AAFC-USDA Collaboration on
Genotyping and Monitoring of High Risk Plant Pathogens
(Aug. 03, 2019)

Enhanced efficiency and accuracy in HTS-based phyto-pathogen/pest diagnosis -- *the role in Biovigilance continuum*

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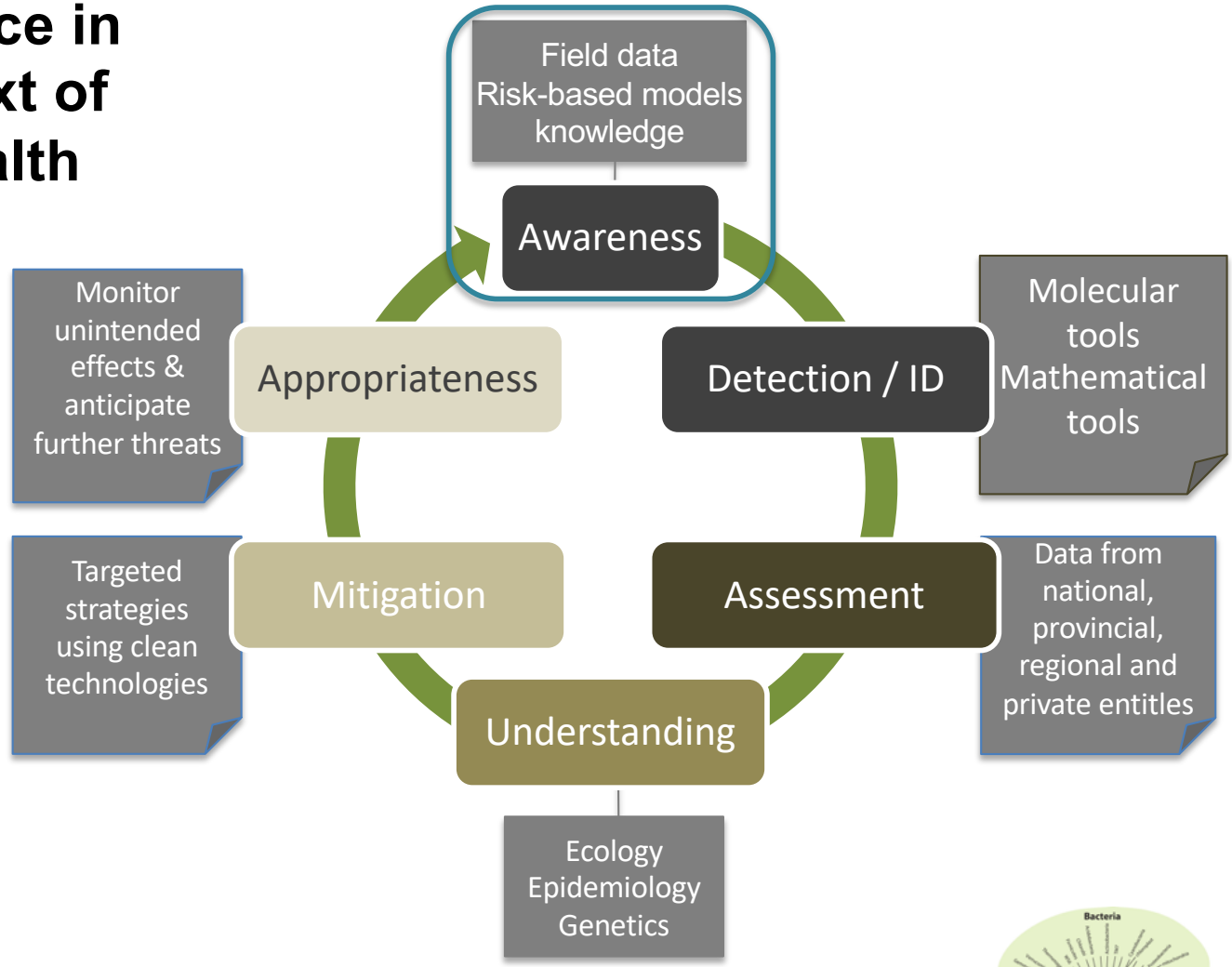
Biovigilance in the context of plant health



