Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Genome-enhanced detection and identification of plant pathogens

Guillaume J. Bilodeau[1]

[1]*Canadian Food Inspection Agency (CFIA), Ottawa, ON, Canada.*

EPPO/Euphresco Scientific Colloquium

Plant Health at the Age of Metagenomics

UNESCO, Paris, France, 26th of September 2019

Canada

Presentation Overview

http://www.formavox.com/

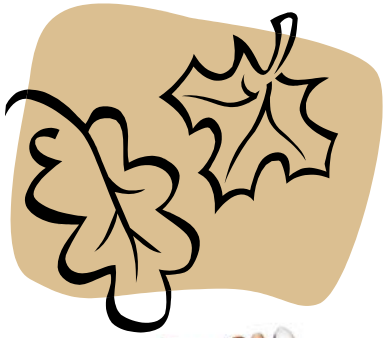
1. Bio/Background
2. Genomics Applications in regulatory research at CFIA
    A. Fungi ID and detection: 2 cases
    B. Metagenomics (briefly)
3. Other research activities

https://towardsdatascience.com/dna-sequence-data-analysis-starting-off-in-bioinformatics-3dba4cea04f

Ottawa Plant Laboratory (OPL)

<u>Ottawa Plant Laboratoy (Fallowfield)</u>

- Nematology
- Genotyping / Botany
- Molecular Identification Research (MIRL)
- Seed Science
- Entomology (Carling)
- Plant Pathology Diagnostic
- ***Plant Pathology Research (PIRL)***



Plant-Pathogen Identification Research Lab (PIRL)

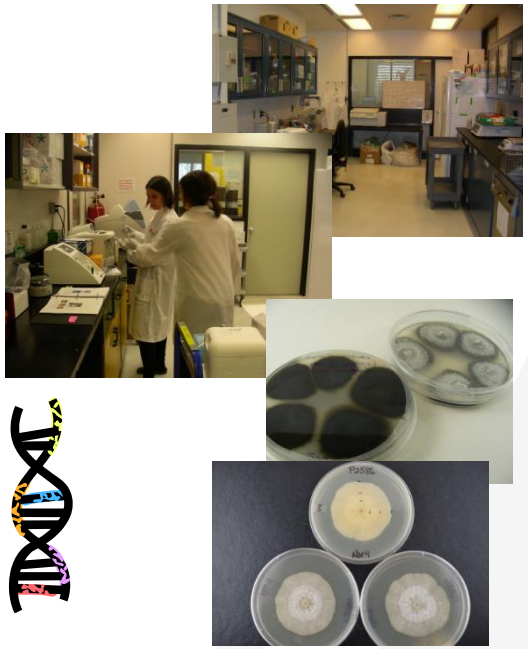
- Support the OPL diagnostic section through research and technology transfer
- Provide scientists and other professionals with information in support of regulatory decision making.

- Detection and identification of plant pests (fungi-oomycetes) of regulatory significance (Forestry and Agriculture)

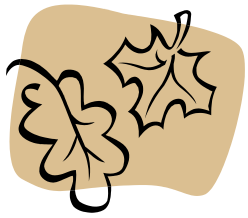
- Expertise: Fungal detection and genotyping, *Phytophthora, Verticillium*, Molecular biology (PCR, Real-time PCR, qPCR, Isothermal amplification, Sequencing, ASO, SSR), genomics, metagenomics and microfluidics

Quarantine organisms

Import/Export materials



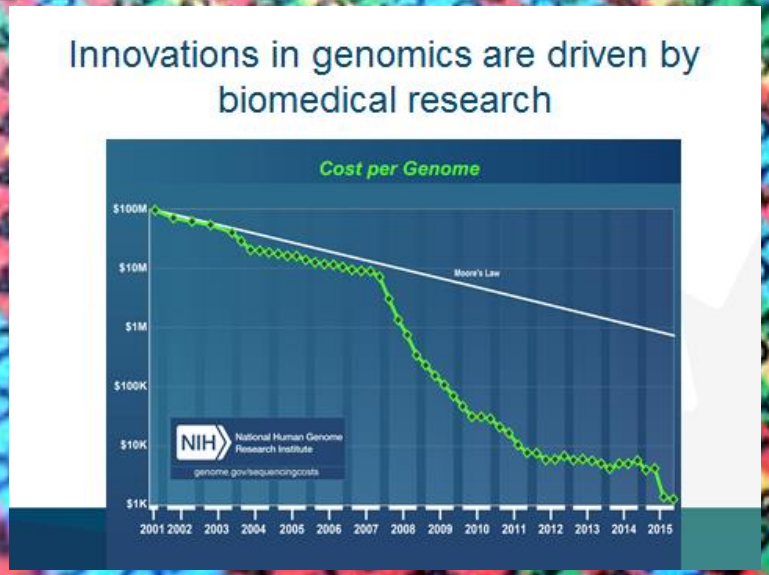
Plant Protection Act & associated regulations

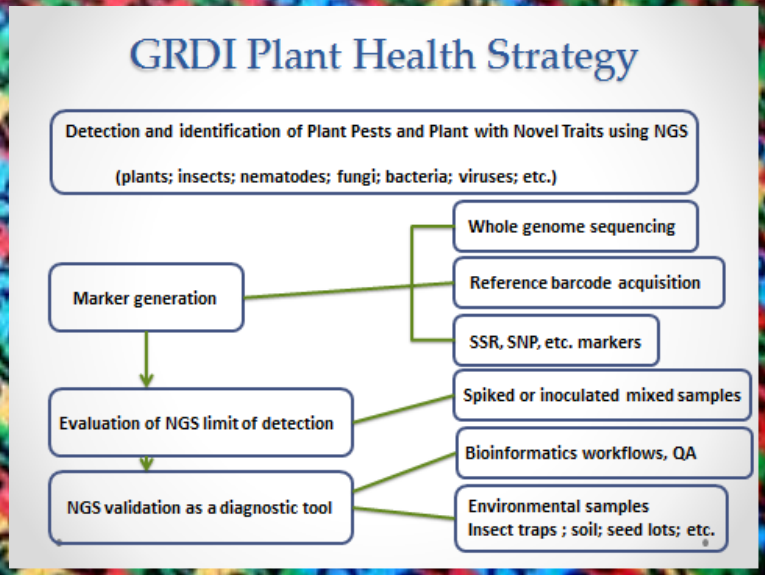


Objectives are;

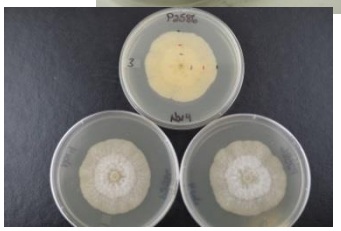
- To prevent the introduction and spread within Canada of plant pests of quarantine significance
- To detect and control or eradicate designated plant pests in Canada
- To certify plant and plant products for domestic and export trade

The remarkable advances in genomics offer a solution to diagnostics





A: Fungi detection & ID



Project Objectives:

1. Demonstrate proof of concept for HTS:
   - Useful for detection and genotyping of targeted species
   - Can be used for the development of specific markers for use in Plant Pathology diagnostic lab.

2. Demonstrate the proof of concept for some pathways and sampling methods as source of targeted pathogenic fungi to provide metagenomic info and ID hotspot areas useful for the Agency

3. Support diagnostic lab work with those newly-developed qPCR and HTS methods to facilitate the identification and detection

Proactive

Assay development

- Sequencing of the Internal Transcribed Spacer (ITS) and Intergenic Spacer (IGS) of ribosomal rDNA usually region for Fungi for designing PCR primers.

Figure : Ribosomal rDNA regions.

- *However it does not work for all fungi, species or subspecies.*
- *Species complex.*

Genomic resources

- Genomics applies next-generation sequencing methods and bioinformatics to sequence, assemble, and analyze the function and structure of genomes.
- Provides tons of data that can be used to answer biological questions and to develop diagnostic assays.

- Possible applications in plant pathology:
  - Development of large number of markers and assays.
  - Search for avirulence/resistance genes.
  - Large-scale ID and detection.
  - Metagenomics.

