Gizmos, genomes, PCR and stuff

Rebecca (Beccy) Ganley and Rebecca McDougal

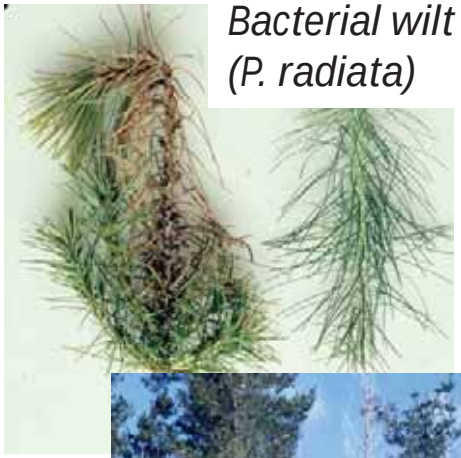
Overview

- Importance of diagnostics
- Traditional diagnostics
- Case studies
- Balancing traditional and molecular diagnostics
- Molecular diagnostics
- Collections
- New diagnostic techniques
- Diagnostics of the future

Importance of diagnostics

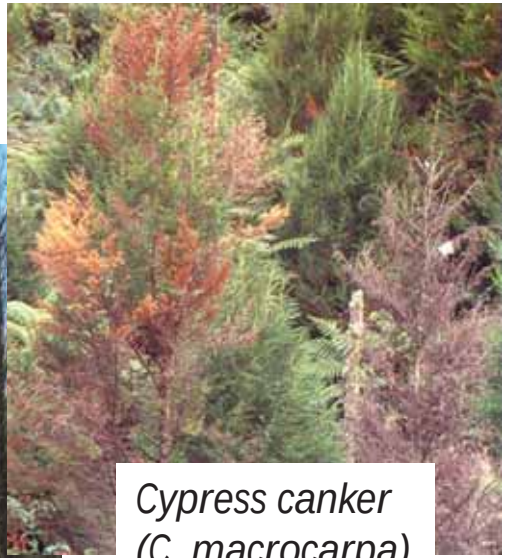
Diagnostics can answer many questions:

- “My trees are not looking good, what’s causing the problem?”

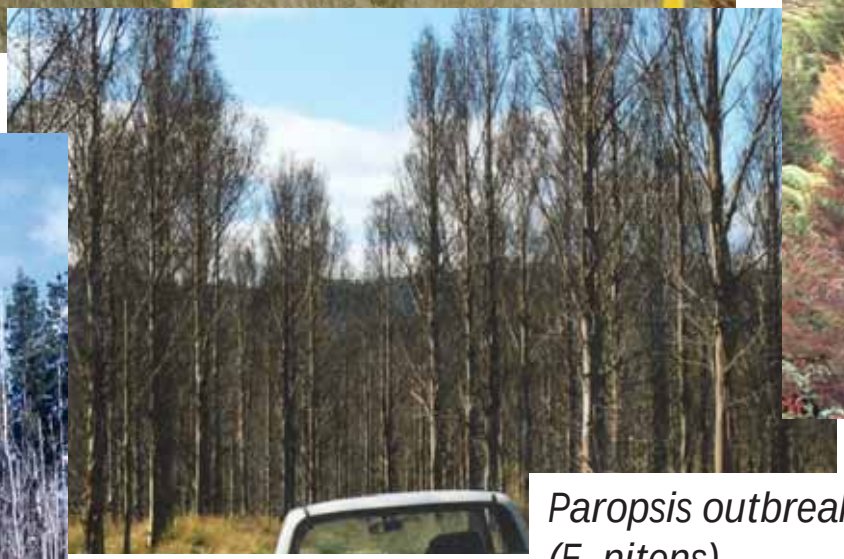


Bacterial wilt (P. radiata)

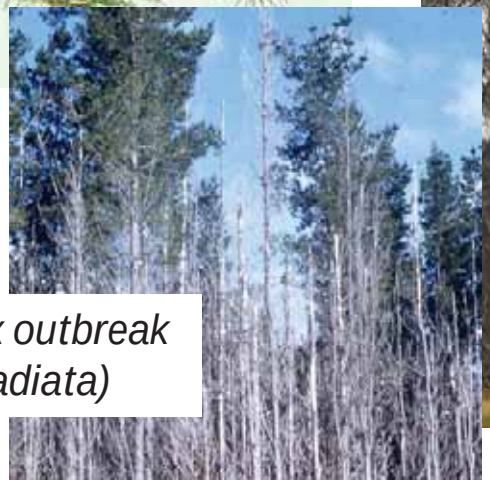
Armillaria root rot (P. radiata)



Cypress canker (C. macrocarpa)



Paropsis outbreak (E. nitens)



Sirex outbreak (P. radiata)

Importance of diagnostics

- Abiotic vs biotic, insects vs pathogens?