Air Sampling

1800/yr

Genetic material

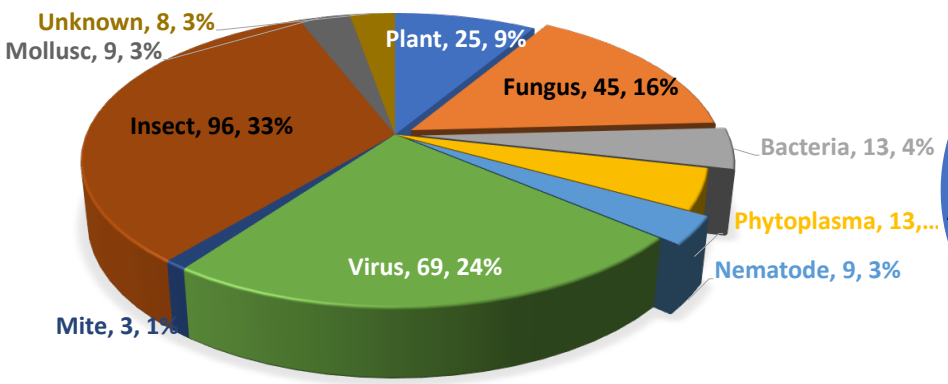
Fungi
bacteria
viruses

Lib. Prep. & HTS

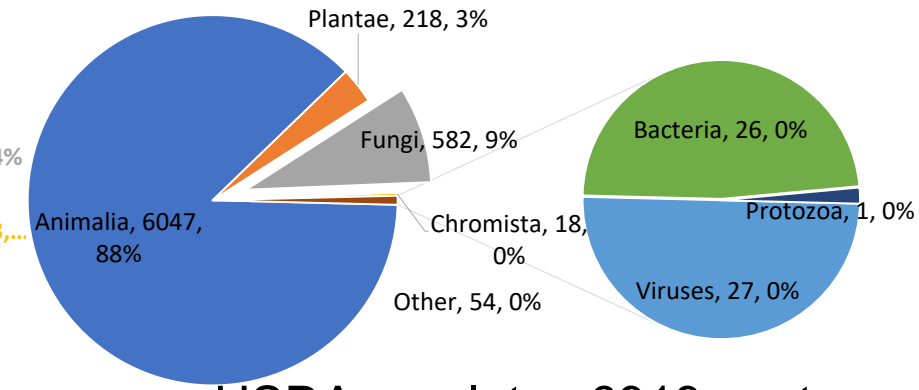
Informatics

Taxonomy ID

Pathogens/pests are identified at species/subspecies levels

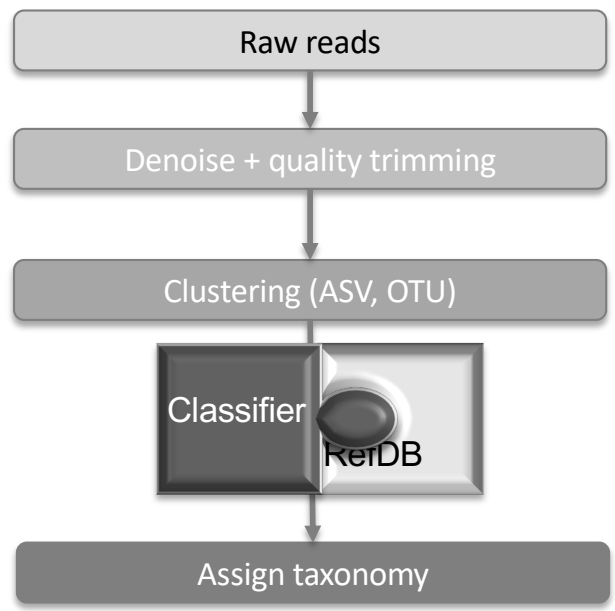


CFIA regulates > 290 pests



USDA regulates 6919 pests

- Commonly used DNA markers have **LOW GENETIC VARIATIONS** between pathogens and close relatives
- Taxonomy annotation in large **PUBLIC** databases are unreliable predictions (Edgar 2018 10.7717/peerj.5030)
- Prediction accuracy by **OFF-THE-SHELF** tools at the genus level is 1.7 – 50.3% for 16S rRNA gene V4, 47.3 – 86.7% for fungal ITS sequences (Edgar 2017&2018, 10.7717/peerj.3889; 10.7717/peerj.4652)