I-Genome-enhanced detection and identification (GEDI)

Feau et al. (2018), Genome-Enhanced Detection and Identification (GEDI) of plant pathogens. PeerJ 6:e4392; DOI 10.7717/peerj.4392

PeerJ

Genome-Enhanced Detection and Identification (GEDI) of plant pathogens

Nicolas Feau[1], Stéphanie Beauseigle[2], Marie-Josée Bergeron[3], Guillaume J. Bilodeau[4], Inanc Birol[5], Sandra Cervantes-Arango[1], Braham Dhillon[6], Angela L. Dale[1,7], Padmini Herath[1], Steven J.M. Jones[5,8,9], Josyanne Lamarche[3], Dario I. Ojeda[10], Monique L. Sakalidis[11], Greg Taylor[5], Clement K.M. Tsui[12], Adnan Uzunovic[7], Hesther Yueh[1], Philippe Tanguay[3] and Richard C. Hamelin[1,13]

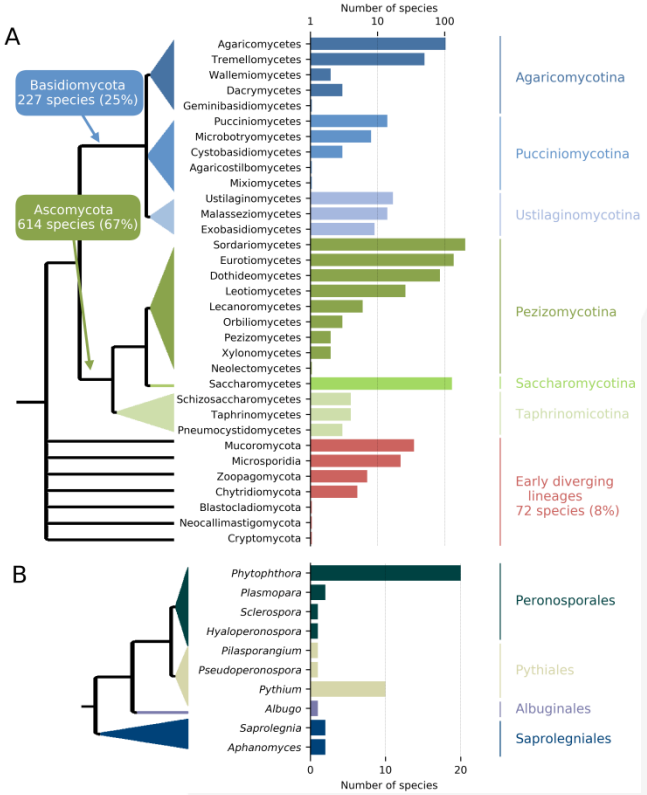
[1] Department of Forest and Conservation Sciences, Forest Sciences Centre, University of British Columbia,

- Required access to assembled and annotated genomes of the targeted organisms as well as closely related taxa.

- Genomes of target and related non-target taxa are required
(e.g., same genus and order).

- Moreover, de novo genome assemblies and full protein sets are either produced and assembled for the targeted species under investigation and for a group of related species or recovered from public genome data repositories.
    - NCBI & fungal genome sequencing initiatives such as the Mycocosm and the 1000 Fungal Genomes project (http://1000.fungalgenomes.org/home/).

- Next-generation or High throughput sequencing (NGS-HTS) technologies constitute a fast and cost-effective way of obtaining whole genome sequences, particularly in eukaryotic organisms.



Number and phylogenic coverage of fungal (A) and Oomycete (B) genomes available on the
NCBI public database

The TAIGA (Tree Aggressors Identification using Genomic Approaches)
http://taigaforesthealth.com/

I-Genome-enhanced detection and identification (GEDI)

Feau et al. (2018), Genome-Enhanced Detection and Identification (GEDI) of plant pathogens. PeerJ 6:e4392; DOI 10.7717/peerj.4392

- The underlying principle of our method is to compare the protein content within the genomes of phylogenetically related taxa to ensure the selection of targets that are discriminant towards the most closely related known species.

- Our bioinformatics pipeline is divided into four modules (Fig. 1).
  - Module 1: genomic resources
  - Module 2: discovering homologous gene clusters
  - Module 3: filtering false positives
  - Module 4: assay design

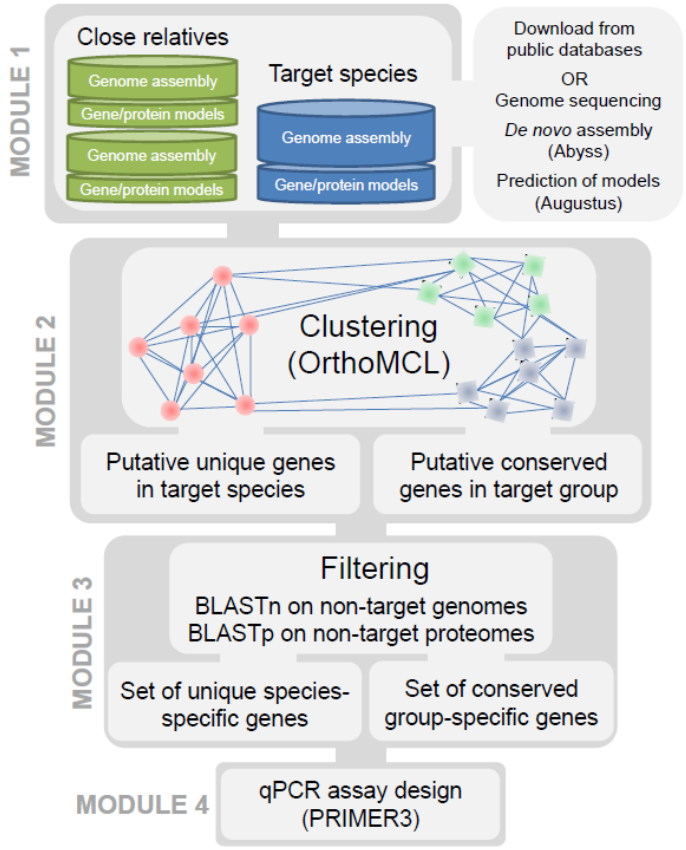


Figure 1 Pipeline for development of qPCR assays using whole genomes.
Full-size DOI: 10.7717/peerj.4392/fig-1

The TAIGA (Tree Aggressors Identification using Genomic Approaches)
http://taigaforesthealth.com/

I-Genome-enhanced detection and identification (GEDI)

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Pipeline run on Phytophthora, Dothideomycetes, Pucciniales

- ***Genus, Species, Lineages-clades***

Ex)
M1:P. ramorum
PINFa, PSOJ, PLAT,
PCAP,PCIN, PHIB, PFOL

M2: # OrthoMCL
Clusters: 52,280
OrthoMCL
unique clusters: 1,624
(3.1%)
# of unique

M3: Clusters:37

Table 3 Experimental screening of the candidate clusters unique to species or group of taxa.

Targeted taxa	# tested targeted taxa	# tested non-targeted taxa	# candidate genes tested	# success
Phytophthora				
P. ramorum	11 *P. ramorum*	40 *Phytophthora* spp.	28	5 (17.9%)
P. lateralis	4 *P. lateralis*	40 *Phytophthora* spp.	16	6 (37.5%)
P. kernoviae	1 *P. kernoviae*	22 *Phytophthora* spp.	12	9 (75.0%)
P. ramorum + *P. lateralis*	11 *P. ramorum*, 4 *P. lateralis*	39 *Phytophthora* spp.	19	5 (26.3%)
Dothideomycetes				
Sphaerulina musiva	2 *S. musiva*	14 *Mycosphaerella* spp.	51	14 (27.5%)
S. populicola	2 *S. populicola*	14 *Mycosphaerella* spp.	65	16 (24.6%)
Phaeocryptopus gaeumannii	10 *P. gaeumannii*	14 *Mycosphaerella* spp.	10	3 (30%)
S. musiva + *S. populicola*	2. *S. musiva*, 2 *S. populicola*	12 *Mycosphaerella* spp.	39	13 (33.3%)
S. musiva + *S. populicola* + *Mycosphaerella* sp. STON1	2. *S. musiva*, 2 *S. populicola*, 1 *Mycosphaerella* sp. STON1	11 *Mycosphaerella* spp.	6	2 (33.3%)
Rusts				
Melampsora larici-populina	13 *M. larici-populina*	15 *Melampsora* spp., 1 *Coleosporium* sp., 1 *Pucciniastrum* sp., 1 *Cronartium* sp., 2 *Chrysomyxa* spp.	10	2 (20%)
M. medusae f. sp. *deltoidae*	10 *M. medusae*	15 *Melampsora* spp., 1 *Coleosporium* sp., 1 *Pucciniastrum* sp., 1 *Cronartium* sp., 2 *Chrysomyxa* spp.	10	2 (20%)
Cronartium ribicola	10 *C. ribicola*	10 *Cronartium* spp., 5 *Melampsora* spp., 3 *Coleosporium* spp., 3 *Pucciniastrum* spp., 2 *Chrysomyxa* spp.	20	3 (15%)
Melampsora genus	19 *Melampsora* spp.	2 *Coleosporium* spp., 3 *Pucciniastrum* spp., 3 *Cronartium* spp., 3 *Chrysomyxa* spp.	5	3 (60%)
Cronartium genus	11 *Cronartium* spp.	8 *Melampsora* spp., 4 *Coleosporium* spp., 5 *Pucciniastrum* spp., 7 *Chrysomyxa* spp.	8	2 (25%)

II- Whole genome sequencing for identification of molecular markers to develop diagnostic detection tools for the regulated plant pathogen

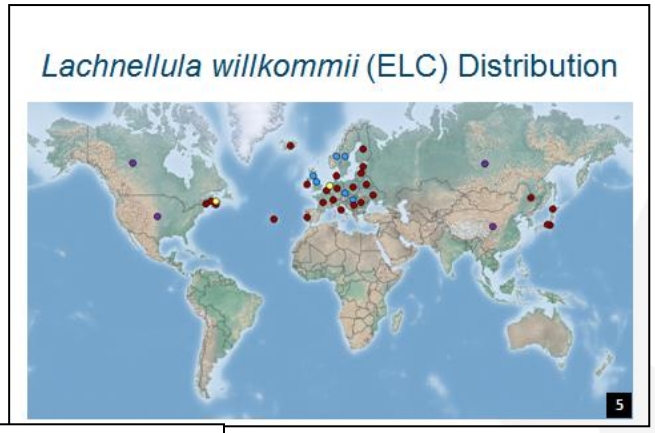
Identification assays have been developed using genomic strategies targeting *Lachnellula willkommii*, European Larch Canker; *Phytophthora ramorum*, Sudden Oak Death; and *Fusarium sporotrichioides* in peas, among others.

