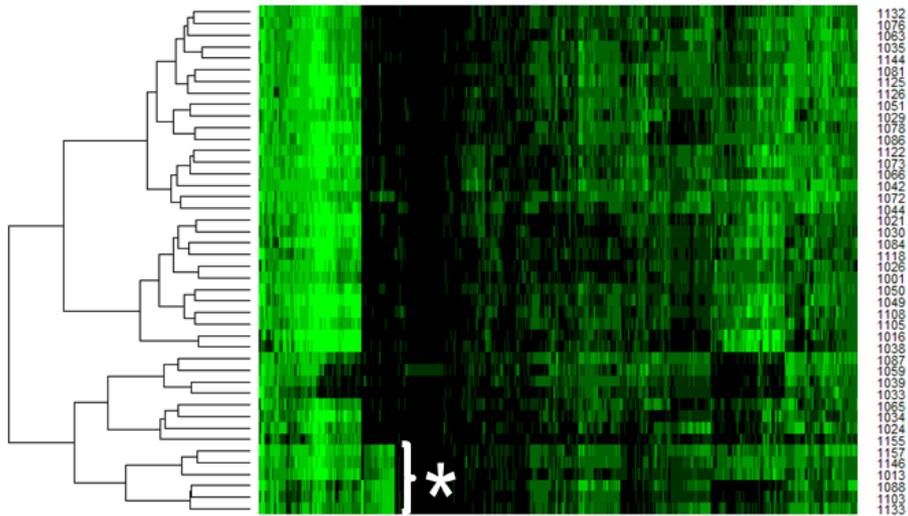
- All Canadian *F. poae* strains made Type A&B trichothecenes.
- Diacetoxyscirpenol (DAS) & beauvericin (BEA) were consistently produced by all Canadian strains.



Secondary metabolite profiling of *F. poae* grown on 4 media

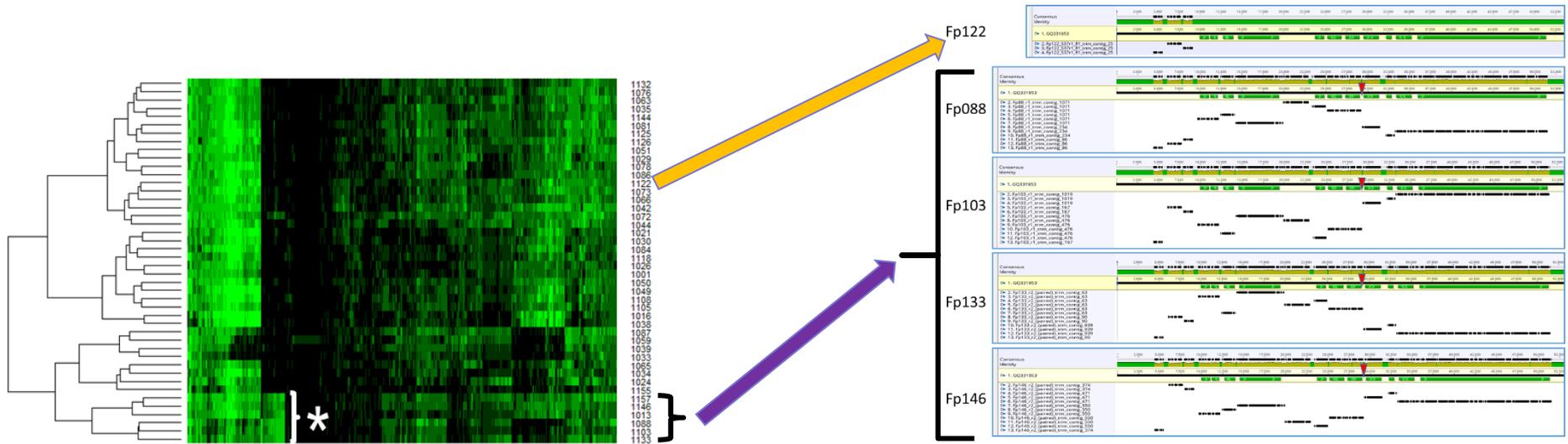


Apicidins produced by a subset of *F. poae* isolates



- Spectral matching of MS/MS fragmentation data identified a metabolite group as apicidins.
- *F. poae* has never before been reported to produce apicidins.
- Apicidins are phytotoxic cyclic tetrapeptides which inhibit histone deacetylase, under development as anti-cancer drugs.

Apicidins produced by a subset of *F. poae* isolates



- *Blastn* *F. incarnatum* apicidin gene cluster - found in all producing *F. poae* (NextSeq genome assemblies) split between 2 small contigs.
- Absent in genomes of non-producers.

