

A New '*Candidatus Liberibacter*' Species Associated with Solanaceous Plants

Lia Liefiting, Bevan Weir, Lisa Ward, Kerry Paice, Gerard Clover

Plant Health and Environment Laboratory
MAF Biosecurity New Zealand

The problem: Tomato

- A new disease observed in glasshouse tomato with following symptoms:
 - spiky chlorotic apical growth
 - general mottling of leaves
 - curling of midveins
 - stunting



The problem: Capsicum (pepper)

- Similar symptoms reported in glasshouse capsicum:
 - chlorotic or pale green leaves
 - sharp tapering of leaf apex (spiky appearance)
 - leaf cupping and shortened internodes
 - flower abortion



Determination of the aetiology

- Plants were tested for a range of pathogens:
 - pathogenic fungi and culturable bacteria
 - generic tests for viruses:
 - herbaceous indexing
 - transmission electron microscopy (leaf dip)
 - dsRNA purification
 - PCR tests for phytoplasmas, viruses & viroids
- All tests negative
- Tomato/potato psyllid observed in association with affected crops

Transmission electron microscopy

- TEM of thin sections of leaf tissue revealed presence of phloem-limited bacterium-like organisms (BLOs)

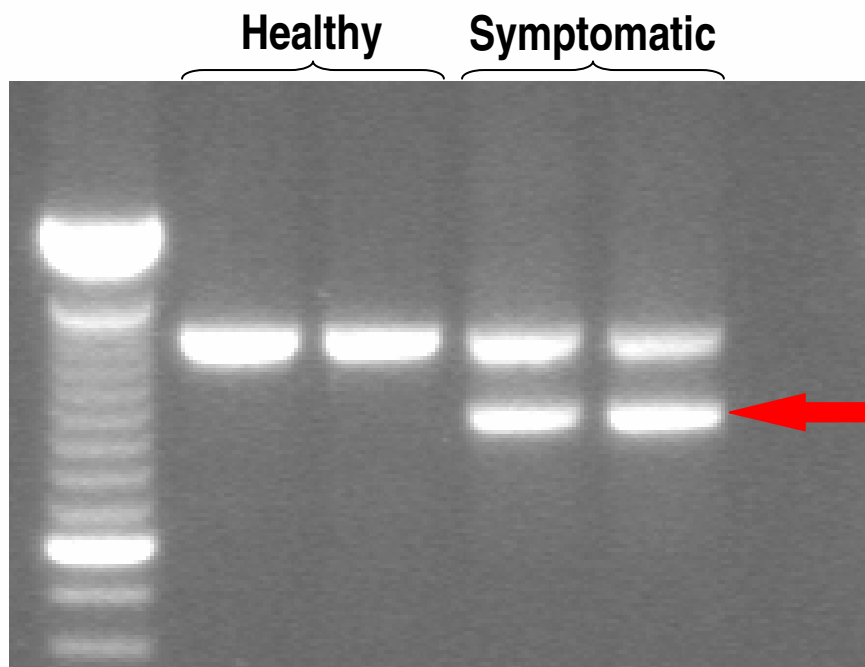


Identification of the BLO

- Range of specific 16S rRNA PCR primers used in different combinations with universal 16S rRNA primers (fD2/rP1)
- Fragments unique to BLO identified by comparing PCR profiles of healthy and symptomatic plants