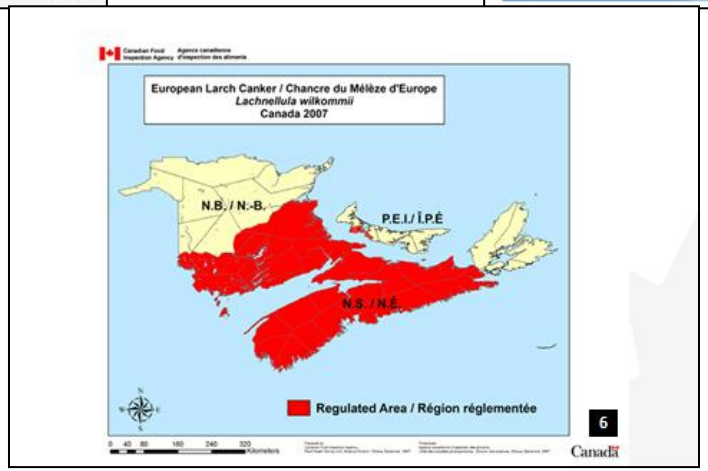
CFIA Ottawa Laboratory Fallowfield (OLF)

- Access to different platform technologies
  - Ion Torrent (PGM and S5)
  - MiSeq, Illumina
  - MinIon, Oxford nanopore
- Identify organism with discussion with diagnostic lab about the need of new markers for easier or high throughput detection.
  - Complex of species, organism not so much info, ITS or region, no much variations. Increase toolbox, ID demand in new export.
  - Ex) *Lachnellula, Fusarium, Colletotrichum, Verticillium, Phytophthora, Synchetrium, Phoma, Phomopsis* …

Test case

Lachnellula willkommii, European Larch Canker (Model test organisms)





3-Andrej Kunca, National Forest Centre - Slovakia, www.forestryimages.org
4-Petr Kapitola, Forestry and Game Management Research Institute - Czechia, www.forestryimages.org
5-Systematic Mycology & Microbiology Laboratory, USDA-ARS, 10300 Baltimore Ave., Beltsville, MD 20705, USA, https://www.cabi.org/isc/datasheet/30017
6-CFIA Plant Health Risk Assessment Unit. 2011. Plant Health Risk Assessment: Lachnellula willkommii (Hartig) Dennis European Larch Canker. Request No. 2009-16.

Diagnostic Identification

- Filamentous ascomycete
  - Family Hyaloscyphaceae (Order Helotiales, Genus *Lachnellula*)
    - Most saprophytic
    - Few parasitic
- Morphology – process of elimination
- Can't differentiate pathogenic *L. willkommii* from saprophyte *L. occidentalis*
- Molecular – unpublished RAPD protocol (K. J. Harrison, L. L. DeVerno, R. C. Hamelin and T. Burton)
  - Limited pathogen differentiation
- ITS primers and probes
  - Genus level detection
  - Region too conserved for development of species-specific probes

Molecular Method Challenges

- Polysaccharide-rich cell walls limits DNA extraction, yield and purity
- Growth media for optimal fungal growth with limited production of polysaccharide-rich fruiting bodies

Growth media and DNA extraction optimizations:

- Growth on PDA with water rinses of mats
- DNA extraction – CTAB method
  - Samples split into multiple subsamples after homogenization
  - 2 $CHCl_3$ extractions
  - Adjustments to incubation times, and [EtOH] for washes

Library preparation optimizations:

- Ion Torrent platform
  - Library preparation optimization
    - Enzymatic shearing $\rightarrow$ mechanical (Covaris)
    - Bead wash, library amplification & quantitation adjustments

Genome Sequencing

Sequenced 7 Lachnellula species

Ion Torrent (PGM) sequencing with Newbler assembly

Species	Raw Reads	Raw Bases	Processed Reads	Processed Bases	Newbler Assembly		
					# Contigs	N50	~ Gen. Size
L. willkommii	3.1 M	0.7 G	2.7 M	0.6 G	13,593 (80,405 bp)	8,685	48.6 Mb
L. occidentalis	6.1 M	1.6 G	5.8 M	1.6 G	3,682 (252,529 bp)	31,280	48.1 Mb
L. subtillissima	4.0 M	1.0 G	3.7 M	1.0 G	2,040 (189,346 bp)	34,100	35.1 Mb
L. suecica	4.4 M	1.1 G	4.0 M	1.1 G	4,381 (115,910 bp)	19,179	43.2 Mb
L. arida	5.4 M	1.5 G	5.2 M	1.5 G	3,229 (187,565 bp)	38,138	42.4 Mb
L. hyalina	6.0 M	1.5 G	5.7 M	1.5 G	585 (531,425 bp)	161,844	33.8 Mb
L. cervina	4.8 M	1.2 G	4.6 M	1.2 G	8,607 (135,220 bp)	23,221	50.0 Mb

