“Candidatus Liberibacter ssp”
and
broom psyllids

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Biosecurity Bonanza
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1) The “host”:
Common or Scotch broom - *Cytisus scoparius*

- Broom is native to Europe
- Spread worldwide as an ornamental
- Major weed in NZ, Australia, USA, India, Japan, Chile, Argentina
- Invades low value pasture, newly planted forestry, and natural habitats (grasslands, sub-alpine scrub, braided rivers).
- Net benefit to NZ if biocontrol successful $10m/yr*

* Jarvis, P. J. et al. 2006. Biol. Control 39, 135-146
2) The “venue”:
Biological Control of Broom in NZ

Existing exotic biocontrol agents:

- Twig miner, *Leucoptera spartifoliella* 1940s
- Broom seed beetle, *Bruchidius villosus*
- **Broom psyllid**, *Arytainilla spartiophila*
- Soft shoot moth, *Agonopteryx assimilella*
- Leaf beetle, *Gonioctena olivacea*
- Gall mite, *Aceria genistae*

Plots established at Hanmer and Waiouru to measure long-term impact using insecticide exclusion
3) The imported “vector”: NZ broom psyllid - *Arytainilla spartiophila*

- Originates from UK
- First release in NZ in 1993, in Australia in 1994
- Agent impact: good in Southland, occasionally elsewhere

http://www.britishbugs.org.uk/homoptera/Psyloidea/Arytainilla_spartiophila.html
Risk Assessment in Weed Biocontrol

• **Host range testing** – with particular focus on NZ natives and valued exotics that are related to broom

• **Purpose**: assess risk of non-target damage

• **Broom psyllid was not tested for “Ca. Liberibacter spp”**,  
  • as it wasn’t a known risk then and  
  • there was no existing technique for identification
“Candidatus Liberibacter ssp”

- Non-culturable, bacterium-like prokaryotes
- DNA techniques needed for identification
- vectored by psyllids
“Candidatus Liberibacter ssp”

- Non-culturable, bacterium-like prokaryotes
- DNA techniques needed for identification
- Vectored by psyllids

- Known “Candidatus Liberibacter ssp”:
  1-3) “Ca. L. asiaticus / africanus / americanus”
      cause huánglóngbìng (citrus greening disease)
  4) “Ca. L. solanacearum” (Lso) causes tomato/potato yellows disease and zebra chip disease
  5) “Ca. L. europeaus” (Leu) in pear – non-pathogenic

- 6) Liberibacter crescens in papaya – non-pathogenic relative to Las
4) The uninvited “guest”: “Ca. L. europaeus” (Leu)

- Plant&Food observe Lso disease symptoms in broom in early in 2012\(^1\)

- DNA sequence analysis (16S rRNA and 16S-23S) of broom and broom psyllids: “Ca. L. europaeus” infestation of both

- first appearance in Italy in 2010 in pear and pear psyllids (behaves as an endophyte) \(^2\)

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2) Raddadi N et al. 2011. ‘Candidatus Liberibacter europaeus’ sp. nov. that is associated with and transmitted by the psyllid Cacopsylla pyri apparently behaves as an endophyte rather than a pathogen. Environmental Microbiology 13, 414-426.
• **Leu imported** with broom psyllid or is it **endemic**?

• **“Host”-specificity**
  
  of Leu - pear, broom, and anything else?
  
  of psyllids - probing on non-hosts?

• **“Vector”-specificity of Leu**
  
  - other than pear and broom psyllids or creeping plants?

• **Impact of Leu**
  
  on NZ broom - reason for successful biocontrol?
  
  on psyllids - plain vector function?
  
  on NZ native flora - pathogenic or asymptomatic
Potential hosts and vectors of Leu in NZ

Hosts:
- NZ native Fabaceae e.g. *Carmichaelia, Clianthus, Sophora*
- Exotics – ornamental species (e.g. *Cytisus* cultivars)

Vectors:
- Endemic psyllids on *Sophora* and *Carmichaelia*
- Other psyllids such as Australian acacia psyllids
Approaching genetic diversity

- DNA sequence analysis – psyllid species identification

- Real-time PCR – Leu infestation testing of host and vector
Psyllid DNA sequence data

*CO1* (mitochondrial DNA region)

http://journalofcosmology.com/Extinction101.html
Sequence data analysis

NZ broom psyllid

TPP psyllid
Sequence data analysis

North Island kowhai psyllid
(Psylla apicalis ?)

