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Fungi in interantional wood trade, in the context of commodities, phytosanitary treatments and genomic-based detection tools

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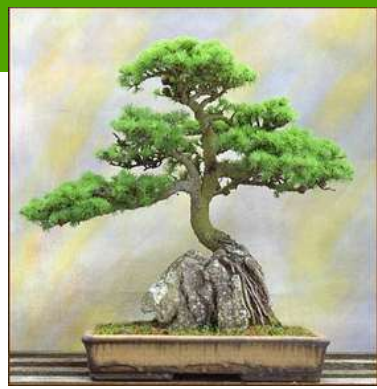
Why the Interest in Fungi?

- Historic focus on insects/nematodes in phytosanitary regulation
- Rapid evolution of international phytosanitary guidelines (ISPM 15 revision: appendix for evaluation of treatments , IPPC and NAPPO Standard for Wood Commodities, IPPC Plants for Planting Standard, NAPPO Standard for Christmas trees)
- More questions and concerns expressed around fungi
- Import regulations with limited scientific information
  - High uncertainty = precautionary principle

Forest Commodities– Different Degree of Pest Risk



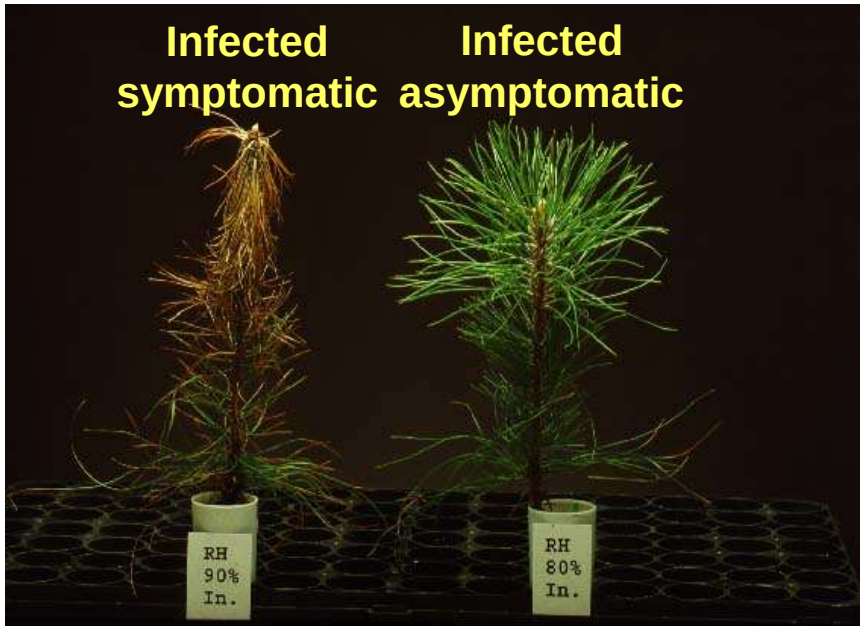
- **EXTRA HIGH – Plants for Planting, Branches**
- **HIGH - Solid wood**
  - Raw logs, round wood, poles, timbers, lumber (green/moist → dry)
- **MEDIUM - Wood fragments**
  - Sawdust, chips, shavings, fuel pellets, wood flour
- **LOW - Wood composites**
  - Plywood, OSB, fiberboard, engineered wood

Phytosanitary Treatments

- ISPM No 28 – Phytosanitary treatments for regulated pests
  - Heat treatment , fumigation, controlled atmosphere, irradiation….
- ISPM No 15 – Regulation of wood packaging (no data for fungi)
  - Heat treatment 56/30 or Fumigation with Methyl bromide
  - Dialectic heating (MW completed, RF on its way)
  - Criteria for new treatments under development
- Plants for Planting – new ISPM adopted March 2012
  - System approaches, integrated measures

The problem: inadequate diagnostics

- Disease diagnosis based on symptoms
- Isolation/detection techniques (visual, limited DNA)
- Target known pathogens and presumed pathways