Genome and Assembly Pipeline

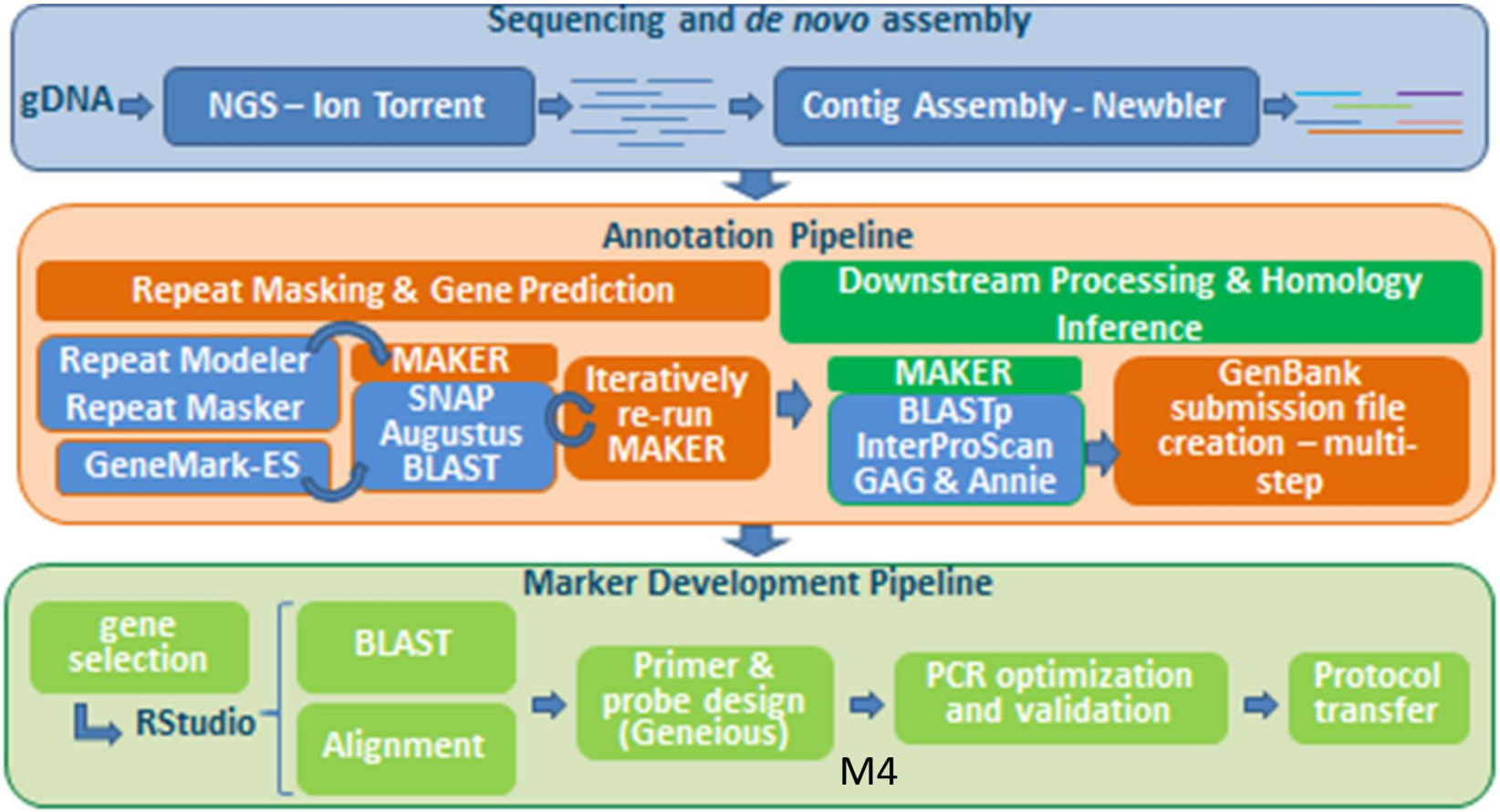
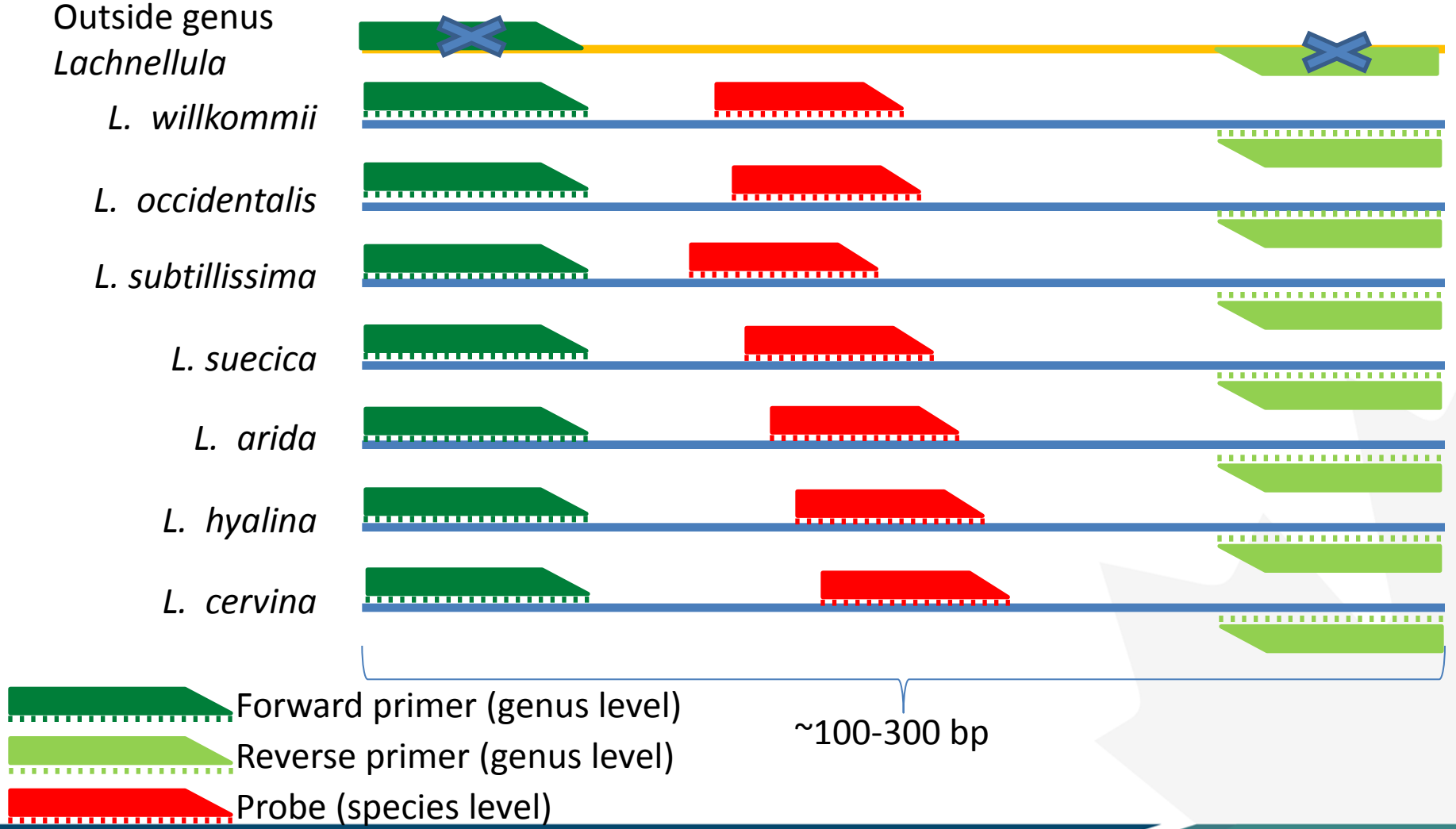


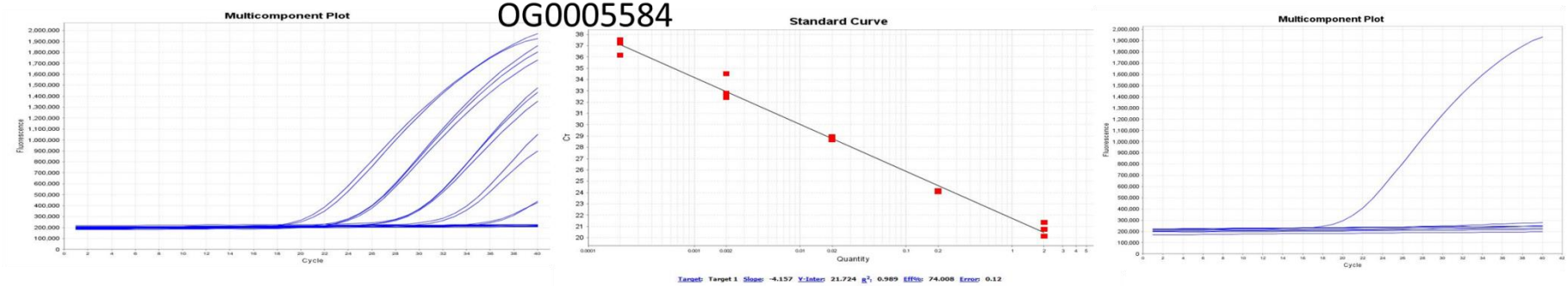
Figure 2: Genome Sequencing, Assembly and Annotation Pipeline.

