Manaaki Whenua
Landcare Research

Molecular detection of associated organisms

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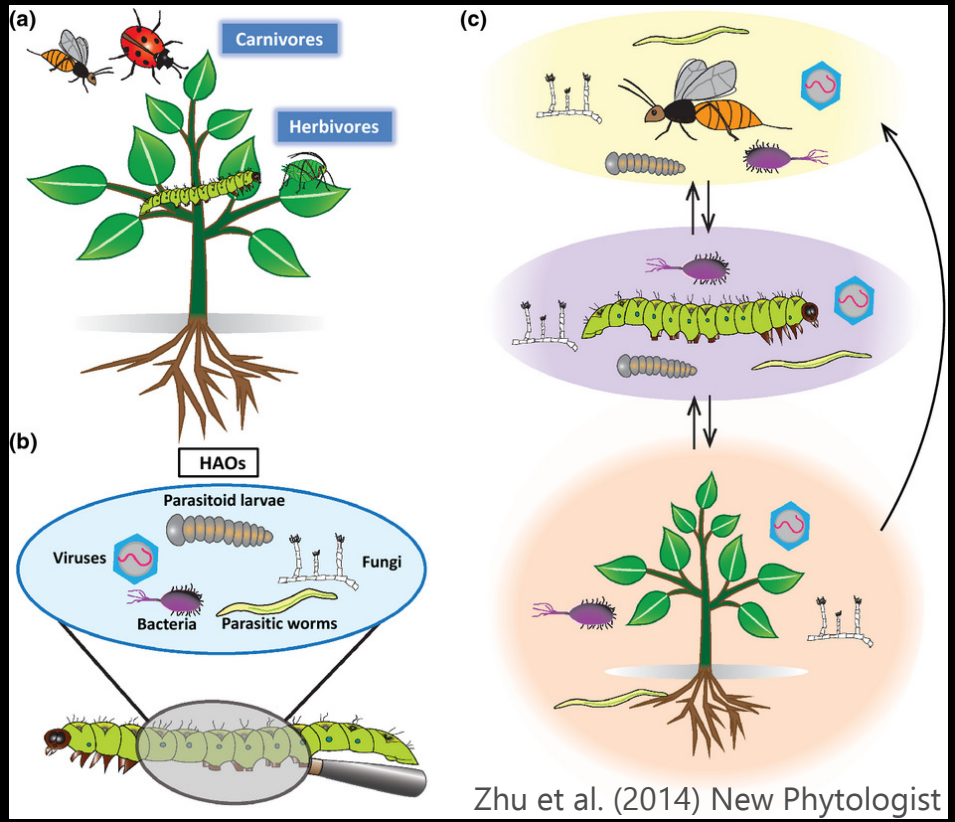
National Biocontrol Collective

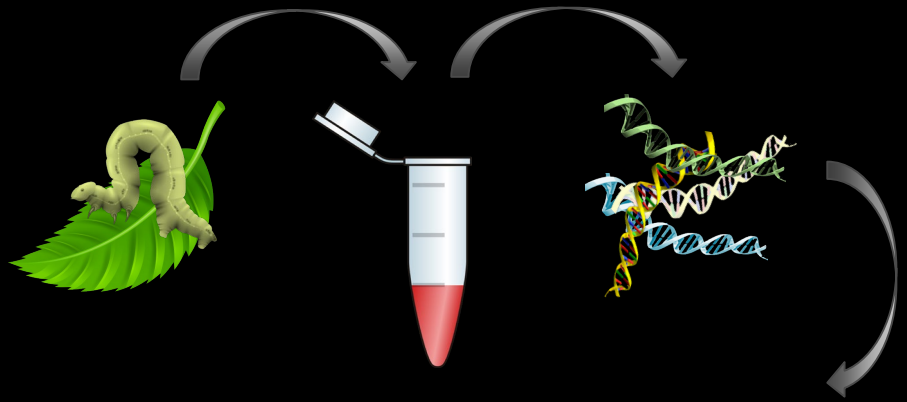
What are “associated organisms”?

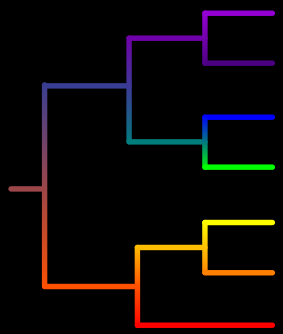


Zhu et al. (2014) New Phytologist

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

What is “molecular detection”?