- 40% records in Index Fungorum DB **WITHOUT ANY LOCI** in GenBank (Hibbett, 2016. Mycologia 10.3852/16-130)

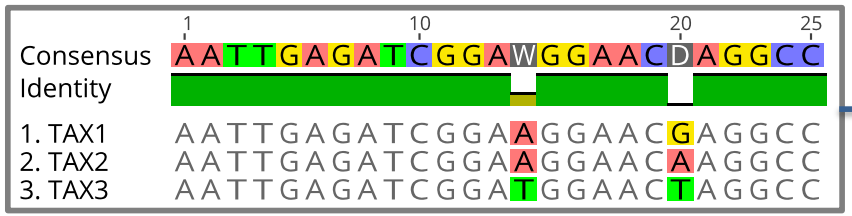


Automated Oligonucleotides Design Pipeline (AODP)

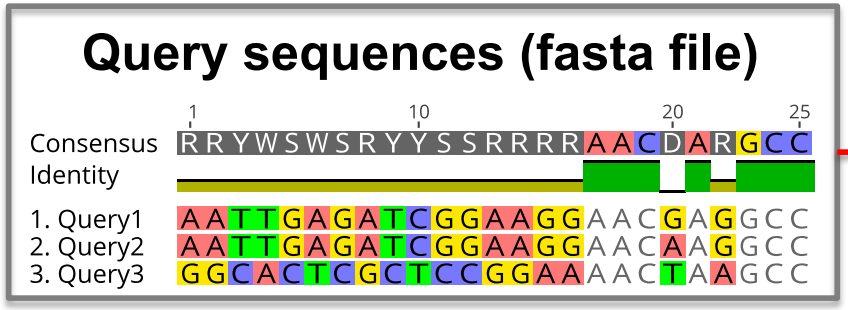
https://bitbucket.org/wenchen_aafc/aodp_v2.0_release

- ❑ Command-line
- ❑ Open source
- ❑ Workshops (CPS)
- ❑ Design signatures (primers and probes)
- ❑ Strain-typing: novel Cluster Signature matching algorithm

Reference DB (fasta file)

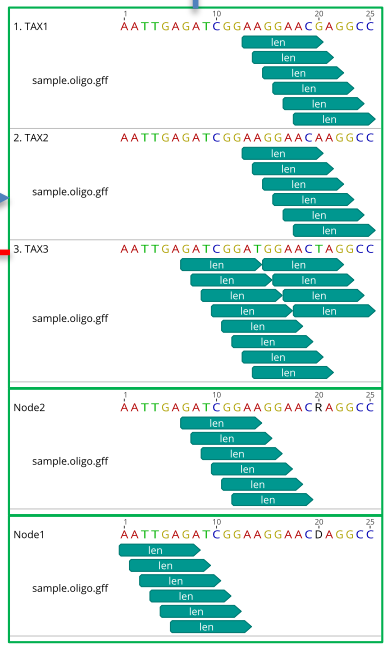


Query sequences (fasta file)



AODP Diagnostic Metagenomics

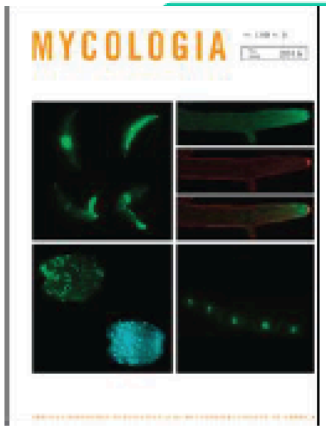
Signatures



Target	Source	prct_identity	cov.	T-len	min-cluster	max-cluster
Query3	-	0.0%	25	25	0	0
Query2	TAX2	100.0%	25	25	1	3
Query1	TAX1	100.0%	25	25	1	3