Targeted pathogen groups

- **Most important pathogens of trees and crops**
- **History of invasiveness**

Oomycetes: Sudden oak_Death, Jarrah dieback, Chilean Pine Blight

Pucciniales: White pine blister rust, poplar and willow rusts

Dothideomycetes: Septoria canker of poplar, pine needle blight

The TAIGA Project – Started July 2011
Tree Aggressor Identification using Genomics Approaches

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Kermit Ritland, Faculty of Forestry, University of British Columbia

Steven Jones, Canada's Michael Smith Genome Sciences Centre

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Stéphan Brière, Canadian Food Inspection Agency

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

Natural Resources Canada | Ressources naturelles Canada

UBC

NOUS SOMMES PRÊTS

BC Cancer Agency
CARE + RESEARCH
An agency of the Provincial Health Services Authority

Canada's Michael Smith Genome Sciences Centre

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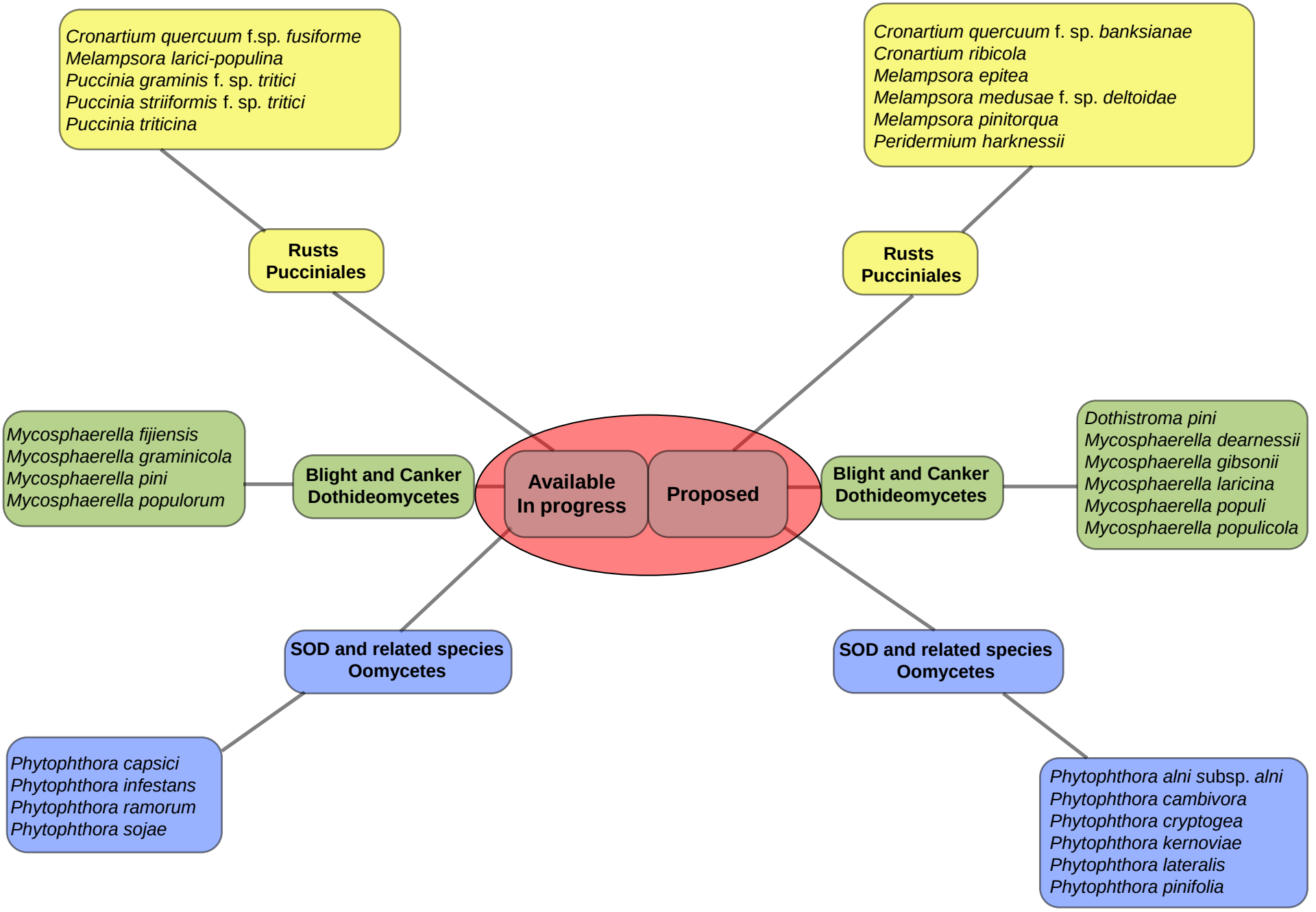
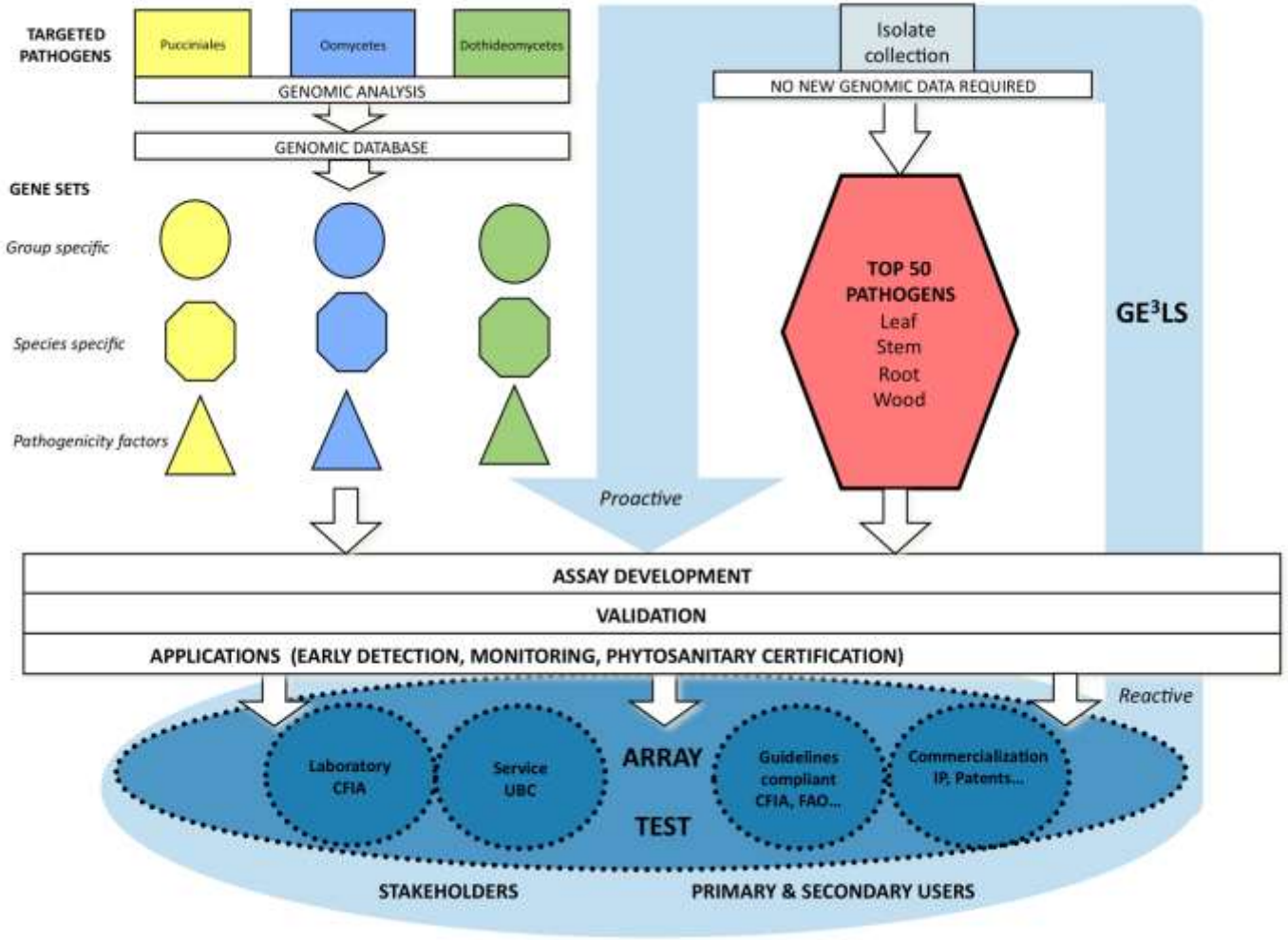
Genomics-Enhanced Diagnostics Will:

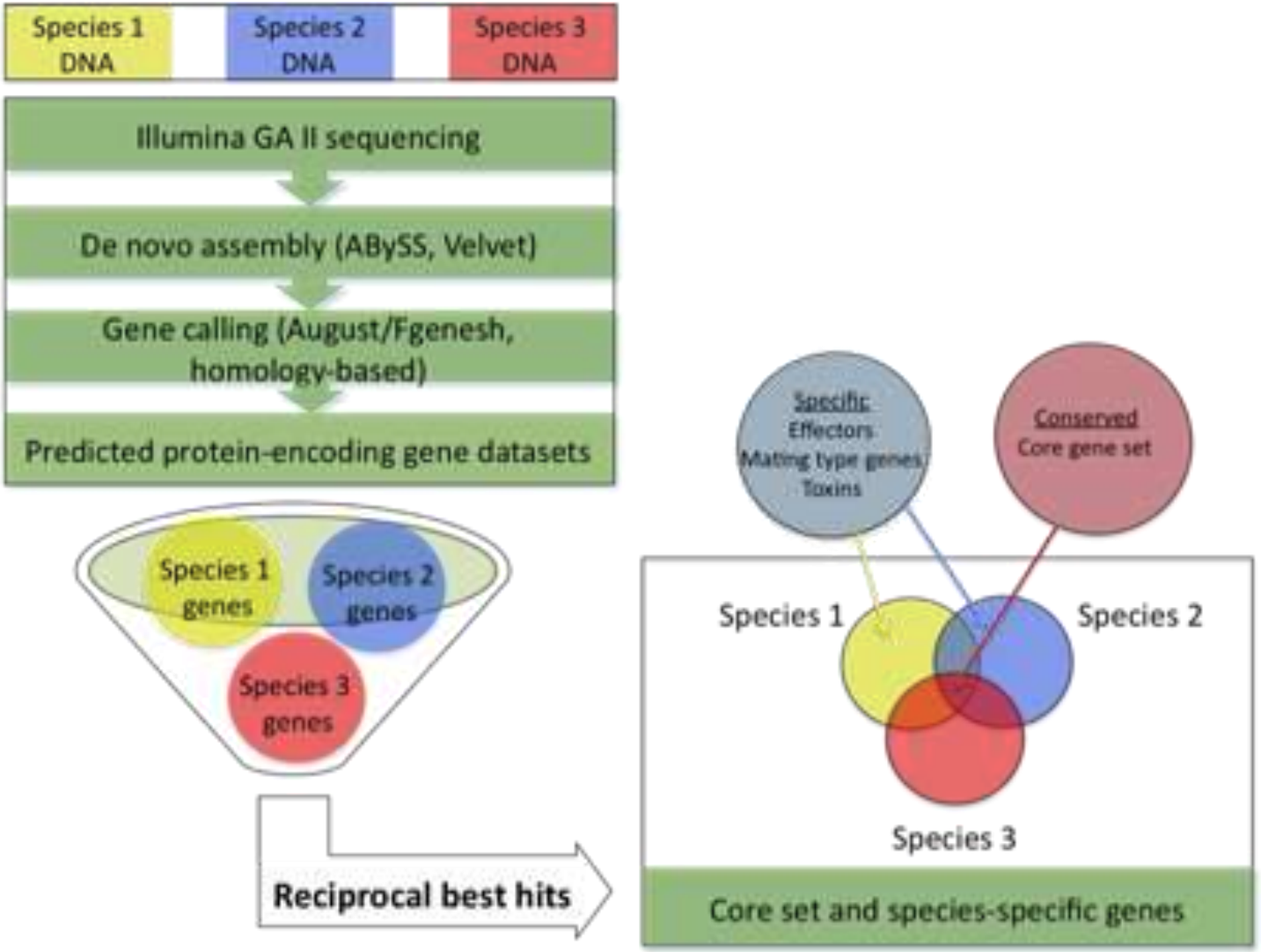
- Accurately detect small amounts of fungal mass of known and unknown pathogens
- Determine introduction and dispersal routes
- Develop diagnostic and monitoring tools to be used to test imports and provide certification of exports