Red needle cast

Upper mid-crown yellowing

Cyclaneusma needle cast

Nitrogen deficiency

Dothistroma needle blight

Importance of diagnostics

- Early detection of a pest or pathogen
  - Eradication
  - Slow the spread
  - Introduce effective management early

Early detection of Fusarium circinatum *(pitch canker) in Douglas fir scions in 2003 allowed destruction of the quarantine material before it entered New Zealand.*

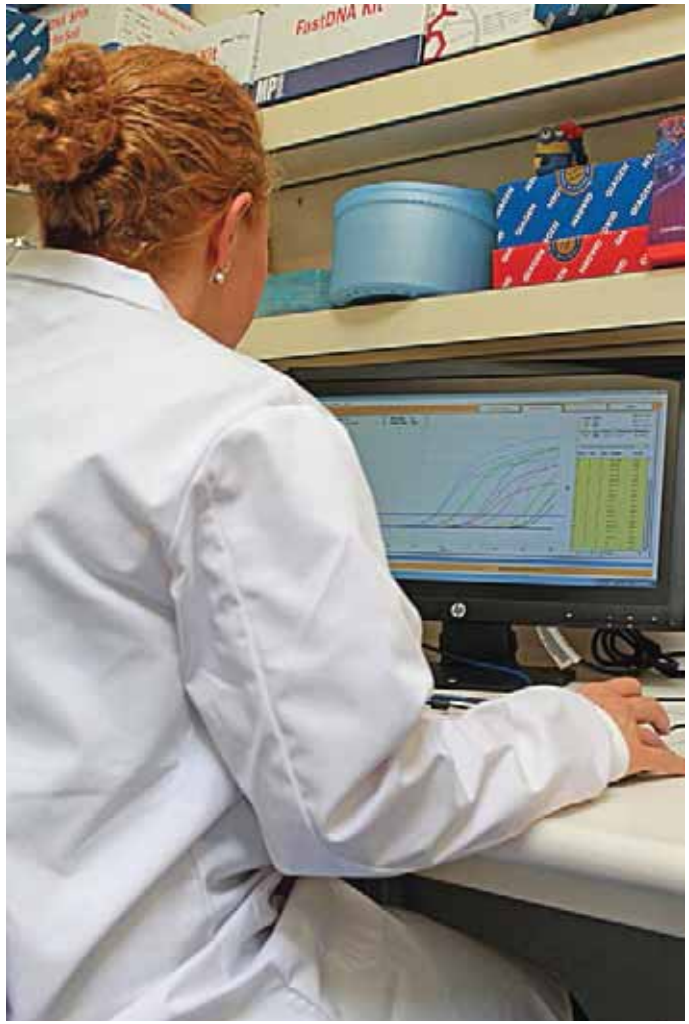

Ultimately diagnostics can allow growers to maintain and improve forest productivity, prevent or reduce market access issues, and prevent or minimize economic impacts of unwanted pests.

Traditional diagnostics

- Plant pathology – around since the 1600's
- At Scion since 1947
- Still use the same techniques…just changed how we use and apply them

Molecular diagnostics

- In use at Scion since 2000’s
- Speeds up traditional diagnostics
- Allows us to do our job better and faster
- Works in partnership with traditional diagnostics



Identifying *Phytophthora*

Kauri dieback – *Phytophthora* taxon Agathis

- Kauri dieback reported in the early 1970's on Great Barrier Island
- Forest service pathologist isolated a *Phytophthora* sp.
- Identified by Commonwealth Mycological Institute (UK) as *P. heveae*
- Recovered under dead kauri in Northland in 2006
- Molecular techniques showed it was not *P. heveae* but a new-to-science species