Zahariev *et al. BMC Bioinformatics* (2018) 19:395
https://doi.org/10.1186/s12859-018-2363-3

BMC Bioinformatics



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Mycologia

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Phylogeny of Canadian ergot fungi and a detection assay by real-time polymerase chain reaction

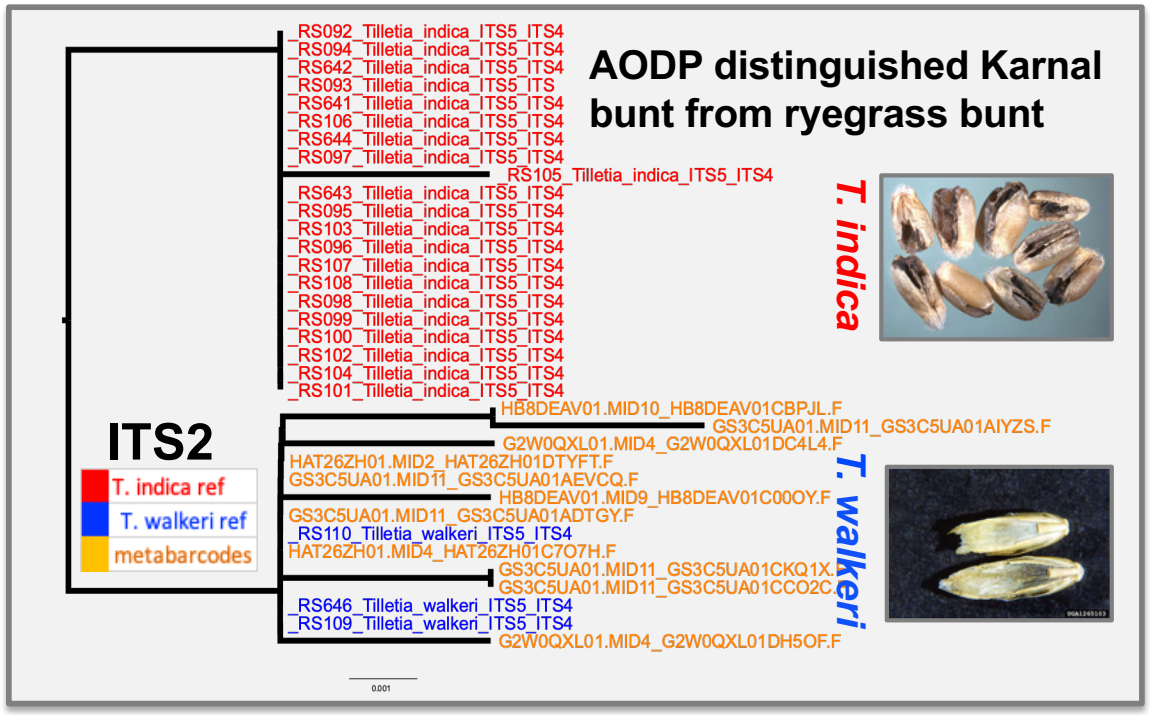
Parivash Shoukouhi, Carmen Hicks, Jim G. Menzies, Zlatko Popovic, Wen Chen, Keith A. Seifert, Rafik Assabgui & Miao Liu

To cite this article: Parivash Shoukouhi, Carmen Hicks, Jim G. Menzies, Zlatko Popovic, Wen Chen, Keith A. Seifert, Rafik Assabgui & Miao Liu (2019): Phylogeny of Canadian ergot fungi and a detection assay by real-time polymerase chain reaction, Mycologia

To link to this article: https://doi.org/10.1080/00275514.2019.1581018

DNA markers and variants from any DNA sequencing dataset. They are particularly useful in discriminating genetic material from closely related organisms and in detecting deleterious mutations in highly or perfectly conserved genomic sites.