Cronartium quercuum f.sp. fusiforme
Melampsora larici-populina
Puccinia graminis f. sp. tritici
Puccinia striiformis f. sp. tritici
Puccinia triticina
Cronartium quercuum f. sp. banksianae
Cronartium ribicola
Melampsora epitea
Melampsora medusae f. sp. deltoidae
Melampsora pinitorqua
Peridermium harknessii
Rusts Pucciniales
Rusts Pucciniales
Mycosphaerella fijiensis
Mycosphaerella graminicola
Mycosphaerella pini
Mycosphaerella populorum
Blight and Canker Dothideomycetes
Available In progress
Proposed
Blight and Canker Dothideomycetes
Dothistroma pini
Mycosphaerella dearnessii
Mycosphaerella gibsonii
Mycosphaerella laricina
Mycosphaerella populi
Mycosphaerella populicola
SOD and related species Oomycetes
SOD and related species Oomycetes
Phytophthora capsici
Phytophthora infestans
Phytophthora ramorum
Phytophthora sojae
Phytophthora alni subsp. alni
Phytophthora cambivora
Phytophthora cryptogea
Phytophthora kernoviae
Phytophthora lateralis
Phytophthora pinifolia

Project at a glance

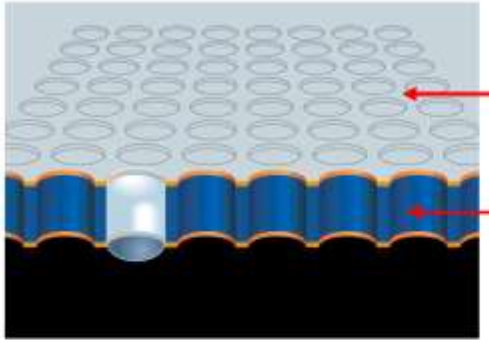


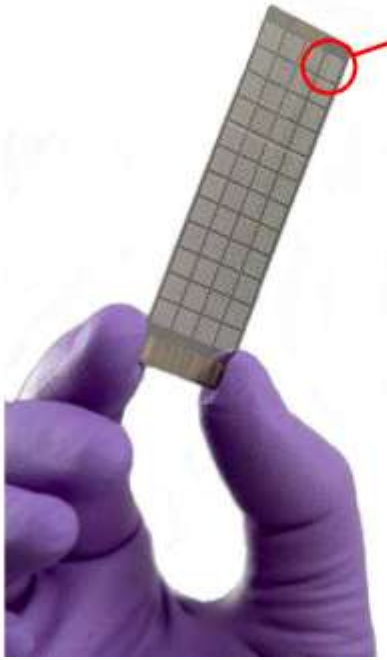
Identification of core gene sets



OpenArray Platform is Chosen

Nanoliter fluidics platform for low-volume, solution-phase reactions.
High throughput platform to analyse >3000 assays on a single slide/ chip.