Nanopore long read sequencing of *F. poae*

3 Canadian *F. poae* isolates sequenced

	<i>Fp122</i>	<i>Fp157</i>	<i>Fp133</i>		<i>Fp2516*</i>
# Nanopore reads	130 K	340 K	389 K		
Mean read length (bp)	17,555	21,465	22,321		
Total fastq (GB)	4.27	13.3	14.2		
Canu assembly - # contigs	32	14	13		176
Total length (MB)	44.5	44.2	45.3		46.3

**Vanheule et al 2016*

Nanopore long read sequencing of *F. poae*

	<i>Fp122</i>	<i>Fp157</i>	<i>Fp133</i>
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# Canu contigs	32	14	13
Total length (MB)	44.5	44.2	45.3

Fp133 Canu assembly (mean coverage 200X)

Contig name	Size (bp)	Annotation
Canu1211	12,007,395	Chrom 1 (11.79MB)
Canu4	9,784,343	Chrom 2 (9.17MB)
Canu1	7,824,537	Chrom 3 (8.78MB)
Canu9	8,497,414	Chrom 4 (8.39MB)
Canu7	939,503	Supernumerary
Canu13	3,789,164	Supernumerary
Canu16	2,076,086	Supernumerary
Canu1212	91,378	Supernumerary
Canu1210	72,143	Supernumerary

Core genome

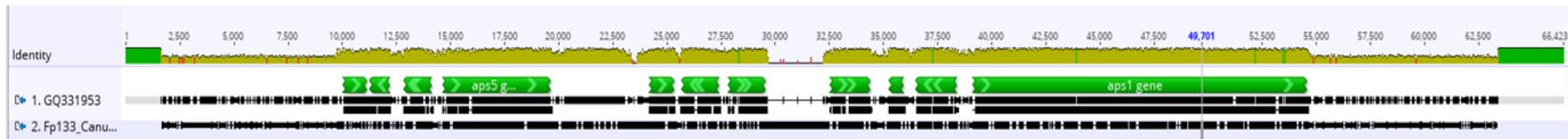
+ 4 contigs = 1 mitochondrial, 3 plasmids

Apicidins produced by a subset of *F. poae* isolates

Apicidin gene cluster found on predicted *Fp133* supernumerary contig 13 (flanked by characteristic TE's).



Apicidin gene cluster (11 genes) is intact within 590,000 to 650,000 bp



F. incarnatum apicidin gene cluster



The Genome of the Generalist Plant Pathogen *Fusarium avenaceum* Is Enriched with Genes Involved in Redox, Signaling and Secondary Metabolism