Identifying *Phytophthora*

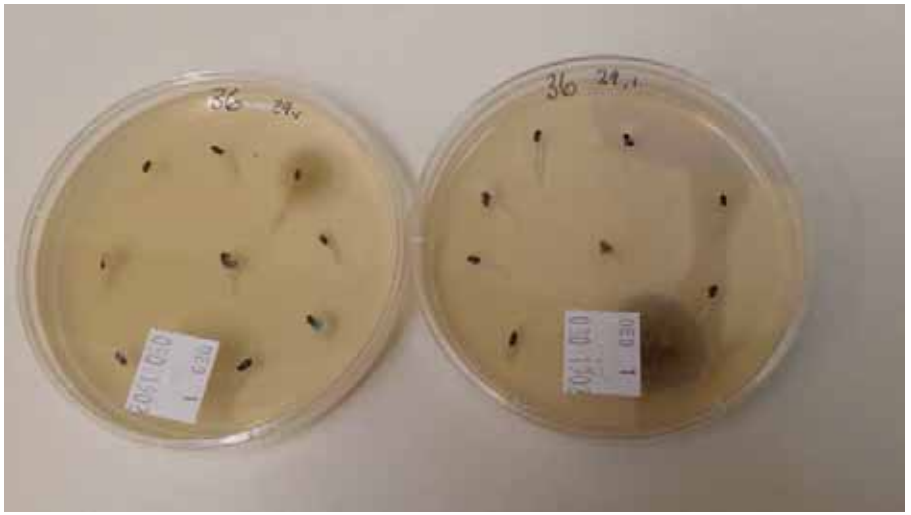
Red needle cast – *Phytophthora pluvialis*

- Reported in mid 2000's in central North Island
- Isolated a *Phytophthora* sp.
- Unable to identify species using traditional diagnostic techniques
- Molecular techniques able to identify it was a new-to science species
  - ➢ within 6 months determined likely origin as Oregon, USA.

Process required the use of both molecular and traditional tools

Balancing traditional and molecular techniques

- Still lots of unknown species using both techniques
- Quality of molecular information available online is variable
- Molecular diagnostics is not always faster or cheaper
  - Dutch elm disease – 20 years on since introduced, still using traditional techniques over molecular ones



Identifying insects

- Scion routinely identifies insects using traditional techniques
- Molecular identifications are limited
- Particularly helpful for identification of larvae or identifications from frass
- Molecular databases have limited New Zealand associated species – this is changing!
- Cerambycidae barcoding project, other barcoding efforts underway

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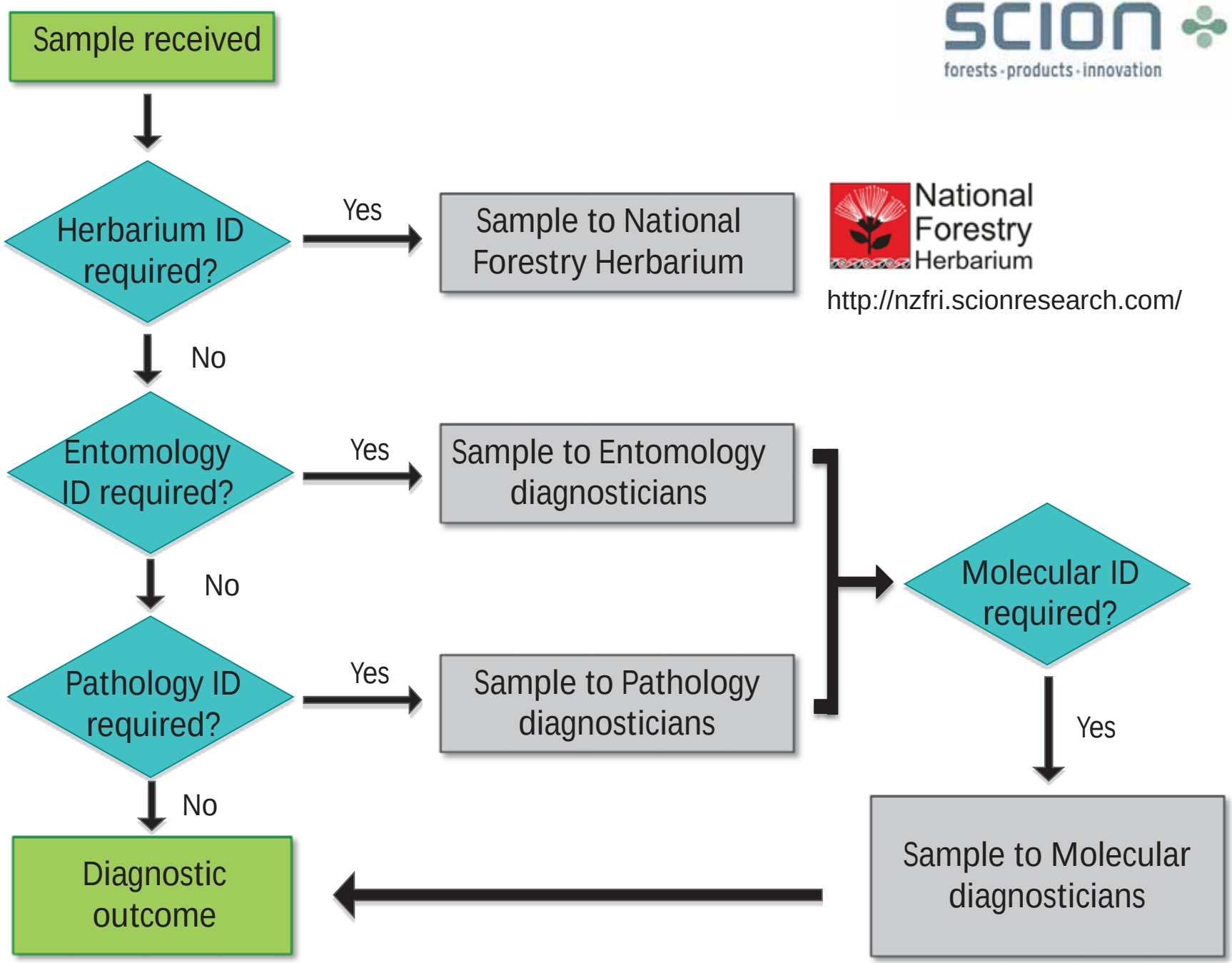