OpenArray Plate Layout	
Subarrays	48 (12 x 4)
Through-holes	64 per subarray (8 x 8)

Data Points per OpenArray Plate	
Genotyping	3,072
Real Time PCR	2,688

http://www.appliedbiosystems.com/absite/us/en/home/applications-technologies/real-time-pcr/real-time-pcr-instruments/openarray-real-time-pcr-system.html

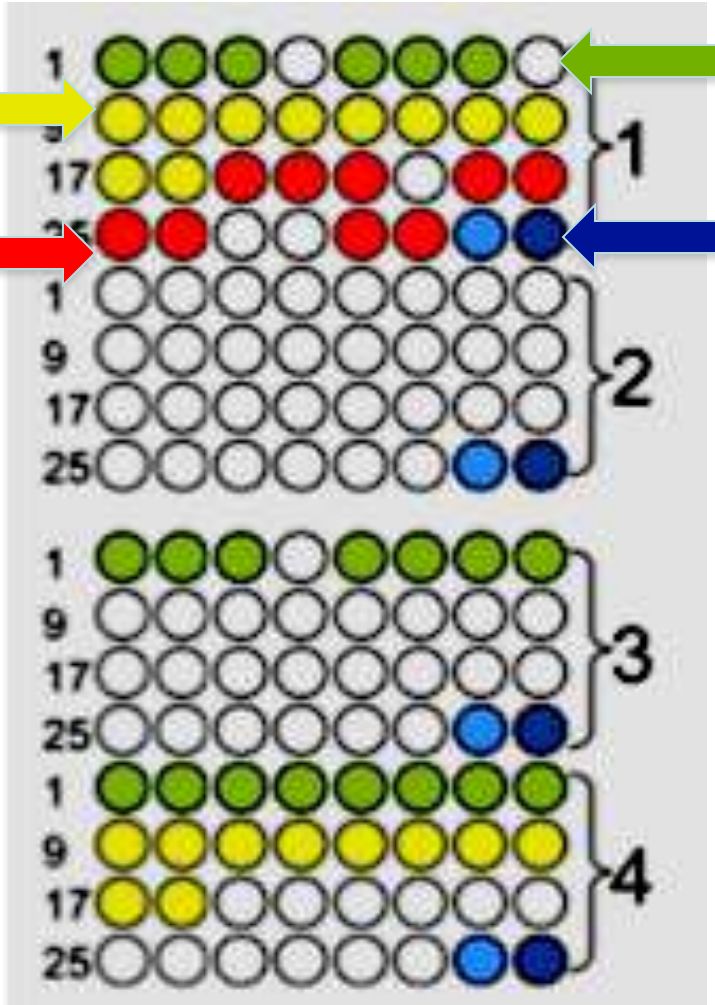
Hierarchical assays

Species probes

Pathogenicity probes

Genus probes

Control probes



Results interpretation

1) Positive for genus, species, and pathogenicity
2) Negative for genus, species and pathogenicity
3) Positive for genus
4) Positive for genus and species

Current Update

- Isolates being obtained, DNA collected, sequencing started
- Top 50 most unwanted pathogen list discussed and created
- 6 Phytophthoras, 5 Mycosphaerella and 8 rusts are sequenced.
- We also have RNA seq for transcriptomic for all those fungi.
- The population re-sequencing for P. ramorum and Mycosphaerella are in the Genome Science Centre pipeline and the Cronartium ribicola will be submitted this summer.
- Next steps: data processing, genome comparisons and analyses

So What?
(Validation, Deployment and Tech transfer)