Marker Identification



Multilocus marker Validation

OG0005584



OG0004581

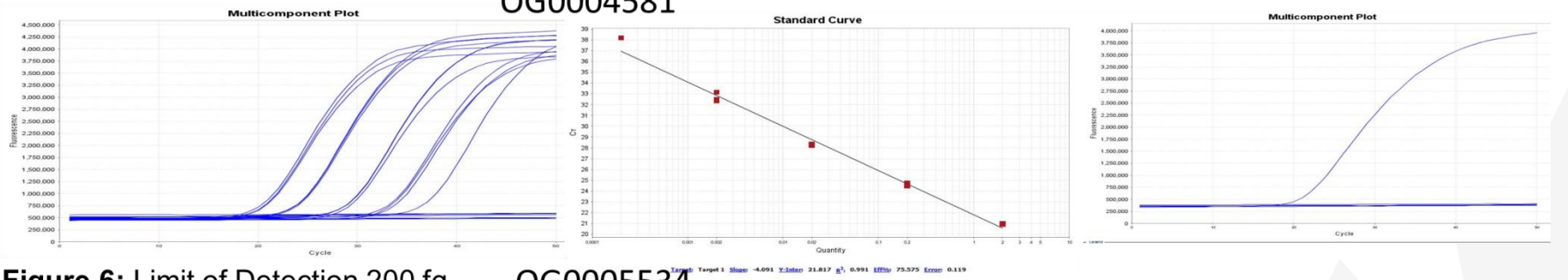


Figure 6: Limit of Detection 200 fg

OG0005534

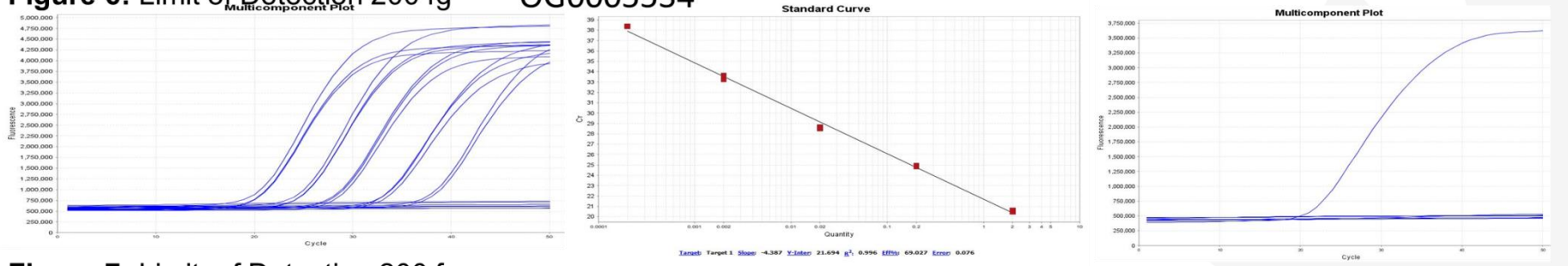
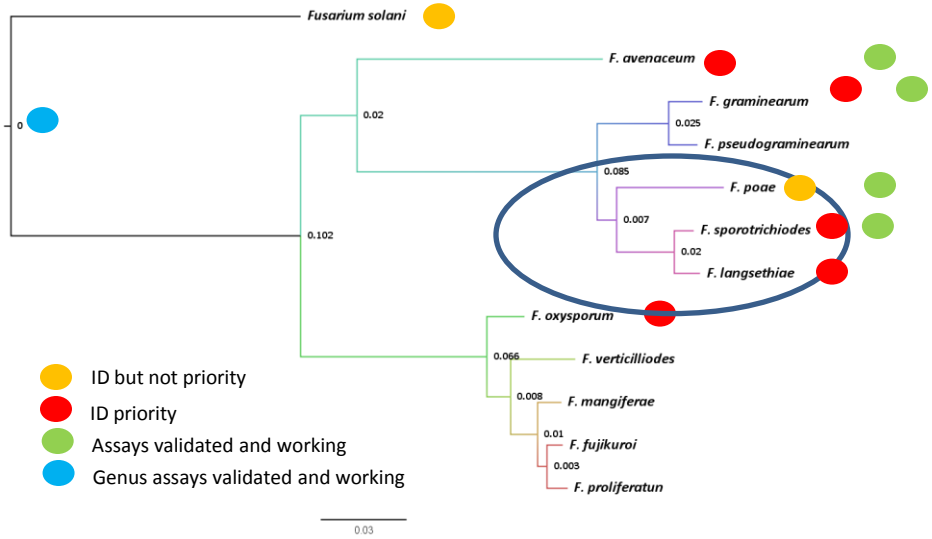
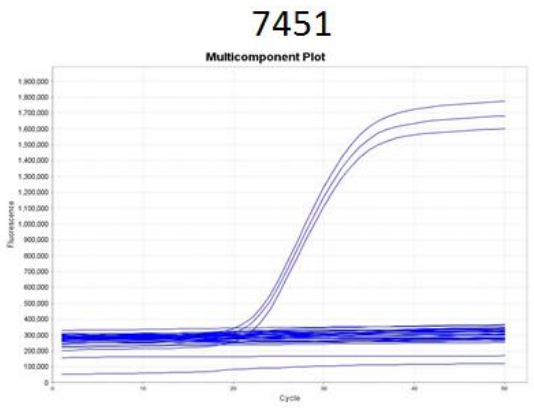
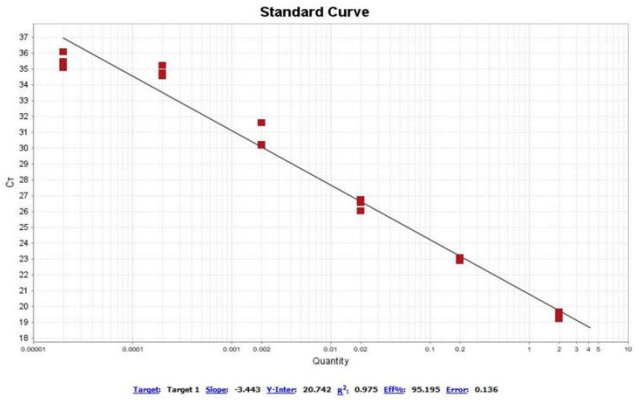


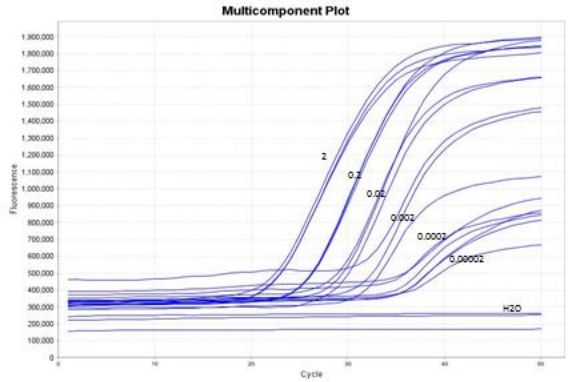
Figure 7: Limit of Detection 200 fg

Fusarium sporotrochiodes and others









Summary

- Molecular marker development for *Lachnellula* spp. detection and identification = test case/ Same for the 3 other forest pathogens group
- Demonstrate the efficiency and efficacy of WGS for marker identification as a reliable approach for developing diagnostic tools for our agency
- Provide us with an established method for the design of molecular markers for other plant pathogens, DNA prep and bioinfo. pipelines

Conclusions

- Focus on development of molecular markers by exploiting WGS data
  - Rapid, sensitive, fills the gap
  - DNA yield and quality no longer an issue
- Addresses bioinformatics bottleneck
- Method transferrable to detection of other pathogens (ex:Colletotrichum, Tilletia, …)
- Prepared for pathogen identification and diagnostic need
- <u>Preparedness</u>

B-Metagenomics

- Genomics, sequences and information of an organism; A genome is an organism's complete set of DNA (nucleic acid), including all of its genes.

- Metagenomics, is the study of genomic content in a complex mixture of microorganisms. The field of metagenomics has also been referred to as environmental genomics, ecogenomics, and community genomics.

- Study of genetic material recovered directly from environmental samples.

SciGenom

Objective 2

Proof of concept for some pathways and sampling methods as source of targeted pathogenic fungi to provide metagenomic info and ID hotspot areas useful for the Agency.

- *Difficult to survey for fungi not attracted like insect in insect traps with attractant.*
- *But now with HTS…*

DNA-based bio-surveillance

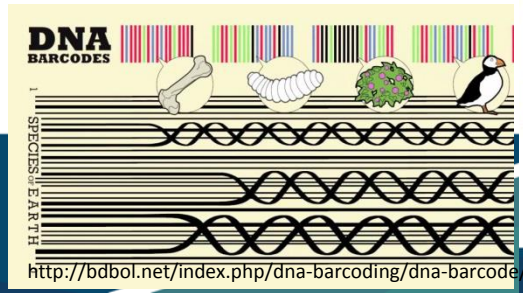
Environmental or commodity samples

Next generation sequencing

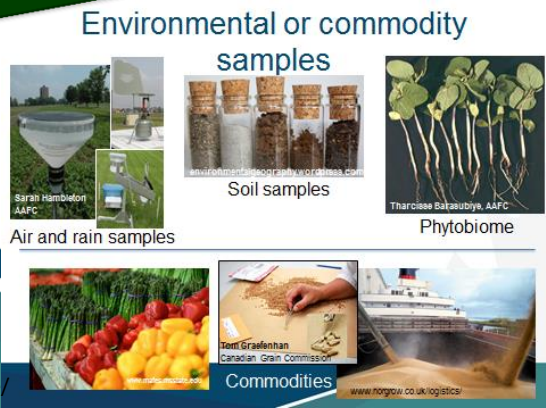
Data analysis & Bioinformatics

Matching Reference collections

Regulatory decision or recommendation



http://bdbol.net/index.php/dna-barcoding/dna-barcode/



NOVEL METHOD OF DETERMINING POTENTIAL INVASIVE FUNGAL PHYTOPATHOGENS AND PLANTS BY METABARCODING

Dre. Émilie Tremblay, CFIA and U. Laval

Phytopathology • 2018 • 108:1509-1521 • https://doi.org/10.1094/PHYTO-02-18-0028-R

Techniques

e-Xtra*

Screening for Exotic Forest Pathogens to Increase Survey Capacity Using Metagenomics

Émilie D. Tremblay, Marc-Olivier Duceppe, Jean A. Bérubé, Troy Kimoto, Claude Lemieux, and Guillaume J. Bilodeau†

First, second, and sixth authors: Canadian Food Inspection Agency (CFIA), 3851 Fallowfield Road, Ottawa, Ontario, K2H 8P9, Canada; third author: Natural Resources Canada, Laurentian Forestry Centre, 1055 Du P.E.P.S. Street, P.O. Box 10380 Québec, Québec, G1V 4C7, Canada; fourth author: CFIA, 4321 Still Creek Dr, Burnaby, British Columbia, V5C 6S7, Canada; and fifth author: Institut de biologie intégrative et des systèmes, 1030 avenue de la Médecine, Québec, Québec, G1V 0A6, Canada.