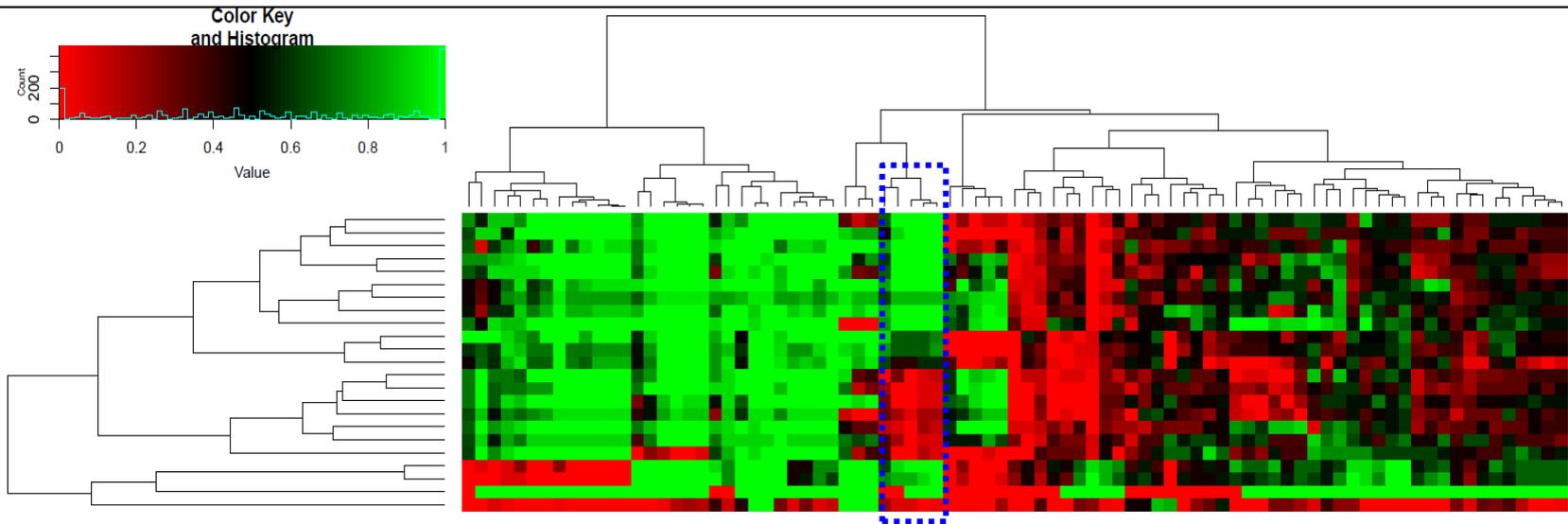


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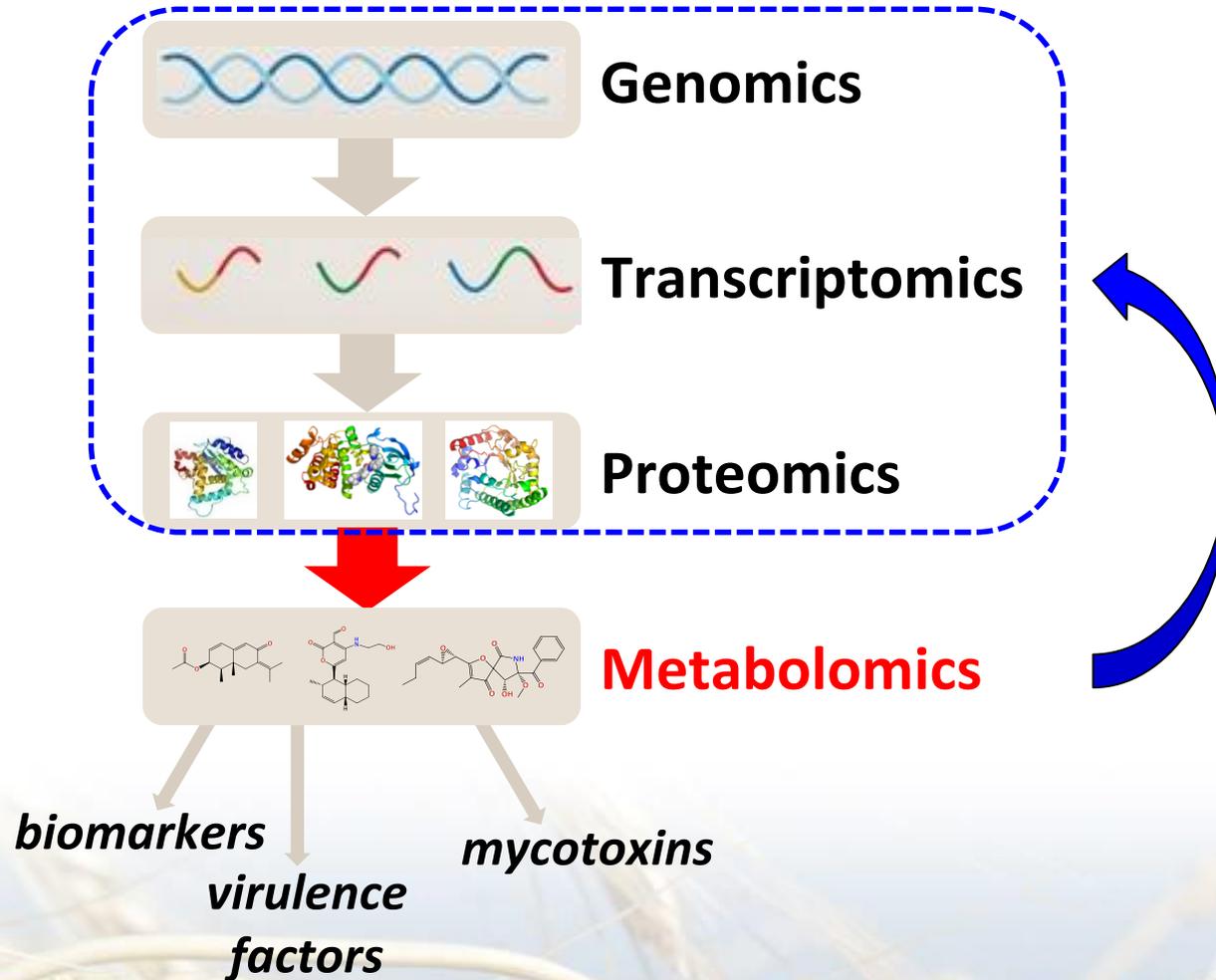
- Recently assembled genome sequences (Illumina Nextseq) and conducted metabolomics profiling (D. Overy) of 17 Canadian *F. avenaceum* isolates.

