Molecular detection of associated organisms

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What are “associated organisms”?  

- Symbionts or pathogens  
- Testing new agents  
- Risk/benefit assessment  
- Regulations  
- Scientific interest  
- Clean rearing, disease assessment, microscopy, molecular detection

Zhu et al. (2014) New Phytologist
What is “molecular detection”?

- Technical diagnostics
- Biological markers
- DNA, RNA and proteins
- Genus, species, genotype identification
- Detection of unculturable organisms
- Quantification and monitoring

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Candidatus Liberibacter europaeus (Leu) in New Zealand

- Ca. Lib. spp. vectored by psyllids, often cause plant diseases
- Leu asymptomatic in Italian pear
- Disease symptoms in scotch boom

http://www.britishbugs.org.uk/homoptera/Psylloidea/Arytainilla_spartiophila.html
Leu established only in scotch broom and broom psyllids (no other Ca. Lib. spp.)
Widespread but only where broom psyllids are found
Transmitted via psyllids and broom seeds
Unclear/unconfirmed disease symptomatic

= Low biosecurity risk to NZ
16S ribosomal RNA gene: Encodes prokaryotic ribosomal subunit

Used in identification and reconstructing phylogenies

→ Genus specific amplification of Liberibacter 16S DNA
Universal amplification of all bacterial 16S DNA in 171 samples (plant tissue and seeds)

- Massive parallel sequencing and bioinformatics (20 million reads @ 300 bp, 122,000 reads per sample)
Leu specific test and quantification

→ Species specific amplification of Leu 16S DNA

Presence/absence PCR test:

Quantitative PCR to assess Leu titre and detection limit:
Leu distribution in New Zealand

52 sites:
- Northland
- Waikato
- Bay of Plenty
- Hawke’s Bay
- Manawatu-Wanganui
- Tasman
- Westcoast
- Canterbury
- Southland

- **Specificity:** Scotch broom, gorse, white broom, tree lucerne, weaver’s broom and *Carmichaelia* (New Zealand brooms) and kowhai

- **Transmission and persistence:** Other psyllids, seeds, seedlings, persistence after insecticide treatment, cuttings (ornamental broom) and grafting (pear)

- **Disease symptoms:** Leu positive and negative broom seedlings in nursery, “sick” and “healthy” looking plants

- Uneven distribution in plant parts
Summary

- Constantly update risk assessment/testing protocols
- Associated organisms play important roles in environmental interactions
- Genetics tools needed to detect associated organisms
- Molecular tools essential for species/genotype ID
- Range of methods available
Our Land, Our Future
Tō tātou whenua, mō āpōpō