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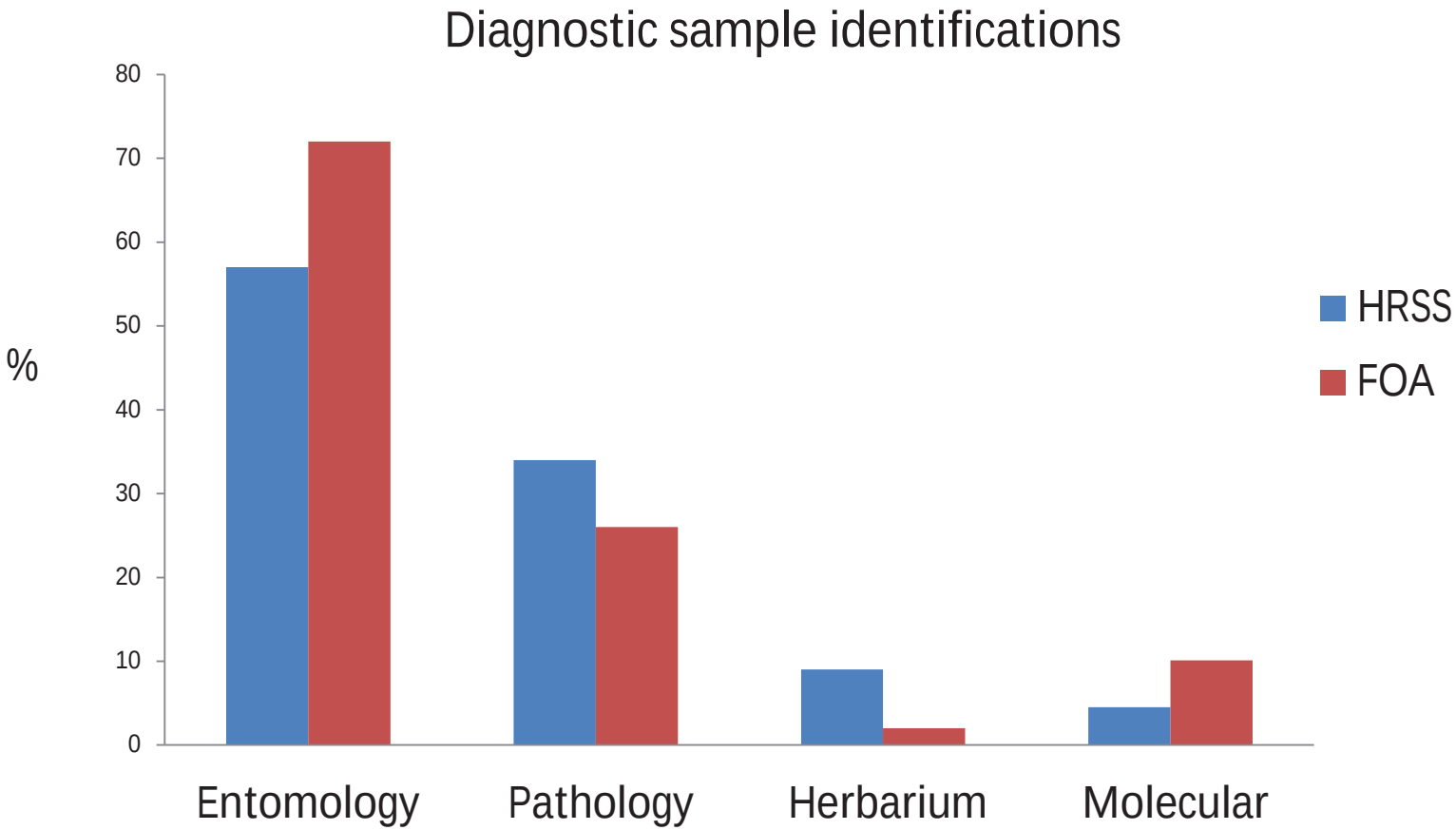
National Forestry Herbarium