Metabolomic profiling of various *F. avenaceum* strains on multiple media

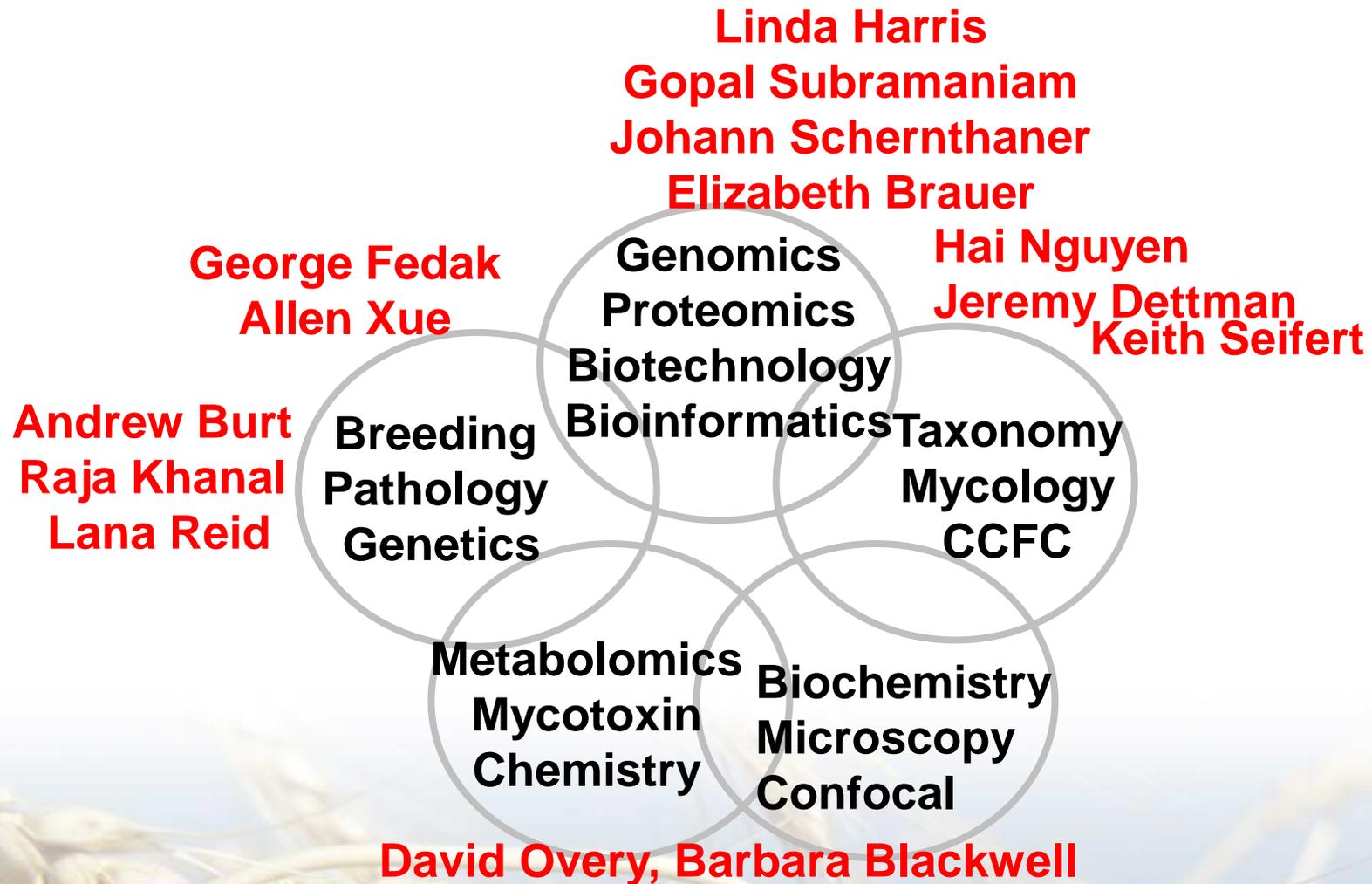


- Identified one novel 2^o metabolite produced by a subset of strains, biosynthesized by a gene expressed *in planta*.

Omics Pipeline for Studies of Mycotoxigenic and Pathogenic Potential of Fungi



Fusarium collaborators within Ottawa RDC



Danielle Schneiderman
Anne Johnston

Whyynn Bosnich



Funding

- AAFC Emerging Mycotoxin network.
- AAFC Genomics Research & Development Initiative.
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