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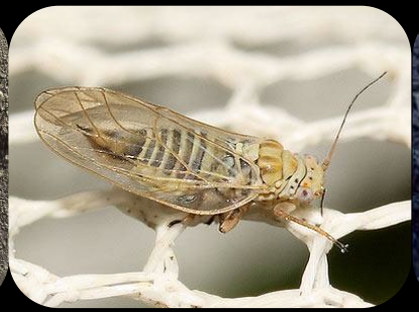
ACGTCTATCATGCCG--GTATCGATCGTCGA
ACGTCTATCATGCCG--GTATCGCTCGTCGA
ACGTCTGTCATGCCG--GTATCGGTCGTCGA
```

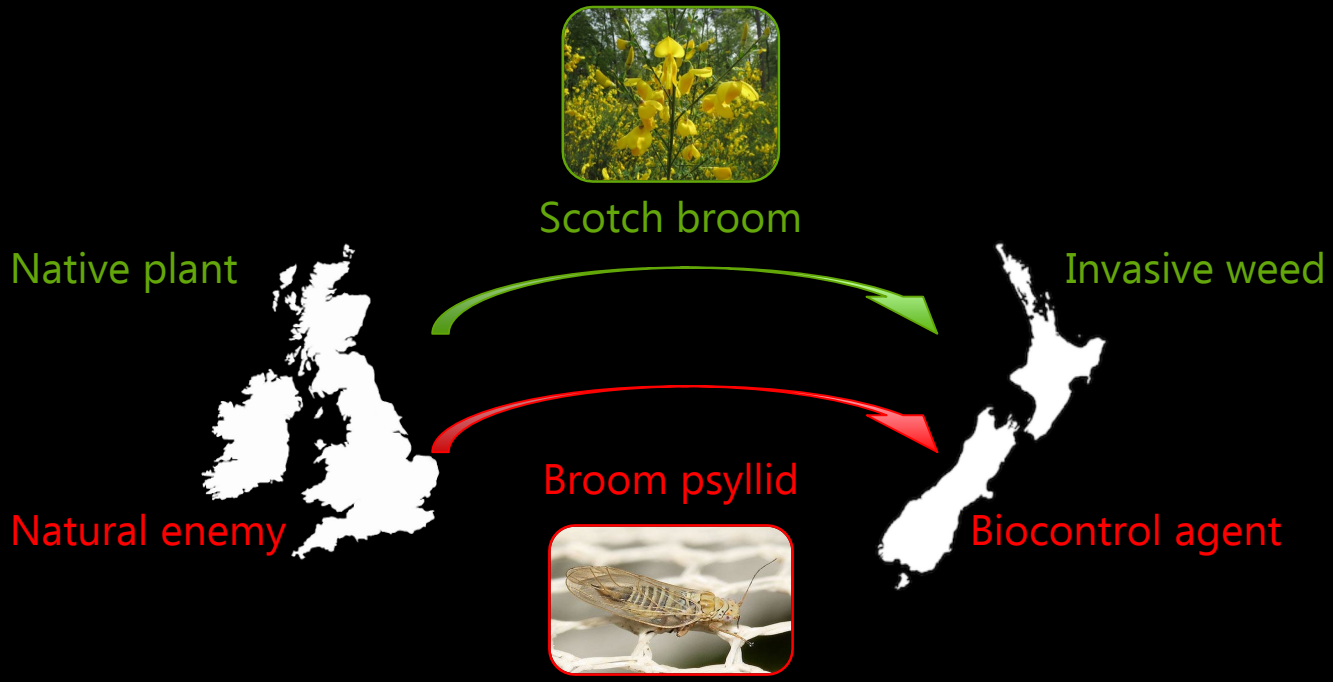
- Technical diagnostics
- Biological markers
- DNA, RNA and proteins
- Genus, species, genotype identification
- Detection of unculturable organisms
- Quantification and monitoring

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


Thompson et al. 2013. New Disease Reports 27, 6.; Raddadi et al .2011. Env. Microb. 13, 414-426.
http://www.britishbugs.org.uk/homoptera/Psylloidea/Arytainilla_spartiophila.html



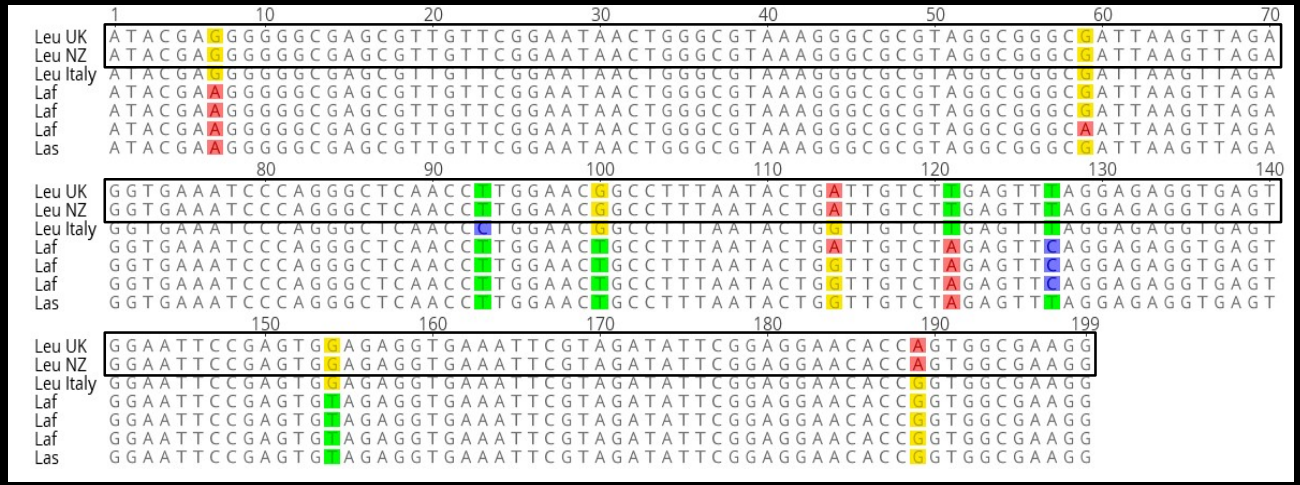
- Leu established only in scotch broom and broom psyllids (no other *Ca*. Lib. spp.)