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ABSTRACT

Anthropogenic activities have a major impact on the global environment. Canada's natural resources are threatened by the spread of fungal pathogens, which is facilitated by agricultural practices and international trade. Fungi are introduced to new environments and sometimes become established, in which case they can cause disease outbreaks resulting in extensive forest decline. Here, we describe how a nationwide sample collection strategy coupled to next-generation sequencing (NGS) (i.e., metagenomics) can achieve fast and comprehensive screening for exotic invasive species. This methodology can help provide guidance to phytopathology stakeholders such as regulatory

using customized fungi-specific ribosomal internal transcribed spacer 1 barcoded primers was performed. Likewise, *Phytophthora*-specific barcoded primers were used to amplify the adenosine triphosphate synthase subunit 9–nicotinamide adenine dinucleotide dehydrogenase subunit 9 spacer. Several *Phytophthora* spp. were detected by NGS and confirmed by species-specific quantitative polymerase chain reaction (qPCR) assays. The target species *Heterobasidion annosum* sensu stricto could be detected only through metagenomics. We demonstrated that screening target species using a variety of sampling techniques and NGS—the results of which were

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DOI: 10.1002/edn3.17

ORIGINAL ARTICLE

Environmental DNA

WILEY

High-resolution biomonitoring of plant pathogens and plant species using metabarcoding of pollen pellet contents collected from a honey bee hive

Émilie D. Tremblay | Marc-Olivier Duceppe | Graham B. Thurston | Marie-Claude Gagnon | Marie-José Côté | Guillaume J. Bilodeau

Canadian Food Inspection Agency, Ottawa, Ontario, Canada

Correspondence
Guillaume Bilodeau, Canadian Food Inspection Agency, 3851 Fallowfield Road, Nepean, Ontario, Canada K2H 8P9.
Email: Guillaume.Bilodeau@canada.ca

Funding information
Canadian Food Inspection Agency, Grant/Award Number: OLF-P-1606 and OLF-P-1411

Abstract

The Canadian beekeeping industry is spread across the country, with the greatest proportion of managed honey bee colonies occurring in the Prairie Provinces. Nationally, the number of beekeepers has recently been trending upwards. Simultaneously, agronomic and environmental plant pest incidents are increasing due to a number of factors, including the introduction of exotic organisms through international trade, which is a major pathway for the introduction of potentially invasive alien species and quarantine pests. Therefore, regulatory agencies are interested in developing high-throughput tools to achieve earlier detection of unwanted species in order to

Journal of Fungi

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Article

High-Throughput Sequencing to Investigate Phytopathogenic Fungal Propagules Caught in Baited Insect Traps

Émilie D. Tremblay [1], Troy Kimoto [2], Jean A. Bérubé [3] and Guillaume J. Bilodeau [1,*]

[1] Canadian Food Inspection Agency, 3851 Fallowfield Road, Nepean, ON, K2H 8P9, Canada; Emilie.Tremblay@canada.ca
[2] Canadian Food Inspection Agency, 4321 Still Creek Dr, Burnaby, BC, V5C 6S7, Canada; Troy.Kimoto@canada.ca
[3] Natural Resources Canada, Laurentian Forestry Centre, 1055 Du P.E.P.S. Street, P.O. Box 10380 Québec, QC, G1V 4C7, Canada; Jean.Berube@canada.ca
* Correspondence: Guillaume.Bilodeau@canada.ca; Tel.: +1-343-212-0283

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Abstract: Studying the means of dispersal of plant pathogens is crucial to better understand the dynamic interactions involved in plant infections. On one hand, entomologists rely mostly on both traditional molecular methods and morphological characteristics, to identify pests. On the other hand, high-throughput sequencing (HTS) is becoming the go-to avenue for scientists studying phytopathogens. These organisms sometimes infect plants, together with insects. Considering the growing number of exotic insect introductions in Canada, forest pest-management efforts would benefit from the development of a high-throughput strategy to investigate the phytopathogenic fungal and oomycete species interacting with wood-boring insects. We recycled formerly discarded preservative fluids from the Canadian Food Inspection Agency annual survey using insect traps and analysed more than one hundred samples originating from across Canada. Using the Ion Torrent Personal Genome Machine (PGM) HTS technology and fusion primers, we performed metabarcoding to screen unwanted fungi and oomycetes species, including *Phytophthora* spp. Community profiling was conducted on the four different wood-boring, insect-attracting semiochemicals; although the preservative (contained ethanol) also attracted other insects. Phytopathogenic fungi (e.g., *Leptographium* spp. and *Meria laricis* in the pine sawyer semiochemical) and oomycetes (mainly *Peronospora* spp. and *Pythium* aff. *hypogynum* in the General Longhorn semiochemical), solely associated with one of the four types of semiochemicals, were detected. This project demonstrated that the insect traps' semiochemical microbiome represents a new and powerful matrix for screening phytopathogens. Compared to traditional diagnostic techniques, the fluids allowed for a faster and higher throughput assessment of the biodiversity contained within. Additionally, minimal modifications to this approach would allow it to be used in other phytopathology fields.

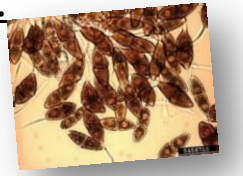
Keywords: insects; vectors; forest; fungi; metagenomics; HTS; oomycete

1. Introduction

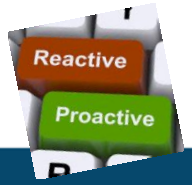
The Era of Globalization has dramatically and consistently increased international cargo shipments since 1970 [1,2]. Solid wood packaging material (SWPM), such as pallets, crates, and boxes are used to transport products all over the world. Bark and wood-boring insects, such as bark beetles, long-horned beetles, wood wasps, jewel beetles, weevils, and ambrosia beetles are often intercepted in SWPM [2–6]. Even with the implementation of International Standards for Phytosanitary Measures (e.g., ISPM No. 15), which states the need to treat wood products shipped abroad, in order to prevent

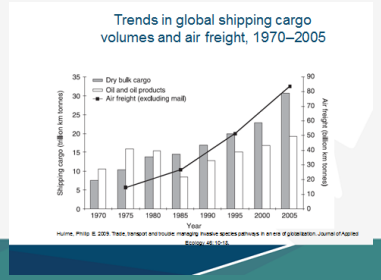
Problematic: Introduction IAS

- Emerging forest diseases caused by invasive alien pathogens represent an important threat to Canadian forests / Entry of spore materials?:
  - International trade (Sawmills, compost, wood storage, agricultural activities, ports, importation, etc. Imported live ornamental plant material.)
  - Environmental factors
    - Wind, rain, air flow. Insect vectored.
  - Phytopathogenic fungal (Fungi) and pseudo-fungal (Oomycetes) spores spreading



- Difficult to ID due to cryptic nature
  - Better understanding of introduction: Origin country, Introduction mode. Establish a molecular method of Biosurveillance:
  - Few methods available, accurate and rapid to detect fungi, specific to single target species.
  - Culture, morphology and on host: slow and not specific.
  - Prevention of infestations.





= Exotic
= Native
= Introduced

Species Targeted CFIA + CFS*

1	*Bretziella fagacearum*	Oak wilt****
2	*Ceratocystis fimbriata*	Blue stain (Norway Spruce)
3	*Ceratocystis laricicola*	Canker stain
4	*Ceratocystis polonica*	Blue stain (Larch)
5	*Chrysomyxa abietis*	Spruce needle rust
6	*Geosmithia morbida*	Thousand Cankers disease
7	*Gremmeniella abietina*	Scleroderris canker, Brunchorstia disease
8	*Gymnosporangium fuscum*	European Pear rust, Cankers
9	*Gymnosporangium yamadae*	Japanese Apple rust
10	*Heterobasidion annosum*	Annosum root rot
11	*Melampsora pinitorqua*	Pine twisting rust
12	*Ophiostoma novo-ulmi*	Dutch Elm Disease
13	*Ophiostoma ulmi*	Dutch Elm Disease
14	*Phytophthora alni*	Alder's *Phytophthora*
15	*Phytophthora kernoviae*	Holly blight, Root rot, Potato blight
16	*Phytophthora ramorum*	Sudden Oak Death****
17	Other *Phytophthoras spp.*	Cankers

*CFS: Canadian Forest Services

Example 1:

Sudden Oak Death, SOD, SLD

- Severe damage on oak trees in California and Oregon
- 1995 to now
- Symptoms
  - Cankers
  - Brown and reddish bleeding at 18.3 m high
  - Leaves lost during summer season
- Sudden Larch Death (SLD), UK

Diseases caused by *Phytophthora ramorum* (Blight and Dieback)

- Affect many plant species, more than 100 (North America and Europe) (Sequoia, Douglas fir, Rhododendron, Camellias, *Vaccinium*, …)
  - Symptoms are different from different hosts
  - **Many states and countries are affected**
    - **Quarantine measures**
  - **Propagate via nurseries**
  - ***Phytophthora ramorum***
    - **Oomycetes**
    - **Chlamydospores-Sporangia**

Example 2:

