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An update on phytoplasma diseases in New Zealand

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Abstract

'*Candidatus* Phytoplasma australiense' occurs in New Zealand and Australia where it is associated with plant diseases in native, weed and crop plants. Between January 2009 and July 2010, four new diverse hosts of '*Ca*. P. australiense' have been identified in New Zealand: potato, Jerusalem cherry, swan plant and celery, as well as a new disease association in boysenberry. A 1.2 kb region of the 16S rRNA gene of the phytoplasma amplified from the new hosts were identical to each other. Partial *tuf* gene sequence analysis of 29 strains from the new plant hosts revealed that they belong to two separate subgroups, *tuf* variant VII and *tuf* variant IX. Two of the strains, one from potato and the other from celery, contained a mixed infection of both phytoplasma subgroups.

Key words: Mollicute, PCR, detection, epidemiology.

Introduction

'*Candidatus* Phytoplasma australiense' (16SrXII-B) is found in New Zealand and Australia where it is associated with a range of host plants. In New Zealand, the phytoplasma is historically associated with the diseases, *Phormium* yellow leaf, strawberry lethal yellows, *Cordyline* sudden decline, and *Coprosma* lethal decline (Liefting *et al*., 2007). In Australia, this phytoplasma species has been associated with Australian grapevine yellows, Papaya dieback, as well as diseases in a range of other hosts including strawberry, pumpkin and bean (Streten and Gibb, 2006).

Sequence analysis of the *tuf* gene of different strains of '*Ca*. P. australiense' in New Zealand determined that there are nine different *tuf* variant groups (I-IX) that cluster into two distinct clades (Andersen *et al*., 2006). Andersen *et al*. (2006) also analysed the available *tuf* gene sequences from Australian strains and determined that some strains formed a third distinct *tuf* gene clade while other strains clustered into one of the clades formed by the New Zealand isolates.

Here we provide an update on four new diverse hosts of '*Ca*. P. australiense' in New Zealand: potato (*Solanum tuberosum*), Jerusalem cherry (*Solanum pseudocapsicum*), swan plant (*Gomphocarpus fruticosa*) and celery (*Apium graveolens*), as well as a new association of '*Ca*. P. australiense' in boysenberry (*Rubus* hybrid). This phytoplasma was previously detected in boysenberry exhibiting different symptoms that were attributed to the fungus *Cercosporella rubi* (Wood *et al*., 1999). The *tuf* gene sequences of '*Ca*. P. australiense' from these new hosts were analysed extending the work of Andersen *et al*. (2006).

Materials and methods

Total plant DNA was extracted from leaf midribs and petioles, stems or tubers using an InviMag Plant DNA Mini Kit (Invitek, Berlin, Germany) and a KingFisher mL workstation (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions.

Initial screening of samples for phytoplasma infection was performed using the TaqMan real-time PCR assay of Christensen *et al*. (2004). For sequence analysis of the 16S rRNA gene, conventional nested-PCR was performed with the primer pairs, P1 (Deng and Hiruki, 1991)/P7 (Schneider *et al*., 1995) followed by R16F2/R16R2 (Lee *et al*., 1995). The *tuf* gene was amplified using the fTufAY/rTufAY primer pair (Schneider *et al*., 1997).

Conventional PCR products were either sequenced directly or cloned into the pCR 4-TOPO vector (Invitrogen) according to the manufacturer's instructions.

The sequences were assembled and edited using Geneious Pro (Drummond *et al*., 2010). Searches of the GenBank database for homologous sequences were performed using the BLASTn network service available at the National Centre for Biotechnology Information (Bethesda, MD, USA).

Results

Potato (*Solanum tuberosum*) plants exhibited upward rolling and purpling of the leaves. Symptoms in Jerusalem cherry (*Solanum pseudocapsicum*) included witches' broom, foliar yellowing and reduced leaf size. Swan plant, also known as milkweed (*Gomphocarpus fruticosa*), showed abnormal foliar yellowing and slight upward rolling of the leaves that resulted eventually in plant death. Celery (*Apium graveolens*) plants were observed to be showing unusual symptoms of pink and yellow foliage and leaf deformation. Symptoms in boysenberry plants become obvious close to flowering when the lateral branches become stunted and young leaves are chlorotic and smaller than normal. As the disease progresses, the older leaves become purple-bronze in colour, particularly towards the margin.

All symptomatic plants described above produced positive real-time PCR results. In order to identify the

phytoplasma present in these samples, a 1.2 kb region of the 16S rRNA gene generated from the R16F2/R16R2 primers was sequenced directly. BLAST analysis of the 1.2 kb region of the 16S rRNA gene amplified from symptomatic Jerusalem cherry, swan plant, celery and boysenberry showed 100% identity to '*Ca*. P. australiense' 16S rRNA gene sequences in the GenBank database.

Partial sequences (~800-840 bp) of the *tuf* gene of '*Ca*. P. australiense' amplified from 29 strains from the symptomatic plants described above were determined. Twenty-two of these sequences were identical to *tuf* variant VII and five sequences were identical to *tuf* variant IX of Andersen *et al*. (2006). Interestingly, these two *tuf* variants belong to distinct subgroups: *tuf* clade 1 (includes variants VIII and IX) isolates originate from both New Zealand and Australia whereas *tuf* clade 2 (includes variants I to VII) consists exclusively of isolates from New Zealand (Andersen *et al*., 2006). None of the isolates in this study belonged to *tuf* variants I to VI and VIII and no new variants were discovered.