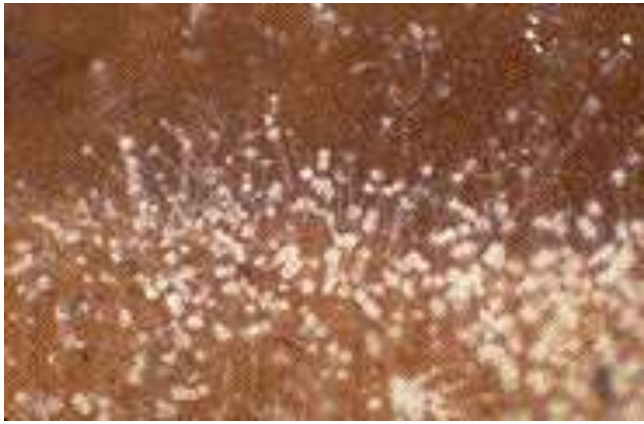
World needs uninhibited trade with

significantly reduced risk of pest introduction

Characteristics of a successful fungal invader

- ease of sporulation
- asexual reproduction
- “resistant” propagules
- competitive ability
- wide host range
- vectored by insects
- adapted to long-distance travel
- high potential for evolutionary change
- pathway considerations – establishment potential

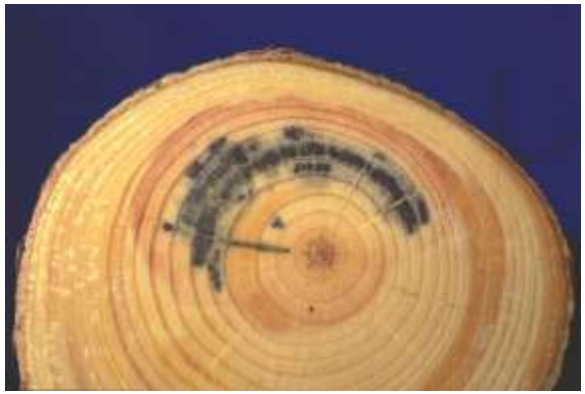
Phellinus pini and *Heterobasidion annosum*

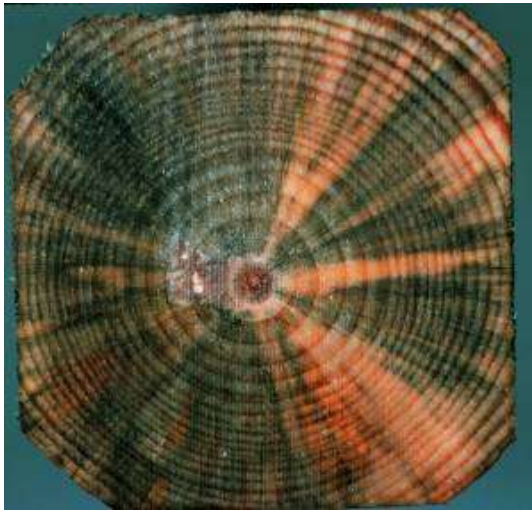


So What?

Phytosanitary treatments combined with other integrated measures may significantly reduce fungal ability to spread and establish even though some fungi survived the treatment

Pathogenic fungi present in a commodity (not P4P)but may have reduced risk of establishment e.g. they lack of competiveness, or ability to produce spores





Input from Science Advisory Board and Key Stakeholders

- End users and scenarios not clearly defined (who would use it and how)
- The top 50 list contains mix of fungi (pathogens, saprotrophs, exotic, native, widely spread, endemic)
- Nature of trade and traded commodities need to be considered:
  - Plants for planting are serious threat and focus on this pathway
  - Wood in trade often processed or treated (KD, HT), current inspection requirements for wood do not require pathogen ID,
  - Detection using DNA does not indicate if pathogen is alive; possibiliy for unecessary regulatory actions
  - Even if alive, pathways not necessarily established (e.g. wood decay fungi need to produce conk to spread)
- Screening for key genera, also for race, pathovar or mating type may be needed
- If a list of fungi is too specific we may miss unknowns

Actions Considered

- Focus on exotic pathogens (imports) moving via live plants
  - Focus primarily on CFIA list of unwanted/monitored fungi (40-45)
  - Also include 5-10 native (established or endemic) pathogens for surveys, monitoring and export certification if needed
- The final list will include the three groups (rusts, cankers/blights and oomycetes) plus *Armillaria*, *Heterobasidion (*pathogenic decay*)*, a few ophiostomatoid fungi, bacterial cankers, needle casts…
- The list remains dynamic and with increasing knowledge and additional need, can be adjusted


Thank You