Oak wilt: *Bretziella fagacearum*

http://www.inspection.gc.ca/plants/plant-pests-invasive-species/insects/plant-pest-cards/eng/1548085757491/1548085933224#s13

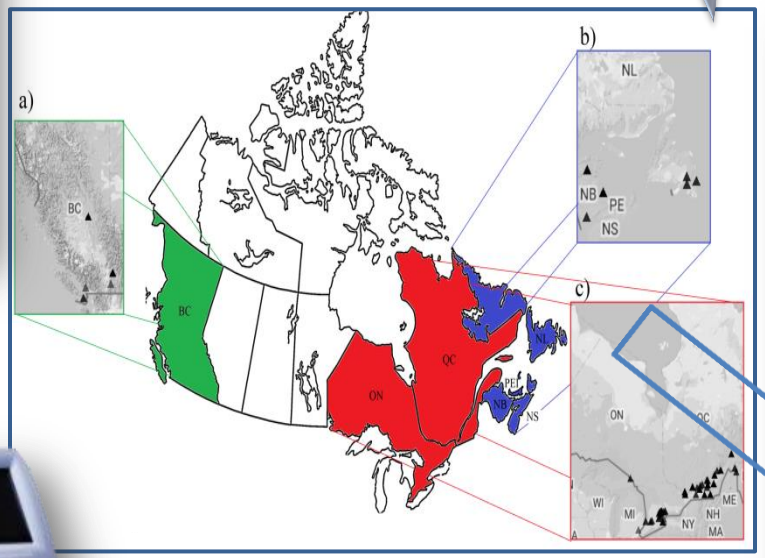
Workflow in brief

Samples collection & processing using different semiochemicals

Fusion primers PCR: ITS1 and ATP9-NAD9 amplicons appended with barcodes

NGS using Ion Torrent PGM platform

Bioinformatics



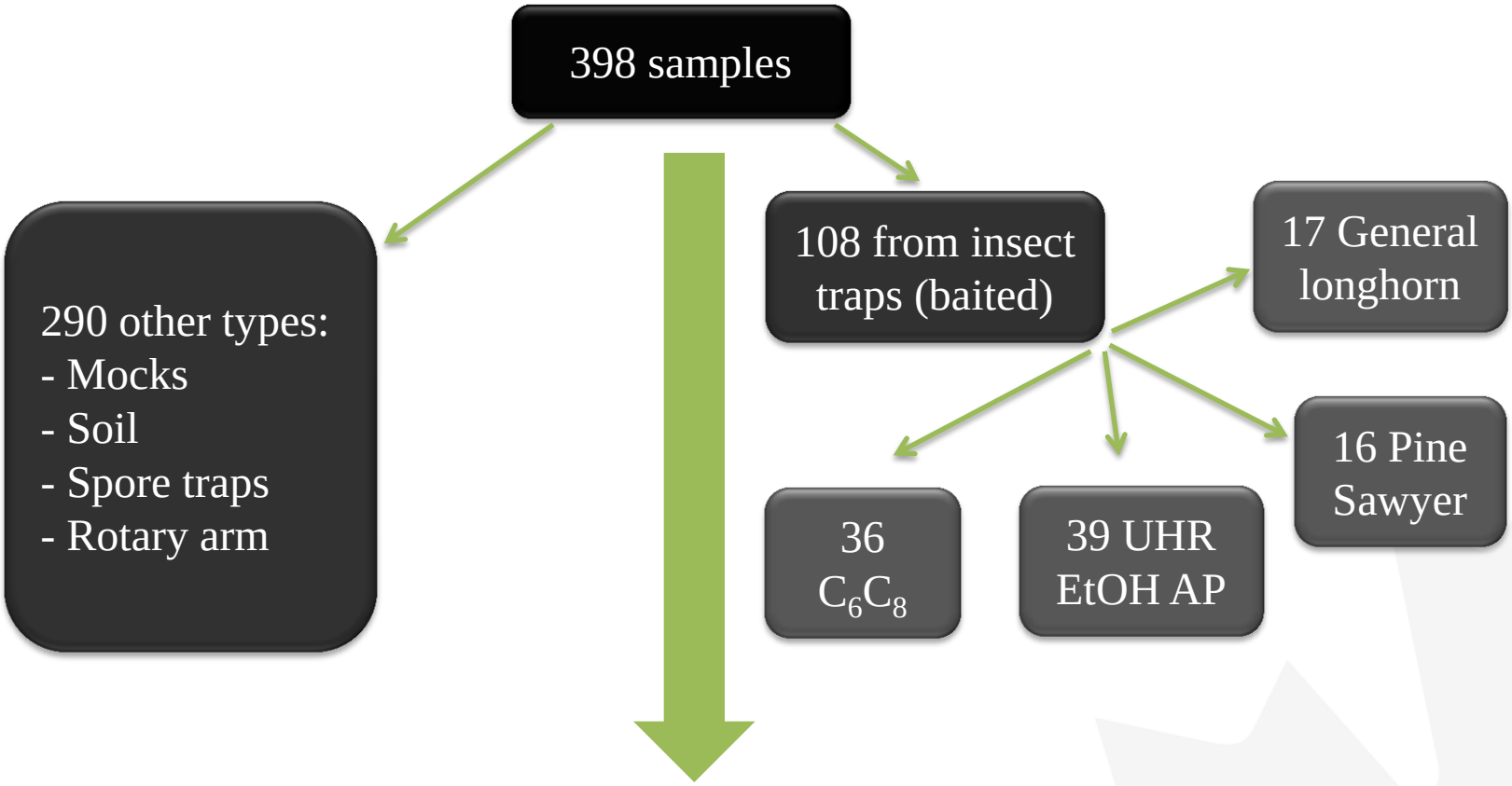
Insect traps

- Stacked funnels
- Lure:
  - Attracts specific insects
  - Preservative (PEG)
- Donations from entomology surveys done by CFIA throughout Canada:
  - Recycling of unused liquids from **existing** traps

Species-specific qPCR validation

Detailed protocol: Tremblay, É. D., Duceppe, M.-O., Bérubé, J. A., Kimoto, T., and Bilodeau, G. J. (2018). Screening for exotic forest pathogens to increase survey capacity using metagenomics. Phytopathology, DOI: 10.1094/PHYTO-02-18-0028-R.

Results: Samples & Next-Generation Sequencing (Ion Torrent) output



	Run number	Number of sequences	Number of amplicons
Total	15	45,068,371	1151
Average per run		3,466,797	77

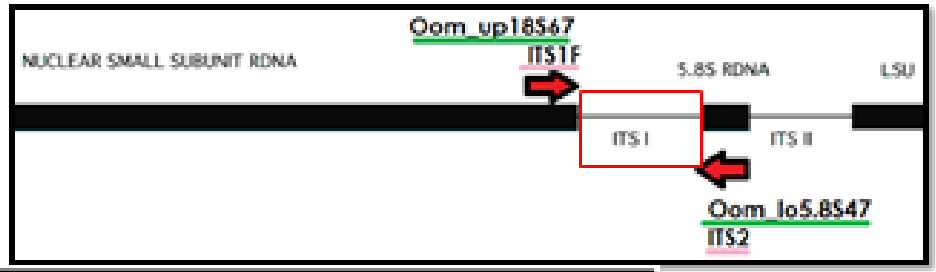
Next generation sequencing evaluation for detection of potential threats or emerging forest pest, entry point from spore and insect traps

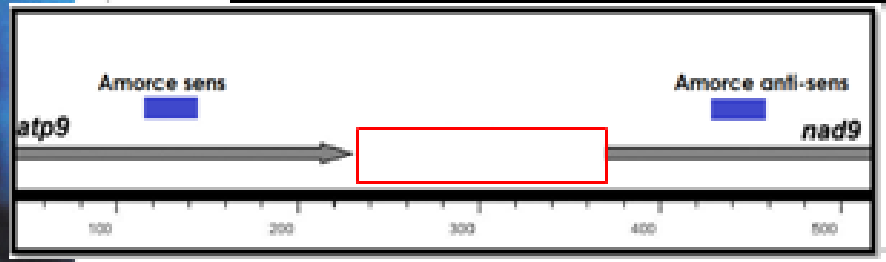
Regions of PCR amplification

Fungi and **oomycetes**: ITS1.

Phytophthora *sp.*: ATP9-NAD9*.

*Phytopathology. 2014 Jul;104(7):732-48. Development of a multiplex assay for genus- and species-specific detection of Phytophthora based on differences in mitochondrial gene order. Blideau GJ, Martin FN, Coffey MD, Bilodeau GJ.