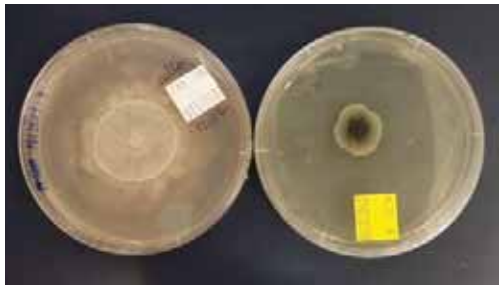
http://nzfri.scionresearch.com/

Diagnostic Samples: 2013-2014 season



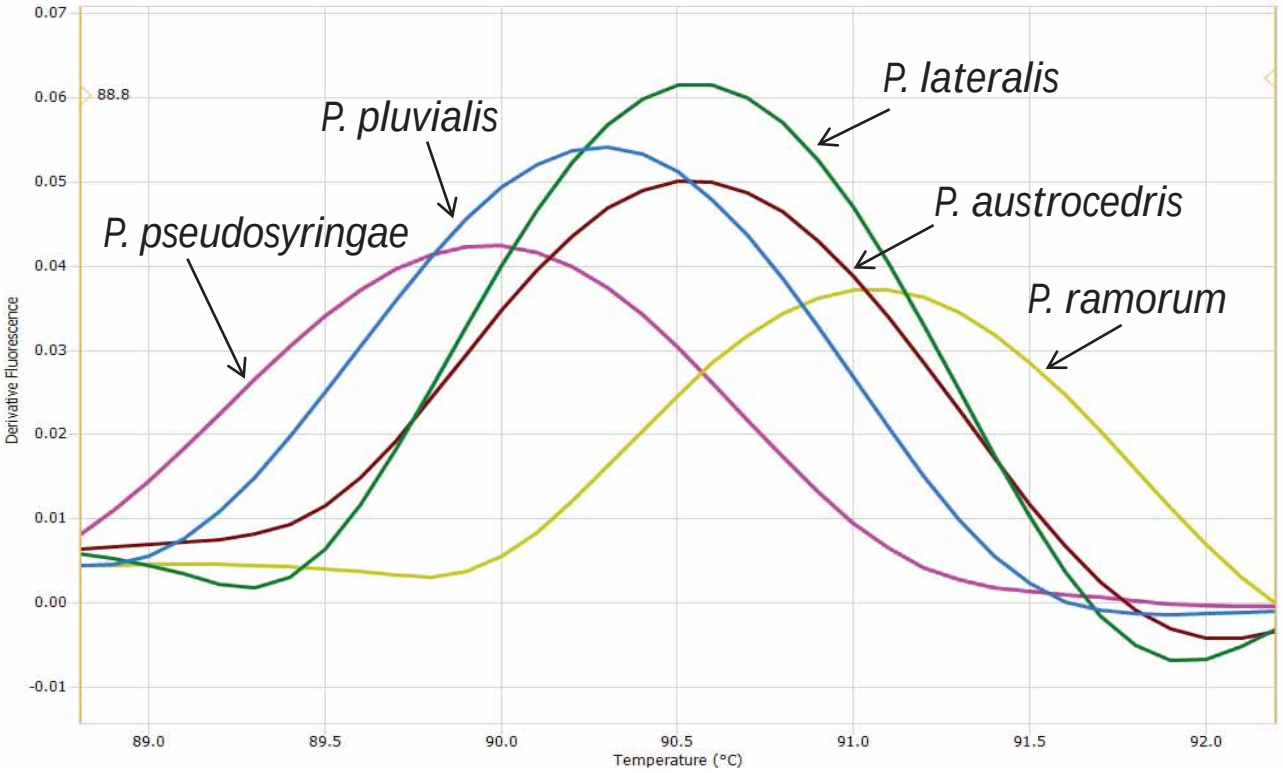
Molecular Diagnostics at Scion

- What kinds of samples do we receive:
  - Pure cultures
  - Needles (e.g. field trials)
  - Immunostrips (from forest owners and diagnostic samples tested)
- As part of some research projects we also test woody tissues and soils



Biosecurity threats

- Keeping abreast of the literature
- Importing DNA, testing molecular assays published
- Inclusion in our assay designs i.e. HRM

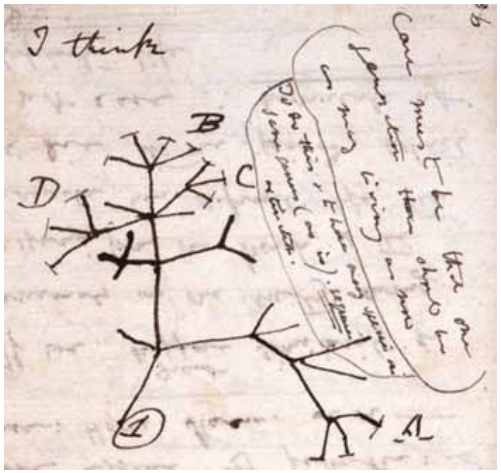


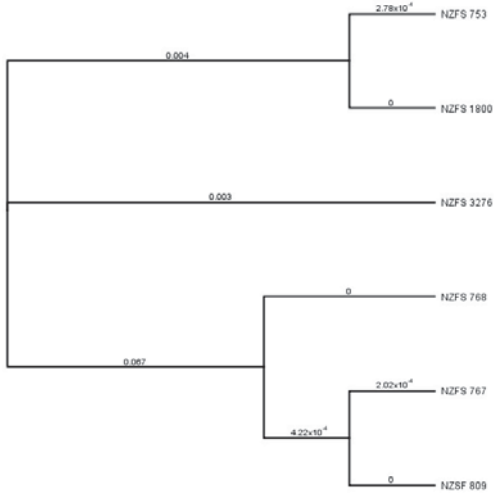
Also received:

- *P. pinifolia*
- *P. nemarosa*
- *P. ilicis*
- *P. psychrophila*
- *P. pluvialis*

Is this something “new”?

- Morphology and/or DNA sequence seems **different** to others
- Use sequences we generate and those from databases to make phylogenetic trees:
  - is it related/similar to something already known or is it something new?
- Can we determine the **origin**? What is the **genetic diversity**?





Herbarium material & culture collections

CS-8.5

MOLECULAR DETECTION OF *PHYTOPHTHORA* SPECIES IN HERBARIUM SPECIMENS

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Red needle cast, caused by *Phytophthora pluvialis*, is a new disease in *Pinus radiata* in New Zealand. Other *Phytophthora* species are also associated with
including *Phytophthora kernoviae*. In the past diagnosis of foliar di

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Barcoding Myrtaceae for rapid identification of plant species in preparation for myrtle rust

Anna R. Caird, Matt H. Buys, Heather J. Flint, and Rebecca J. Ganley
Scion, National Forestry Herbarium, Private Bag 3020, Rotorua 3046, New Zealand. Email: anna.caird@scionre