- Widespread but only where broom psyllids are found
- Transmitted via psyllids and broom seeds
- Unclear/unconfirmed disease symptomatic

= Low biosecurity risk to NZ

Bacterial species identification

- 16S ribosomal RNA gene: Encodes prokaryotic ribosomal subunit
  Used in identification and reconstructing phylogenies

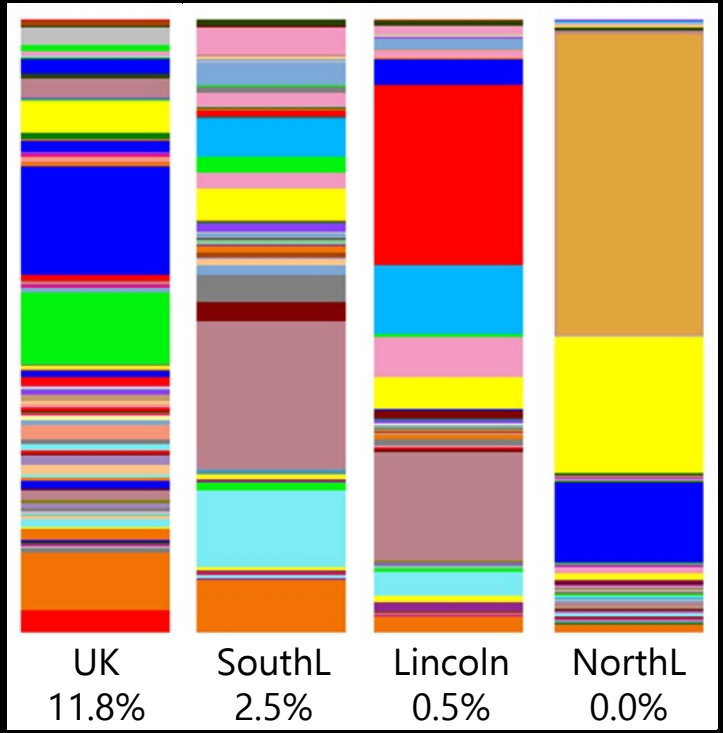
→ Genus specific amplification of Liberibacter 16S DNA



k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Candidatus Liberibacter
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;Other
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__Rickettsia
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Hymenobacter
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Tremblayales;f__Tremblayaceae;g__Carsonella