Identification of the BLO

- A unique 1-kb fragment was amplified from symptomatic plants only

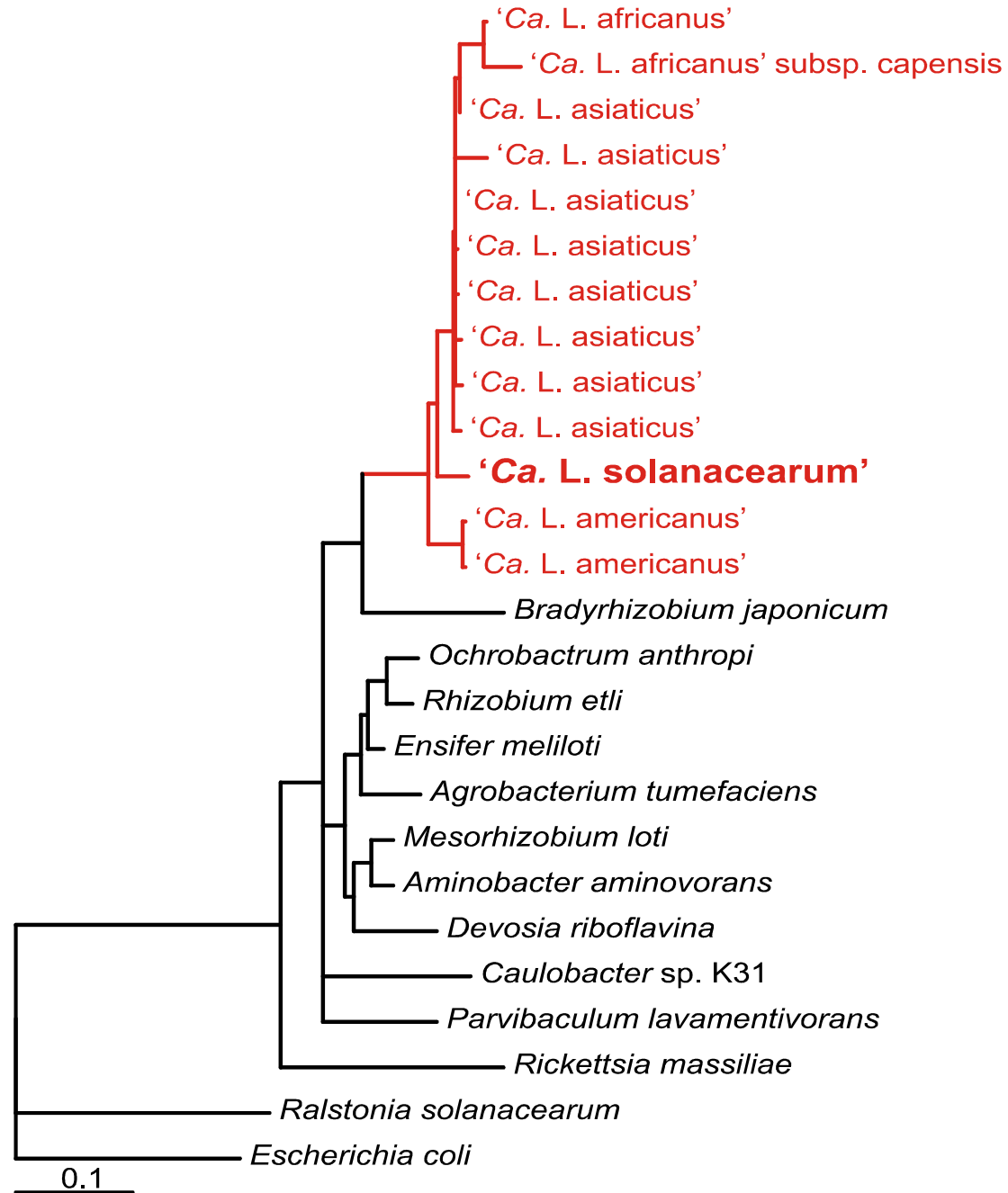


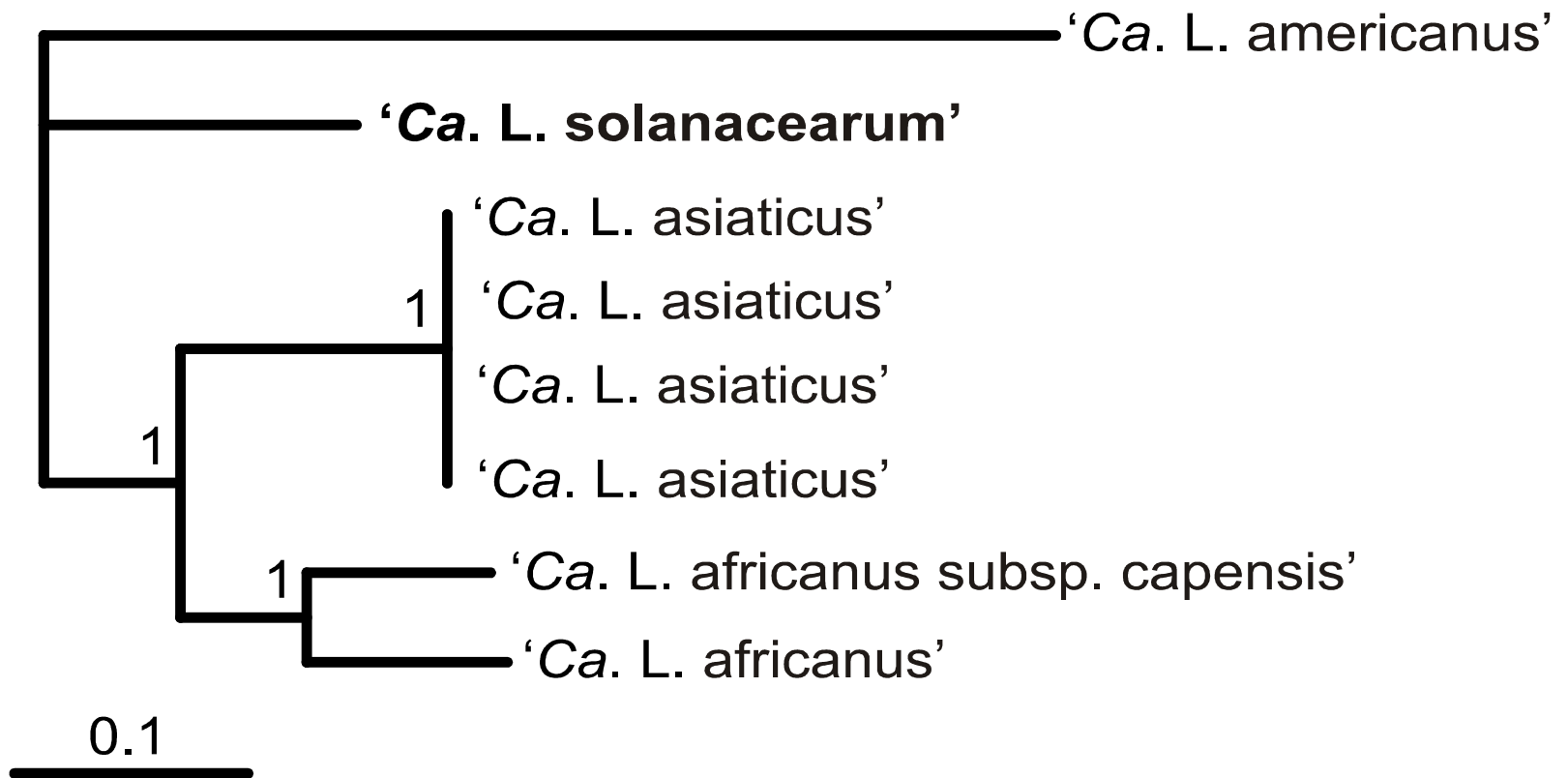
97% identical to
16S rRNA gene of
'*Candidatus*
Liberibacter asiaticus'

Characterisation of the liberibacter

- Sequence and phylogenetic analysis of:
 - 16S rRNA gene
 - 16S-23S intergenic spacer
 - β operon

16S rDNA





β operon

(1.7-kb fragment of the *rpIKAJL-rpoBC* operon)

Characterisation of the liberibacter

- Member of the genus '*Candidatus Liberibacter*'
- Phylogenetically distinct from the three currently described liberibacter species
- '*Candidatus Liberibacter solanacearum*'
- Specific conventional & real-time PCR tests developed