Introduction

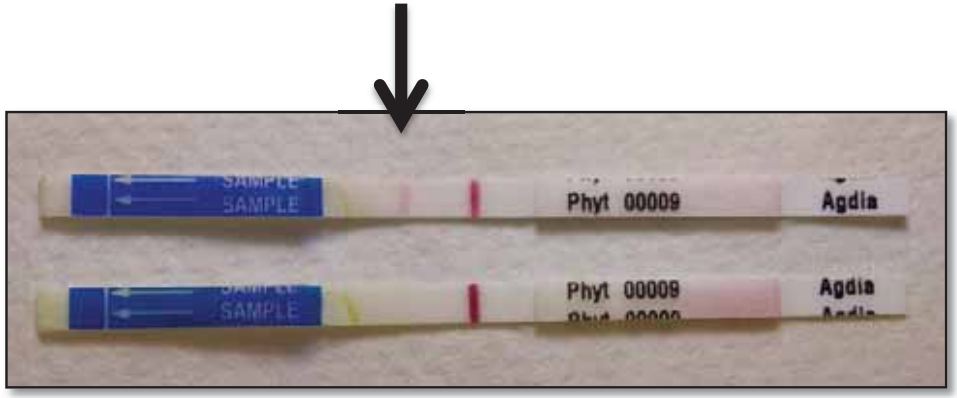
Puccinia psidii is a rust fungus that infects many species of Myrtaceae (Fig. 1). The fungus is present in Australia and it is highly likely that airborne spores of this pathogen will cross the Tasman Sea to New Zealand as other pathogens have in the past e.g. wheat rust, or arrive via anthropogenic vectors.

The management of plant pathogens is dependent on a

The utility of the DNA
well in the genus Me
5A) and *M. glomerata*
plantings in New Zea
morpho-logically. As
sequences (Fig. 6) th
reliable way of identif

Phytophthora diagnostics: current methods

1. Sample arrives via diagnostic service
2. Immunostrip or culturing
3. Goes to molecular team for PCR and/or sequencing
4. Time = one week or more.



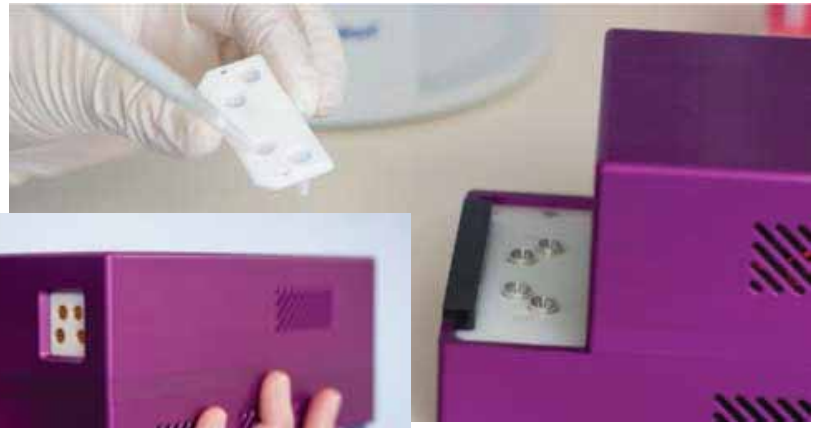
Positive → confirm species with PCR

Negative

Limitations of molecular analyses

- The identification is only as good as:
  - the database compare sequences to
  - the molecular assay using to identify
- Taxonomic resolution is not clear for all groups of plant pathogens
- Viability (if direct from soil or plant material)
  - Methods are available to look at live vs dead e.g. viability PCR, RNA-based methods
- DNA titre can be very low from environmental samples

In-field diagnostics: portable qPCR



Ubiquitome Bio ▸ **The Kauri Dieback Management Programme**
February 17 at 2:38pm ·

How mobile molecular testing can help in the fight against Kauri Dieback. Scion scientist Rebecca McDougal presented at the Kauri dieback symposium over the weekend http://www.ubiquitomebio.com/.../how-mobile-molecular-testing...

How mobile molecular testing can help in the fight against Kauri Dieback

Over the weekend, Freedom For You grants program

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Freedom for You grant finalists announced at http://www.ubiquitomebio.com/.../ubiquitome-announces-freedom.... Let us know which one is your favorite project!