Target	Fungi and Oomycete	*Phytophthora*
Region	ITS1	ATP9-NAD9
size (pb)	350-400	322-355
Type	Ribosomal	Mitochondrial

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https://www.wur.nl

Pipeline

https://isugenomics.github.io

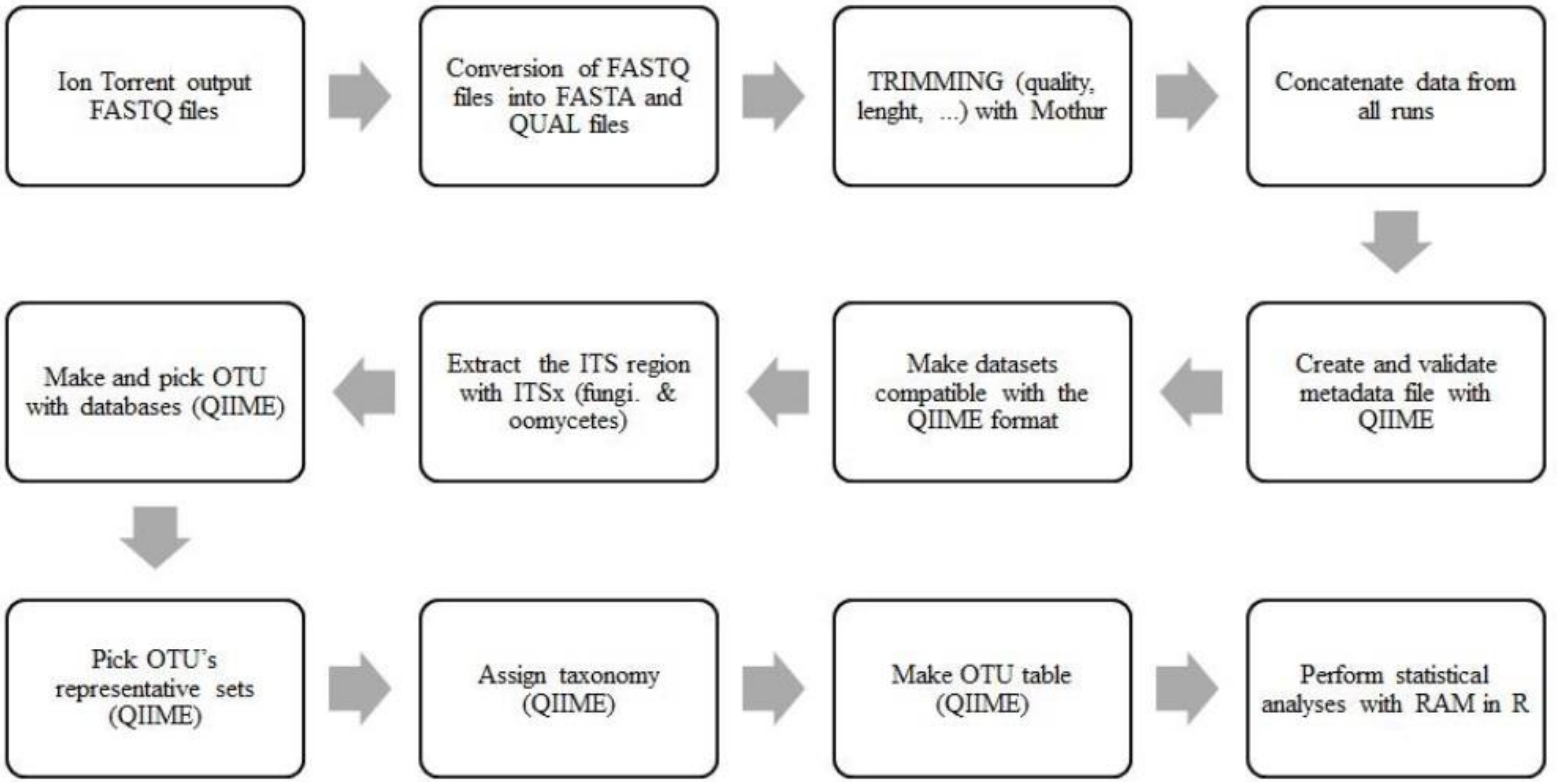
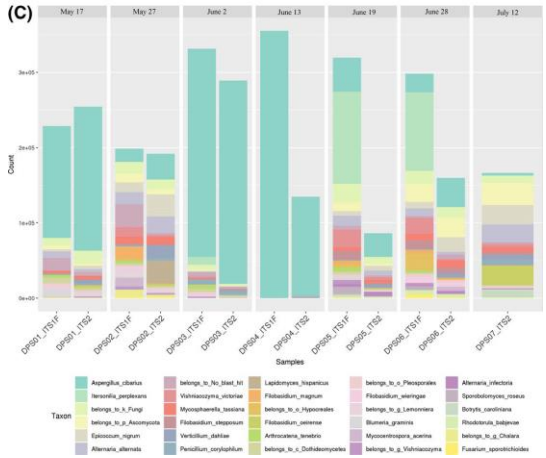


Figure 2.2 **Bioinformatic pipeline and tools used for next-generation sequencing analysis.**

Findings



- *Phytophthora* species
- *Heterobasidion* species
- Genera including phytopathogenic species found in the pollen samples comprised *Fusarium* sp., *Ophiostoma* sp., *Peronospora* sp., *Phytophthora* sp., and *Pythium* sp.
- Other potential host for some pathogens
- Correlation some insects that might vector some pathogens
- New area to refine sampling and inspections.
- Baseline on potential presence of some organisms

Challenges

Challenges

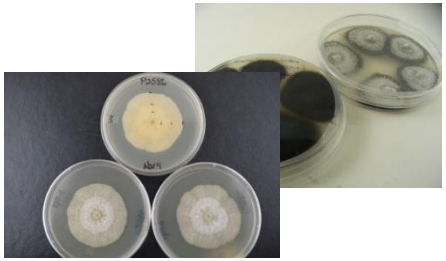
- Custom pipeline development is time and resource consuming
- Massive amounts of data to analyze
- Identification of DNA is sometimes impossible beyond the family or genus levels
- NGS error rates can exceed the genetic differences between species
- Databases (improvement)
- Severity of the results if invasive species are identified

Summary

This technology is proving to be an effective detection tool:

- Sampling in high risk areas using insect and spore traps is fairly new
- Same from pollen from honey bee for invasive fungi and plants
- Multiplexing of 3 genic regions
- High-throughput sample processing is possible
- Results may provide guidance for CFIA biosurveillance surveys
- Potential identification of hotspots and high risk areas
- Correlation between both methods (NGS & qPCR) validates data and demonstrates robustness of the concept
- Detection of closely related species demonstrates the ability to resolve pathogen species
- Possibility of transferring the method for diagnostic utilization in the future
- *Be careful, this is spores detection and no first report, no Koch postulates*

III-Other activities related



1. *GRDI multidepartments : "**Ecobiomics**" Metagenomics Based Ecosystem Biomonitoring (CFIA, AAFC, NRCAN, EC, DFO, PHAC and NRC) Soil and water microbiomes*
2. *Genome Canada LSARP : (BioSAFE) BioSurveillance of Alien Forest Enemies: Developing genomics-enhanced tools to detect forest invasive* http://www.biosafegenomics.com/
3. *CFIA RPS OLF-P-1803: ID markers for tools for regulated plant pathogens. – G&O, Potato, support to diagnostic lab. Collaboration AAFC.*
4. *CFIA RPS 2485 OLF-P-1901 Detection of Oak wilt using metagenomics and qPCR in insect traps*
5. *GRDI CFIA mandated: 2546 : Genomics, pest & pathogen detection*
6. *Other collaborations (Forestry & Agriculture)*
   - *Biovigillance in organic soil (spore traps for fungi and Phytopthora)*
   - *NGS of bees and pollen for biosurveillance of agricultural pathogens and invasive species*
     - *Targeting viruses, bacteria, fungi and plants*
   - *Evaluation of Phytophthora species in Christmas tree plantations*

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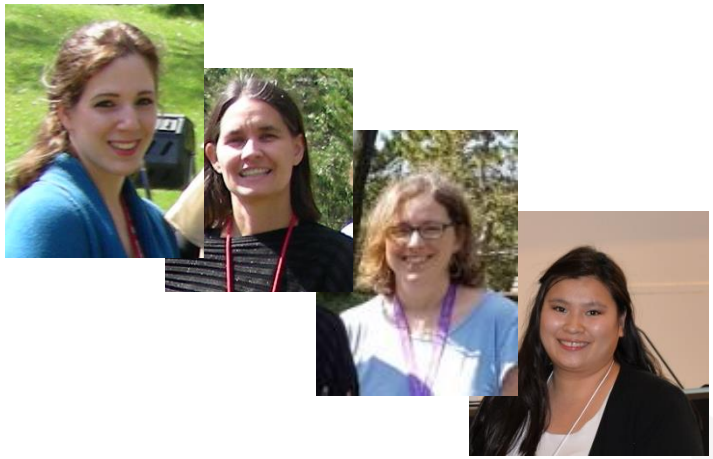

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Guillaume.Bilodeau@canada.ca
Twitter: @GuillaumeBilod2


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