New Disease Reports

First report of '*Candidatus* Liberibacter solanacearum' causing leaf discoloration and wilting in tamarillo and cape gooseberry in Ecuador

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'*Candidatus*' Liberibacter species are unculturable bacteria associated with economically devastating diseases of citrus, potato and other crops (Liefting *et al*., 2009). In North and Central America and Oceania, '*Candidatus* Liberibacter solanacearum' (CLso) haplotypes A and B, are associated with emerging diseases in solanaceous plants, including potato, tomato, pepper, tamarillo and cape gooseberry (Liefting *et al*., 2009). Recently in Ecuador, CLso haplotype A was detected in potato and its vector *Bactericera cockerelli* (Caicedo *et al*., 2020). In Ecuador, tamarillo and cape gooseberry are widely grown and are important commodities for their nutritional value and export potential.

During August to December 2019, symptoms resembling those of CLso infection were observed in commercial fields of tamarillo (*Solanum betaceum*) and cape gooseberry (*Physalis peruviana*) (approximately 2 hectares per crop) in Pichincha and Imbabura provinces of Ecuador. Symptoms in tamarillo were pink coloration of new leaves (<10% incidence; Fig. 1) and shoot proliferation (c. 30 % incidence; Fig. 2), similar to those described in New Zealand (SPHDS, 2017), and wilting in young plants (c. 40% incidence; Fig. 3), a symptom previously undescribed. Symptoms in cape gooseberry were purpling (c. 20% incidence) and yellowing leaves (c. 50% incidence) (Fig. 4). No symptoms were described in cape gooseberry previously (Liefting *et al.*, 2008). High population densities of *B. cockerelli*, were observed in both crops.

Diseased and asymptomatic tamarillo and cape gooseberry were tested for presence of CLso. Six composite diseased samples (10 plants per sample) were collected, three tamarillo (M3-T-Pi, M6-T-Pi and M7-T-Pi) and one cape gooseberry sample (M8-C-Pi) from Pichincha, and two tamarillo samples from Imbabura (M4-T-Im and M5-T-Im). All samples were taken from different symptomatic trees. For each crop in both provinces one composite asymptomatic sample (5 plants per sample) was tested. DNA was extracted from 0.2 g of petioles and midribs using the Fungi/Yeast Genomic DNA Isolation Kit (Norgen Biotek Corp., Canada) (Caicedo *et al*., 2020). To amplify the partial sequence of 16S rRNA gene, conventional PCR was done using the CLipo-F/OI2c primer pair (Secor *et al*., 2009). Amplicons of the expected size for CLso (1.1 kb) were observed from all diseased samples. No amplification was observed for asymptomatic samples. Six positive amplicons were sequenced in both directions using the Sanger method. BLAST analysis of the 16S rRNA gene showed 100% nucleotide identity to the corresponding sequence of CLso taxid: 556287 (GenBank Accession No. EU834131) and with strains from potato in Ecuador (MN396642-43). Alignment of all sequences of CLso from this study revealed three conserved single nucleotide polymorphism mutations (g.212T>G, g.581T>C and g.1049A>G) (Nelson *et al*., 2011), confirming that CLso haplotype A infects tamarillo and cape gooseberry in Ecuador. DNA sequences were deposited in GenBank (MN396639, M3-T-Pi; MT036058, M6-T-Pi; MT036059, M7-T-Pi; MN396640, M4-T-Im; MN396641, M5-T-Im; and MT036060, M8-C-Pi). A phylogenetic tree was constructed based on partial 16S rRNA sequences by the maximum likelihood method (Tamura Nei Model) with 2000 bootstrap replications. The tree revealed that all sequences in this study were grouped with the clade belonging to CLso with a bootstrap of 100% (Fig. 5).

This is the first report of CLso haplotype A in tamarillo and cape gooseberry in Ecuador, and its association with wilting in tamarillo, and yellowing and purpling leaves in cape gooseberry. These findings will support governmental plans to minimise the impact of the disease.

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Figure 1 Figure 2 Figure 3

Figure 4 Figure 5

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