South Island kowhai psyllid A
(Psylla apicalis ?)

South Island kowhai psyllid B
(Psylla apicalis ?)

NZ broom psyllid

TPP psyllid
Sequence data analysis

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(*Psylla apicalis?*)

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Carmichaelia psyllid A and B?

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Carmichaelia psyllid A and B?

NZ broom psyllid

TPP psyllid

Other psyllids found on kowhai, e.g. acacia psyllid
Approaching genetic diversity

- Sequence data analysis – psyllid species identification

- Real-time PCR – Leu infestation testing of host and vector
Real-time PCR analysis

Test for presence or absence of Leu and quantitation

http://www.bio.davidson.edu/Courses/Molbio/Molstudents/spring2003/Pierce/realtimepcr.htm
Results of first survey in NZ

Broom
• Leu is widespread over NZ
• Leu infestation occurs when psyllid population high
  • so does successful broom biocontrol
• Absence of broom psyllids correlates with absence of Leu
  • psyllids the exclusive vector
• Presence and quantity of Leu within sites highly variable

Kowhai and carmichaelia plant samples Leu negative

Non-broom psyllids Leu negative
Results of first survey internationally

- Original collection sites in UK
  - different broom psyllid species received
  - Leu negative

- Californian samples
  - same as NZ psyllid
  - Leu negative
  - broom psyllid with possibly low impact on invasive broom

- Planning more sampling
  - in NZ/Europe to confirm likely invasion route and
  - in California to understand biocontrol mechanism

- New host-specificity testing + vector-specificity testing
SUMMARY

• What is the origin of Leu?
• What is the role of Leu within broom biocontrol mechanism?
• What are distribution factors of Leu in NZ?
• Is it a biosecurity risk to native flora of NZ?
• Develop new risk assessment/testing protocols for the future
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• Sequencing Team LCR Tamaki
DNA sequence data

Template DNA

Forward primer → PCR

Target gene

Reverse primer

PCR product of the target gene

Cycle sequencing PCR (4 dNTPs + 1 ddNTP★)

Automated sequencing

Alignment and create consensus sequence

Compare consensus sequence to database sequence

Phylogenetic analysis
Real-time PCR analysis

Quantitation:
certain amount of fluorescence achieved after $x$ amplification

http://www.ebioservice.com/eng/show_news.asp?id=506
## Results of pre-liminary survey in NZ

<table>
<thead>
<tr>
<th>Broom collection Location</th>
<th>Psyllids present</th>
<th>Leu present in broom psyllid</th>
<th>Leu present in broom stem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotorua</td>
<td>No</td>
<td>n/a</td>
<td>5/5 negative</td>
</tr>
<tr>
<td>Turangi South</td>
<td>Yes</td>
<td>8/17 positive</td>
<td>3/5 positive</td>
</tr>
<tr>
<td>Taupo</td>
<td>partially</td>
<td>8/17 positive</td>
<td>5/5 negative</td>
</tr>
<tr>
<td>Waihi Town / Beach</td>
<td>No</td>
<td>n/a</td>
<td>5/5 negative</td>
</tr>
<tr>
<td>Kuratau</td>
<td>Yes</td>
<td>6/24 positive</td>
<td>5/5 negative</td>
</tr>
<tr>
<td>Lichfield</td>
<td>No</td>
<td>n/a</td>
<td>5/5 negative</td>
</tr>
<tr>
<td>Waihiti</td>
<td>Yes</td>
<td>5/5 negative</td>
<td>5/5 negative</td>
</tr>
<tr>
<td>10 sites around Lincoln</td>
<td>Yes</td>
<td>24/30 positive</td>
<td>7/10 positive</td>
</tr>
</tbody>
</table>