The sequences of the PCR products from two strains, one from celery and the other from potato indicated that these hosts contained a mixed phytoplasma population due to the presence of ambiguous bases. The *tuf* gene PCR amplicon from these two samples were cloned and the sequence was determined from 10 resulting clones. Sequence analysis of the clones from celery revealed that 7 were identical to variant VII and 3 were identical to variant IX, and for potato, 2 clones were of variant VII and 8 were of variant IX, thereby confirming the presence of two *tuf* gene types in these samples.

Discussion

Between January 2009 and July 2010, four new hosts (potato, Jerusalem cherry, swan plant and celery) of '*Ca*. P. australiense' have been identified in New Zealand, as well as a new disease association in boysenberry. These are the first new hosts of '*Ca*. P. australiense' to be recognised in New Zealand since *Coprosma* in 1998 (Beever *et al*., 2004), although they may have gone unnoticed for several years.

The economic impact that '*Ca*. P. australiense' will have on these newly identified hosts is potentially significant. Already, phytoplasma-infected potato tubers have failed quality control checks at the processing factory. In boysenberry, fruit from infected plants are unmarketable and the plants die within a year from when the symptoms are first noticeable. Although there have been no reports of the phytoplasma in commercially grown celery, the discolouration of the foliage that the phytoplasma induces in this crop would also render it unmarketable.

The polyphagous feeding behaviour of *Zeolarius oppositus*, the insect responsible for transmitting the phytoplasma into *Cordyline* and *Coprosma* (Beever *et al*., 2008), suggests that it may also be moving the phytoplasma into the new hosts described here. *Z. oppositus* is especially abundant in grasses and sedges that commonly grow around crops in New Zealand. These hosts may act as symptomless reservoirs of the phytoplasma and along with weed hosts such as Jerusalem cherry, play an important role in the spread of the phytoplasma. The diversity of the new hosts described in this paper emphasises the potential that '*Ca*. P. australiense' has to spread into additional native plants and horticultural crops.

References

ANDERSEN M. T., NEWCOMB R. D., LIEFTING L. W., BEEVER R. E., 2006.- Phylogenetic analysis of '*Candidatus* Phytoplasma australiense' reveals distinct populations in New Zealand.- *Phytopathology*, 96: 838-845.

BEEVER R. E., WOOD G. A., ANDERSEN M. T., PENNYCOOK S. R., SUTHERLAND P. W., FORSTER R. L. S., 2004.- '*Candidatus* Phytoplasma australiense' in *Coprosma robusta* in New Zealand.- *New Zealand Journal of Botany*, 42: 663-675.

BEEVER R. E., ANDERSEN M. T., WINKS C. J., 2008.- Transmission of '*Candidatus* Phytoplasma australiense' to *Cordyline australis* and *Coprosma robusta*.- *Journal of Plant Pathology*, 90(S2): 459.

CHRISTENSEN N. M., NICOLAISEN M., HANSEN M., SCHULZ A., 2004.- Distribution of phytoplasmas in infected plants as revealed by real-time PCR and bioimaging.- *Molecular Plant-Microbe Interactions*, 17: 1175-1184.

DENG S. J., HIRUKI C., 1991.- Amplification of 16S rRNA genes from culturable and non-culturable Mollicutes.- *Journal of Microbiological Methods*, 14: 53-61.

DRUMMOND A. J., ASHTON B., BUXTON S., CHEUNG M., COOPER A., HELED J., KEARSE M., MOIR R., STONES-HAVAS S., STURROCK S., THIERER T., WILSON A., 2010.- Geneious v5.1, Available from http://www.geneious.com.

LEE I-M., BERTACCINI A., VIBIO M., GUNDERSEN D. E., 1995.- Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy.- *Phytopathology*, 85: 728-735.

LIEFTING L. W., BEEVER R. E., ANDERSEN M. T., CLOVER G. R. G., 2007.- Phytoplasma diseases in New Zealand.- *Bulletin of Insectology*, 60: 165-166.

SCHNEIDER B., SEEMÜLLER E., SMART C. D., KIRKPATRICK B. C., 1995.- Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas, pp. 369-380. In: *Molecular and Diagnostic Procedures in Mycoplasmology, Vol. I* (RAZIN S., TULLY, J. G., Eds).- Academic Press, San Diego, CA, USA.

SCHNEIDER B., GIBB K. S., SEEMÜLLER E., 1997.- Sequence and RFLP analysis of the elongation factor Tu gene used in differentiation and classification of phytoplasmas.- *Microbiology*, 143: 3381-3389.

STRETEN C., GIBB K. S., 2006.- Phytoplasma diseases in subtropical and tropical Australia.- *Australasian Plant Pathology*, 35: 129-146.

WOOD G. A., ANDERSEN M. T., FORSTER R. L. S., BRAITHWAITE M., HALL H. K., 1999.- History of boysenberry and youngberry in New Zealand in relation to their problems with Boysenberry decline, the association of a fungal pathogen, and possibly a phytoplasma with this disease.- *New Zealand Journal of Crop and Horticultural Science*, 27: 281-295.

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