Keywords: DNA hybridization, Oligonucleotide signatures, Metabarcoding, Metagenomics, Regulated pathogens



Biosystematics and RefDB development

- **Pucciniales** (rust fungi)
- **Tilletiaceae** (smut)

- **Oomycetes (USDA-AAFC, Drs. H. Nugyen, F. Martin, NJ Grünwald)**

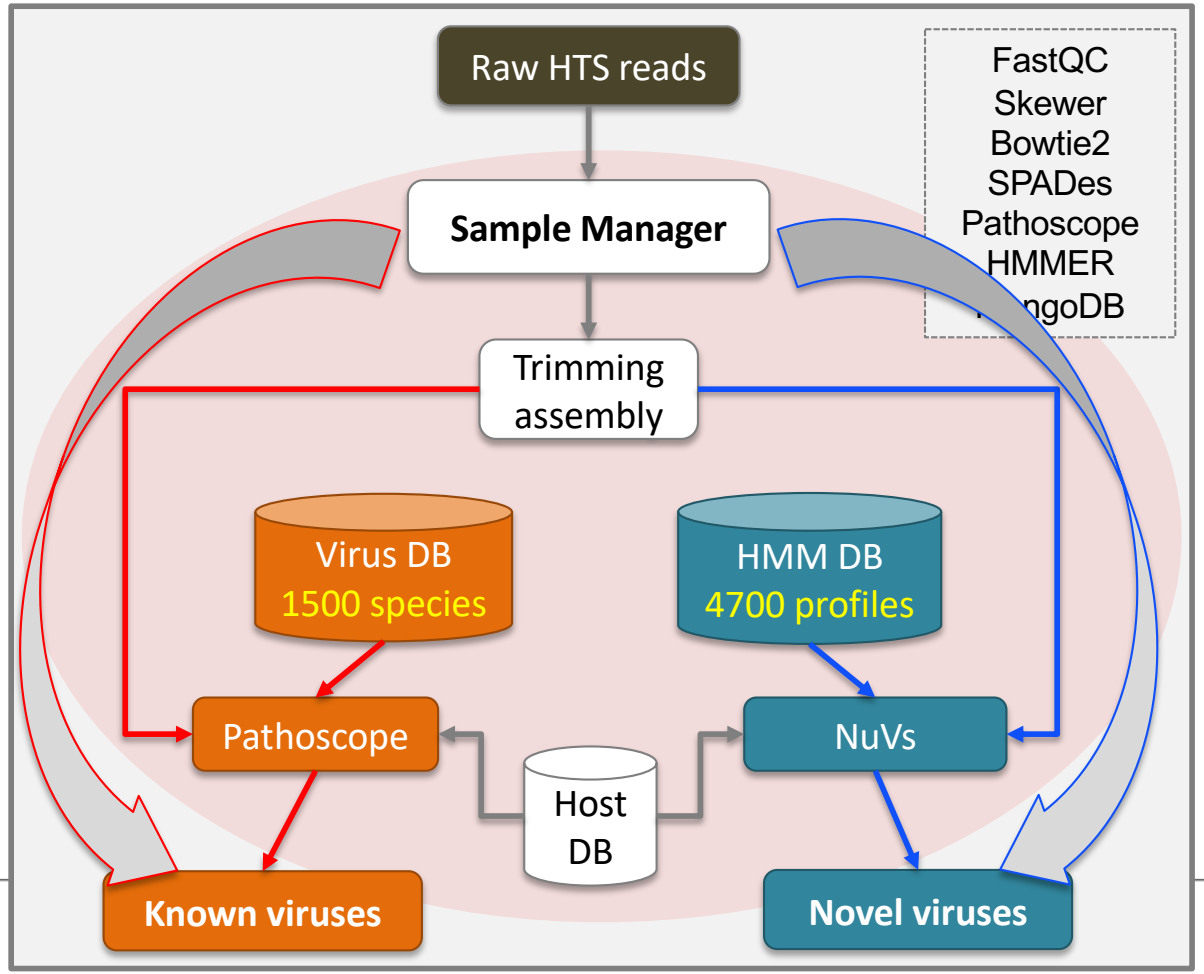
- ***Botrytis* (Carisse, AAFC)**
  - 27 phytopathogenic spp.
  - Broad host range (> 1000)
  - Difficult to control
  - Fungicide-resistance

