



First report of '*Candidatus Liberibacter europaeus*' associated with psyllid infested Scotch broom

S. Thompson¹, J.D. Fletcher¹, H. Ziebell², S. Beard¹, P. Panda³, N. Jorgensen¹, S.V. Fowler⁴, L.W. Liefting⁵, N. Berry¹ and A.R. Pitman^{1,3*}

¹ New Zealand Institute for Plant & Food Research Limited, Private Bag 4704, Christchurch, New Zealand; ² Julius Kühn-Institut, Braunschweig, Germany; ³ Bio-Protection Research Centre, Lincoln University, PO Box 84, Canterbury, New Zealand; ⁴ Landcare Research, PO Box 40, Lincoln 7640, New Zealand; ⁵ Plant Health and Environment Laboratory, Ministry for Primary Industries, PO Box 2095, Auckland 1140, New Zealand

*E-mail: andrew.pitman@plantandfood.co.nz

Received: 01 Oct 2012. Published: 19 Feb 2013. Keywords: *Cytisus scoparius*, *Arytainilla spartiophila*, *Cacopsylla pyri*, biocontrol

In the Canterbury region (mid-South Island), New Zealand, in November 2011, disease symptoms resembling those associated with '*Candidatus Liberibacter*' species were observed in common or Scotch broom (*Cytisus scoparius*), an invasive leguminous exotic shrub. Symptoms included stunted growth of shoots, shortened internodes, and leaf dwarfing and leaf tip chlorosis (Fig. 1). A large population of broom psyllid, *Arytainilla spartiophila*, was noted on the plants. Total DNA was extracted from the stems of 19 Scotch broom plants as well as seven broom psyllid samples collected from infested plants using CTAB. Each DNA sample was tested for the presence of '*Ca. Liberibacter*' by amplification of a partial 16S rDNA sequence using a liberibacter-specific semi-nested PCR based on primers Lib16s01F, Lib16s01R and OA2 (Beard *et al.*, 2012).

A 578 bp partial 16S rDNA fragment indicative of the presence of a '*Ca. Liberibacter*' was amplified from 15 out of 19 plant samples and from five out of seven broom psyllid samples. Amplicons from seven of these samples were directly sequenced, trimmed to 510 bp and subsequently used in a phylogenetic comparison with partial 16S rDNA sequences of '*Ca. Liberibacter*' from other hosts using Mr. Bayes v. 3.0b4. The resulting majority rule consensus tree clustered the partial 16S rDNA sequences from Scotch broom and broom psyllid with that of '*Ca. Liberibacter europaeus*' (Fig. 2). '*Ca. L. europaeus*' has been previously associated with the pear psyllid, *Cacopsylla pyri*, and shown to be transmitted to pear plants where it apparently behaves as an endophyte (Raddadi *et al.*, 2011). Further testing confirmed that '*Ca. L. europaeus*' is widespread in at least South Island, New Zealand. It has also been identified in samples of Scotch broom from sites elsewhere in Canterbury and in Southland (lower end of South Island) where the broom psyllid is common. Plants in these regions showed symptoms similar to those described above, including stunted growth of shoots, leaf dwarfing and leaf curling (Fig. 3).

A larger 2072 bp PCR fragment comprising the entire 16S rRNA and 16S-23S intergenic spacer was then amplified from Scotch broom (GenBank Accession No. JX244259) and a broom psyllid (JX244258) using primers U16a (AGAGTTTGATCCTGGCTC) and ITSREub (GCCAAGGCATCCACC) (Wang & Wang, 1996; Cardinale *et al.*, 2004). DNA sequences obtained from both sources were 100% identical to one another. Subsequent BLAST analysis of this sequence revealed a 99.7% identity between the 16S region and the 1410-bp partial 16S rRNA of '*Ca. L. europaeus*' from pear (FN678792). A total of four single nucleotide

polymorphisms were observed between the European and New Zealand isolates of '*Ca. L. europaeus*'. To our knowledge, this is the first report of an association of '*Ca. L. europaeus*' with the broom psyllid and its host plant. Native to Western Europe, broom psyllids were first released in New Zealand in 1993 to control Scotch broom and subsequently in Australia in 1994 for the same reason (Syrett *et al.*, 1999). The association of '*Ca. L. europaeus*' with the psyllid may explain the ability of this biocontrol agent to restrict growth of Scotch broom when insect populations reach high numbers, as they do in both New Zealand and in Europe.

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Figure 1

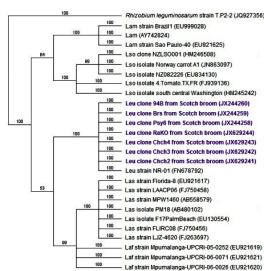


Figure 2



Figure 3

To cite this report: Thompson S, Fletcher JD, Ziebell H, Beard S, Panda P, Jorgensen N, Fowler SV, Liefting LW, Berry N, Pitman AR, 2013. First report of '*Candidatus Liberibacter europaeus*' associated with psyllid infested Scotch broom. *New Disease Reports* **27**, 6. [http://dx.doi.org/10.5197/j.2044-0588.2013.027.006]

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