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae;g__Wolbachia
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;Other

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

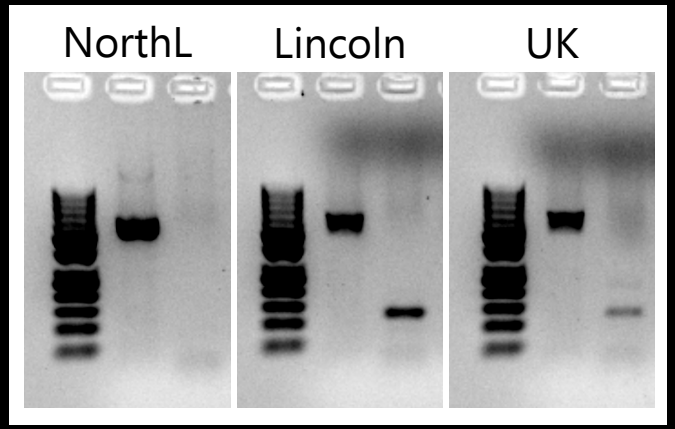
- Massive parallel sequencing and bioinformatics (20 million reads @ 300 bp, 122,000 reads per sample)



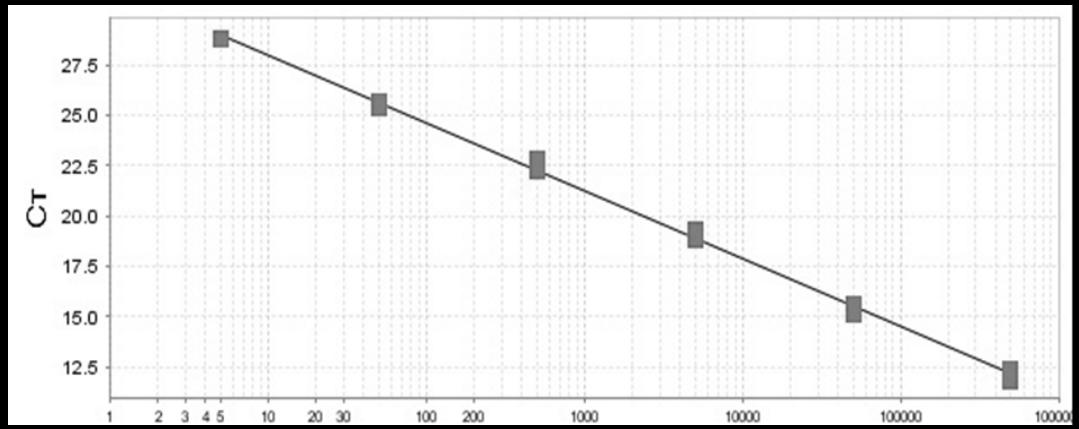
Leu specific test and quantification

→ Species specific amplification of Leu 16S DNA

Presence/absence PCR test:



Quantitative PCR to assess Leu titre and detection limit:

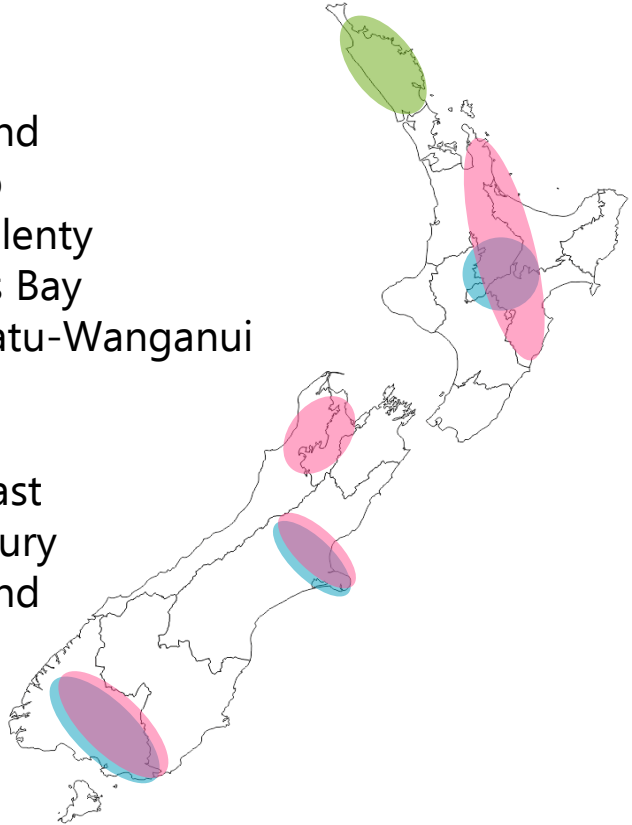


Leu distribution in New Zealand

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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