- ***Sclerotinia* (Chatterton, AAFC)**
  - Broad host range (hundreds)
  - Genetic diversity?
  - Fungicide-resistance in Canada?

gov.mb.ca canolawatch.org naturepl.com

VirTool

- A user friendly **web-based** application for plant virus diagnostics (www.virtool.ca; github.com/virtool/)
- **VirTool - AODP integration** for a broader spectrum detection of phytopathogens (2019, CFIA-funded)



- Tree fruit, grapevines and small fruit
- Border inspections

- dsRNA
- siRNA
- Ribo-RNA
- totRNA

Summary

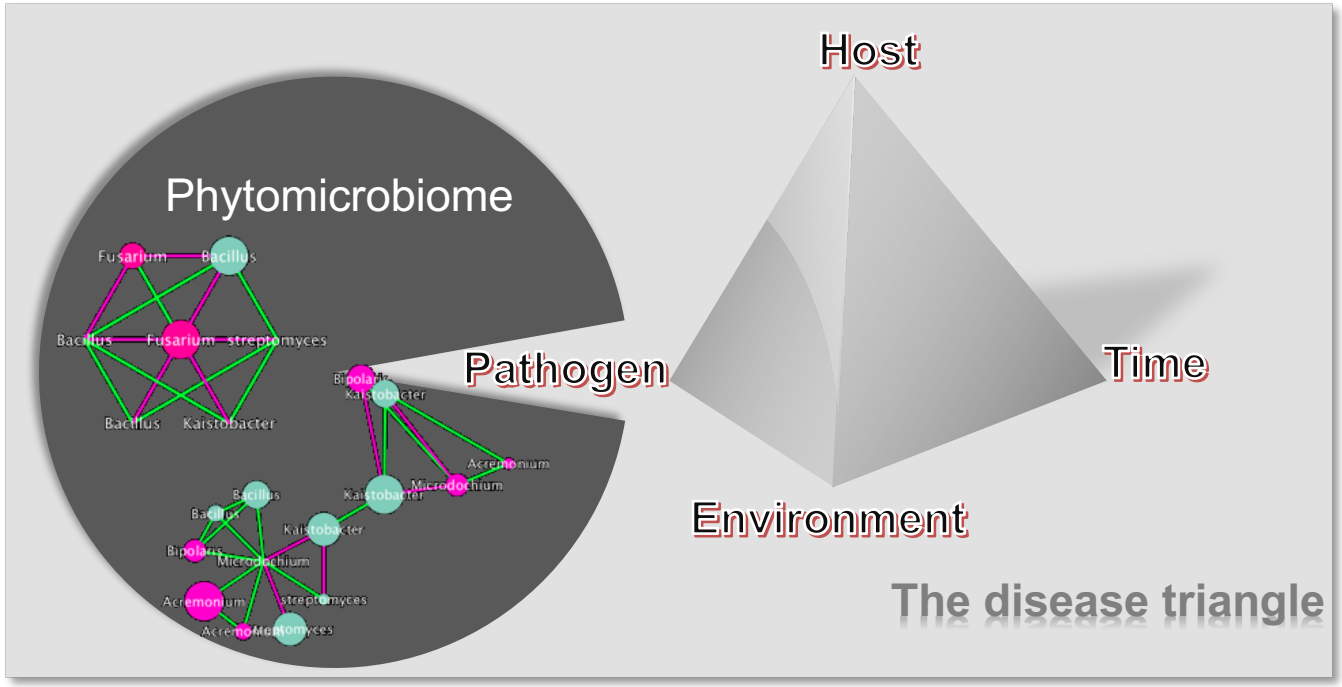
Accurate and rapid detection of known and emerging pathogens

