New Disease Reports

First report of '*Candidatus* Liberibacter europaeus' in the United Kingdom

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'*Candidatus* Liberibacter europaeus' (Leu) was described in Italy from *Pyrus communis* where it seemed to behave as an endophyte (Raddadi *et al.*, 2011). In November 2011, stunting and leaf chlorosis symptoms were observed on *Cytisus scoparius* (Scotch broom) in Canterbury, New Zealand (NZ), leading to the first record of Leu in Scotch broom and the associated broom psyllid, *Arytainilla spartiophila* in NZ (Thompson *et al.*, 2013). To test for the presence of Leu in the UK, a total of 148 broom psyllids and 52 Scotch broom plants were sampled from four sites in Berkshire and Surrey in 2013, 2016, 2017 and 2019. Sites were within 30 km of each other and less than 50 km southwest of London. Fresh twigs were collected and kept chilled in polythene bags with silica gel for no more than ten days after collection. Insect specimens were collected using beating trays or aspirators and preserved in 96% ethanol at 4°C. The insect and plant samples were frozen at -80°C (NZ) or kept at 4°C (France), prior to DNA extraction.

Two methods were used to test for liberibacter. In NZ, DNA from samples collected in 2013 and 2016 was extracted from individual psyllids as described by Beard *et al.* (2013) and from 100 mg plant tissue (multiple 1 mm thick shoot discs from a single plant) using a NucleoSpin® 96 Plant II kit (Macherey-Nagel, Germany) as per the manufacturer's instructions (sample lysis was done with PL1 buffer and RNaseA for 1 hr at 65°C). DNA samples were tested by amplification of 578 bp of the V2-V4 region of the 16S rDNA using a liberibacter-specific semi-nested PCR using primers Lib16s01F, Lib16s01R and OA2 (Beard *et al.*, 2013). Positive amplicons were obtained from five plants and five psyllids (Table 1). In France, a novel PCR was developed for rapid detection of '*Ca.* Liberibacter spp.'. Primers CLibF1 (5′-ACATGGCGAGACGATATCAGAG-3′) and CLibR1 (5′-CAATCCGAACTGAGATGGCTTT-3′) were designed based on alignment of '*Ca*. Liberibacter spp.' sequences to amplify 256 bp of the V6 region of the 16S rDNA gene. DNA was extracted from 20 mg silica-gel dried plant tissue (leaf with petiole) using a Plant DNeasy kit (Qiagen) and insect DNA from 30 individual specimens and 14 batches of five psyllids using a Blood and Tissues kit (Qiagen). PCR was done in 10 µl reactions containing 5µl of 2X SsoAdvanced Universal SybrGreen Supermix (Biorad), 0.3µM of each primer, and 2 µl template DNA. The PCR profile consisted of 3 mins at 95°C, followed by 40 cycles of 5 sec at 95°C, 10 sec at 59°C, and 30 sec at 72°C. DNA from samples collected in 2017 and 2019 tested positive in three batches of psyllids, one individual psyllid and one plant sample (Table 1). Amplicons of positive samples were sequenced and BLAST analysis revealed 100% identity with the Leu sequence (GenBank Accession No. JX244258) reported in NZ from *A. spartiophila*. In total, 12% of the 52 broom samples and 10% of the 92 psyllid samples tested positive for Leu over a seven-year period and in different locations (Table 1). A longer PCR fragment (946 bp) was amplified from one psyllid using primers 16S-341F (5'-CCTACGGGNGGCWGCAG-3') and LG1463R (Morris *et al.*, 2016), and was cloned and sequenced. The sequence was identical to NZ Leu (JX244258) but differed from Italian Leu (FN678792) by three substitutions. This sequence was deposited in GenBank (MN176610), and aligned with partial 16S rDNA sequences of '*Ca*. Liberibacter spp.' and related taxa for a phylogenetic comparison using MEGA6 (Fig. 1).

This is the first report of '*Candidatus* Liberibacter europaeus' in broom psyllid and in Scotch broom in the UK. The broom psyllid *A. spartiophila* is commonly found in the UK (Waloff, 1966) and, in the 1990's, psyllids from Berkshire and Surrey were released in NZ for biological control of Scotch broom (Syrett *et al.*, 2007). The similarity of UK and NZ Leu sequences fits with a scenario where Leu arrived accidentally in NZ with the introduction of *A. spartiophila* for biological control of Scotch broom.

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Figure 1

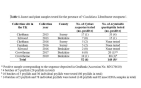

Figure 2

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