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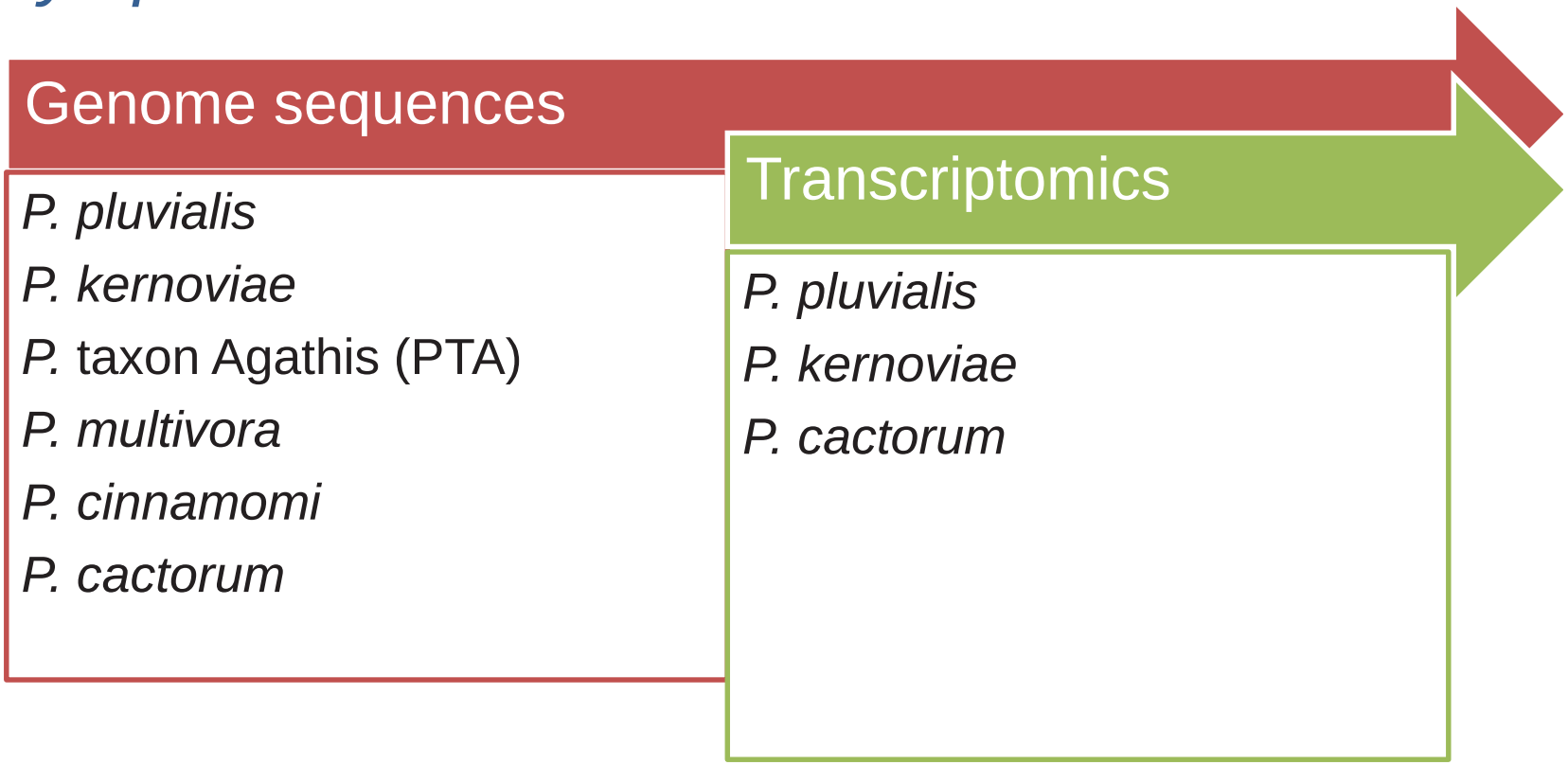
Phytophthora Genomic Resources



Genomics-based resources provide a rich pool of DNA sequence and gene expression information that can be mined for new diagnostic tools.

Healthy trees, healthy future
Enabling technologies to combat *Phytophthora* diseases

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New era of diagnostics is upon us!

Next Generation Sequencing

- Diagnosis of “unknowns”
- Genomics for identification & description of new species
- HUGE amounts of data being generated = pros & cons!

Phone apps

- Spore traps alert growers to increasing levels of disease inoculum in real-time
- Disease identification via photographs & social media, eg TREE TAGGR (Twitter)

LAMP

- FERA (UK) has over 20 assays for oomycetes, fungi, insects and bacteria in use
- New Zealand researchers are developing LAMP assays

www.scionresearch.com

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