Identification of Antagonists / synergists and environmental drivers

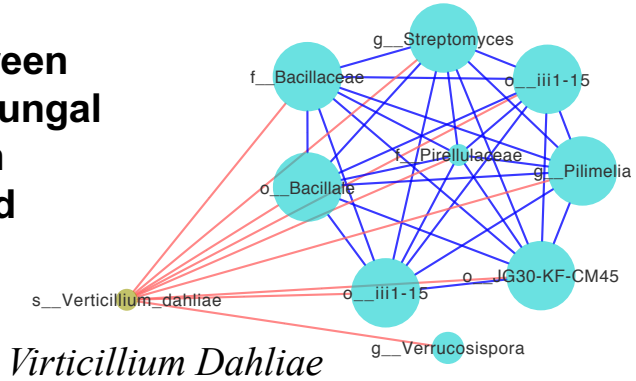
Modeling dissemination of inoculum and disease epidemiology

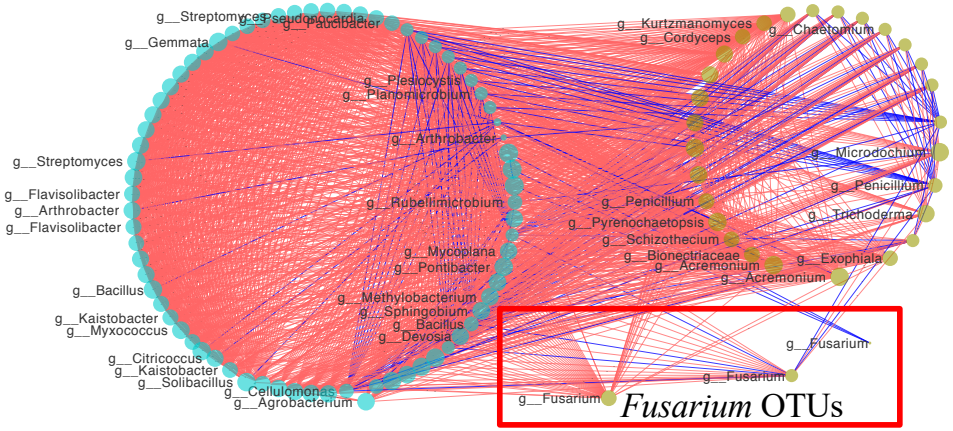
Integrated Pest Management (IPM)

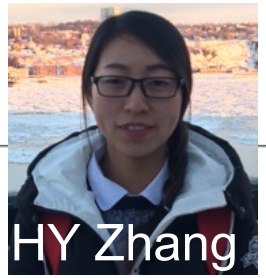
Phytomicrobiome and the disease triangle



- **Associations between bacterial OTUs & fungal plant pathogens in bentonite amended sandy corn fields**







Zhang et al (2019) Amending sandy soils with bentonite induced changes in soil microbiota and fungistasis in continuous cornfields, **ASE** (minor revision submitted)

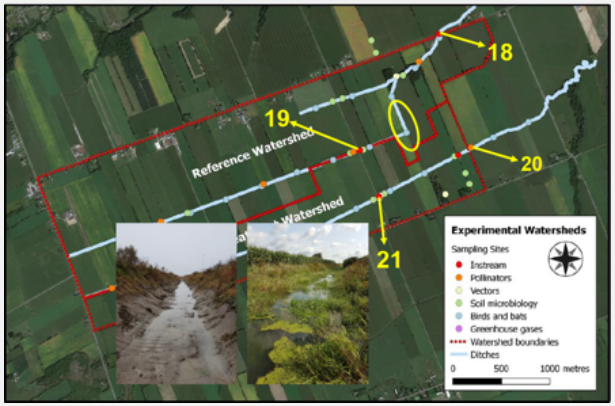
Research foci: Microbiome of Agroecosystems

Soils

Canada (ON)
Northern China
Western France

Bioindicators for
- Soil health
- Agronomy practices
- Climate change
- Plant disease development
- Soil suppressiveness

Water, sediment



Eastern Canada
ON, QC,

Agricultural watersheds
- Ecosystem function
- Agro-ecosys. resilience
- Environmental impacts
- Water quality and one health