Hosts identified to date in New Zealand

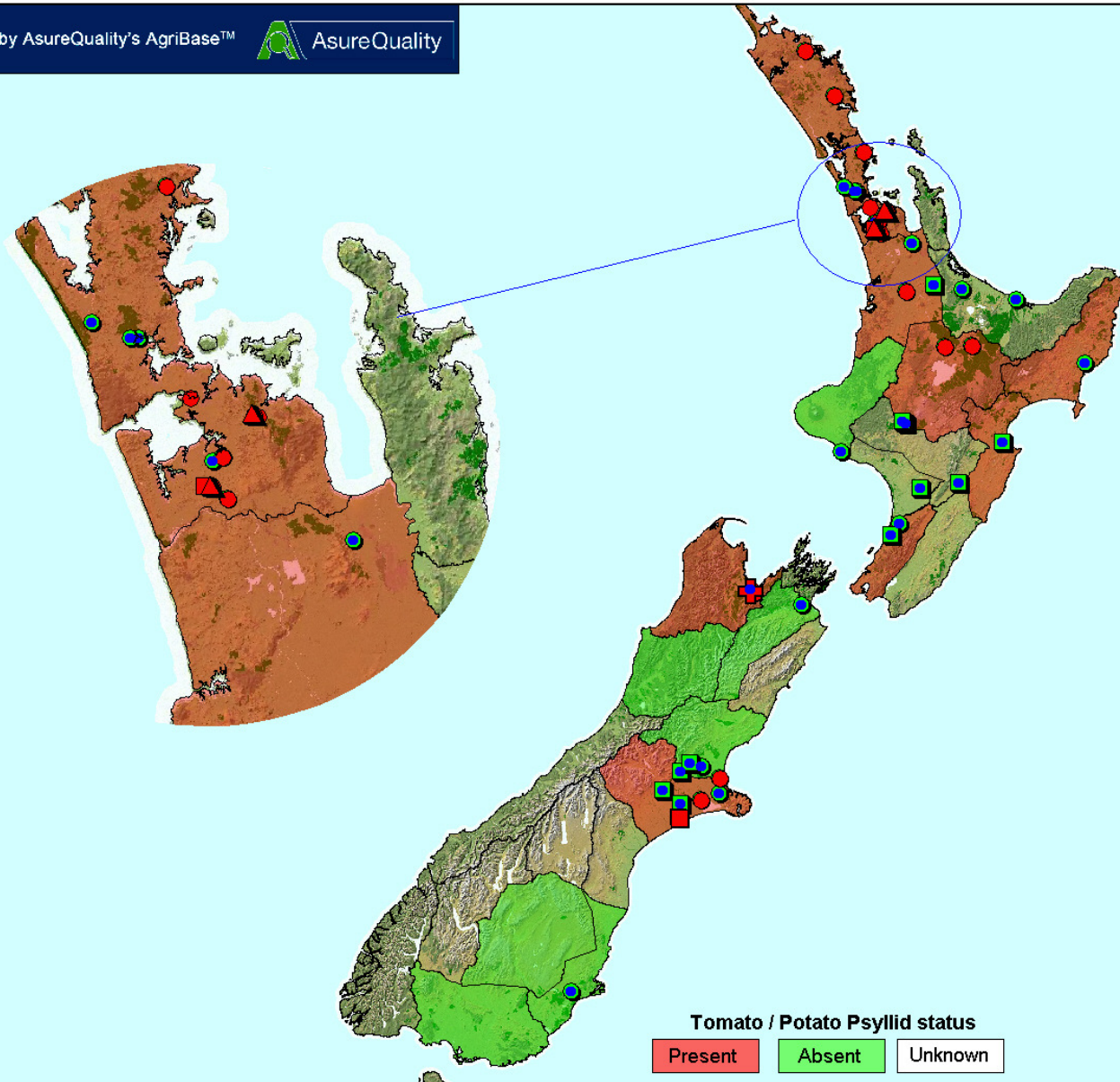
- Tomato (*Solanum lycopersicum*)
- Capsicum (*Capsicum annuum*)
- Potato (*Solanum tuberosum*)
- Tamarillo (*Solanum betaceum*)
- Cape gooseberry (*Physalis peruviana*)

All in the family Solanaceae



Distribution in New Zealand

- 26 commercial tomato & capsicum glasshouses
 - 11 positive
- 12 potato fields (harvested tubers)
 - 2 positive
- 256 plants sampled from each site, 5 plants combined for each test



Tomato / Potato Psyllid status

-
 Present

-
 Absent

-
 Unknown

Date	15 August 2008
Programme	Ca. Liberibacter sp.
TPP Status	

- Survey sites by type
- Potato (12)
 - Psyllid (1)
 - Tomato & Capsicum (26)
 - Tamarillo (2)

- Ca. Liberibacter sp. - positive (20)
- Ca. Liberibacter sp. - Negative at Lab (27)
- Green sites have been sampled
- Crosby boundaries



Worldwide situation

- Detected in potato and tomato in USA
- Possible causal agent of ‘zebra chip’ disease of potato
- Transmitted by tomato/potato psyllid (*Bactericera cockerelli*)
- *B. cockerelli* occurs in:
 - USA
 - Canada
 - Mexico
 - Guatemala & Honduras
 - New Zealand (since May 2006)



Research in progress

- Graft transmission (tomato, capsicum, tamarillo)
- Seed transmission (tomato, capsicum, tamarillo)
- Psyllid transmission from ripe harvested fruit to healthy plants
- Additional plant hosts

Conclusions

- ‘*Candidatus Liberibacter solanacearum*’: a new liberibacter species
- Identified in 5 members of the family Solanaceae
- Reported in New Zealand and USA
- Transmitted by the tomato/potato psyllid, *Bactericera cockerelli*
- First liberibacter to naturally infect non-rutaceous hosts

Acknowledgements

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