Air/rain, endophytes, commodity, food value chain

Across Canada
Northeast/Southern China

Phytopathogens
- Detection of (un)known
- Monitoring and modeling
- Land use changes
- Biocontrol agents
- Decision-making
- Regulatory measures

Microbial Ecology

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frontiers in Microbiology

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Check for updates

Aquatic Bacterial Communities Associated With Land Use and Environmental Factors in Agricultural Landscapes Us Approach

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Geography and agronomical practices drive diversification of the epiphytic mycoflora associated with barley and its malt end product in western Canada

CrossMark

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Relevant publications

Legrand, F., **Chen, W**., Cobo-Díaz, J.F., Picot, A., and Floch, G.L. (2019). Co-occurrence analysis reveal that biotic and abiotic factors influence soil fungistasis against Fusarium graminearum. *FEMS Microbiol. Ecol.* 95(5). doi: 10.1093/femsec/fiz056.

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Legrand, F., Picot, A., Cobo-Díaz, J.F., Carof, M., **Chen, W.,** and Le Floch, G. (2018). Effect of tillage and static abiotic soil properties on microbial diversity. Appl. Soil Ecol. doi: 10.1016/j.apsoil.2018.08.016.

Chen, W*., Wilkes, G., Khan, I.U.H., Pintar, K.D.M., Thomas, J.L., Lévesque, C.A., et al. (2018). Aquatic Bacterial Communities Associated With Land Use and Environmental Factors in Agricultural Landscapes Using a Metabarcoding Approach. Front. Microbiol. 9(2301). doi: 10.3389/fmicb.2018.02301.

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Legrand, F., Picot, A., Cobo-Díaz, J.F.**, Chen, W*** and Le Floch, G. (2017). Challenges facing the biological control strategies for the management of Fusarium Head Blight of cereals caused by F. graminearum. Biological Control 113, 26-38. doi: 10.1016/j.biocontrol.2017.06.011.

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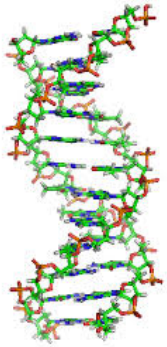
Chen, W.,* Hambleton, S., Seifert, K.A., Levesque, C.A., Carisse, O., Diarra, M.S., et al. (2017). AeroNet: spore samplers in collecting fungal plant pathogens in the air and rain. Oral Presentation. Canadian Journal of Plant Pathology 39(4), 551. doi: 10.1080/07060661.2017.1386378.

Chen, W.*, Turkington, T.K., Lévesque, C.A., Bamforth, J.M., Patrick, S.K., Lewis, C.T., et al. (2016). Geography and agronomical practices drive diversification of the epiphytic mycoflora associated with barley and its malt end product in western Canada. Agriculture, Ecosystems & Environment 226, 43-55

Acknowledgements



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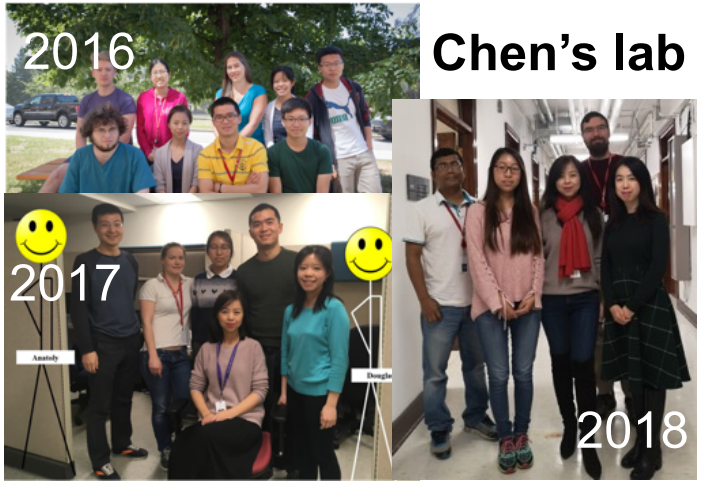

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