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DISEASE NOTES OR NEW RECORDS

First report of the association of phytoplasmas with plant diseases in the territory of Wallis and Futuna

R. I. Davis[A,E], *Y. Arocha*[B,C], *P. Jones*[B] *and A. Malau*[D]

[A]Secretariat of the Pacific Community (SPC), Private Mail Bag, Suva, Fiji Islands.
[B]Plant–Pathogen Interactions Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK.
[C]Present address: Centro Nacional de Sanidia Agropecuaria (CENSA) Apto 10, San Jose de las Lajas, Havana, Cuba.
[D]Service de l'agriculture à Wallis, Services Territoriaux des Affaires Rurales et de la Peche BP 19, Mata'utu, 98600 Uvea, Wallis and Futuna Islands.
[E]Corresponding author. Email: richardd@spc.int

Abstract. Phytoplasmas found in the French territory of Wallis and Futuna were identified by nested polymerase chain reaction (PCR), restriction fragment length polymorphism analysis (RFLP) and sequence analysis. They were associated with diseases of the crop plants cassava (*Manihot esculenta)*, Indian mulberry or noni (*Morinda citrifolia*) and pineapple (*Ananas comosus*) and the weeds *Emilia sonchifolia* (red tassel) and *Polygala paniculata*. The phytoplasmas found were most closely related to members of the '*Candidatus* Phytoplasma asteris' and '*Ca.* Phytoplasma aurantifolia' groups.

Little is known about phytoplasmas in the Pacific Islands, although phytoplasma-like diseases are thought to be widespread in the region [Plant Protection Service, Secretariat of the Pacific Community (SPC), unpublished data]. Some early records have been verified using electron or fluorescence microscopy from Papua New Guinea (PNG) and the Solomon Islands, but only a small number of phytoplasmas from American Samoa (A. J. Dabek 1997, SPC unpublished data) and PNG (Davis *et al*. 2003) have been characterised using molecular techniques.

In May 2004, a survey for plant diseases caused by viruses and virus-like pathogens was made in the French Pacific territory of Wallis and Futuna by the SPC and the Wallis and Futuna Service de l'agriculture. A number of plants were sampled on Futuna Island because they were showing phytoplasma-like disease symptoms. They were *Emilia sonchifolia* (red tassel) and *Polygala paniculata* both showing phyllody (leaf like structures replacing petals and sepals); *Manihot esculenta* (cassava) with symptoms of witches' broom (proliferation of axillary buds), yellowing and little leaf; *Morinda citrifolia* (Indian mulberry or noni in parts of the Pacific) with severe yellowing; and *Ananas comosus* (pineapple) with symptoms of shoot proliferation. The cassava sample came from a plot in which many plants showing similar symptoms were seen in patches. The other samples were isolated crop or roadside (weeds) occurrences.

About 1 g fresh weight of young leaves or shoot tips with symptoms were surface sterilised in 1% available chlorine solution, rinsed in water and blotted dry. Leaf mid-ribs and petioles were excised and together with shoot tips were finely chopped, dried over anhydrous calcium chloride in sealed plastic vials then stored at –20°C until despatch. On receipt at Rothamsted Research, the dried material was ground to a fine powder in liquid nitrogen and placed in hot CTAB buffer at 60°C for 30 min. Deoxyribonucleic acid was extracted and used as template in a nested PCR with universal phytoplasma rDNA primer pairs P1 (Deng and Hiruki 1991)/P7 (Schneider *et al*. 1995) and R16F2n/R16R2 (Gundersen and Lee 1996).

Ribosomal DNA PCR products of 1 250 bp were digested with the restriction endonuclease *Hae*III which distinguishes phytoplasma from other prokaryote 16S rDNA (Cronjé *et al*. 1998). Typical phytoplasma *Hae*III profiles were obtained from all four plant species and no other profiles were seen. PCR products were purified from agarose gels using a QIAquick gel extraction kit (QIAGEN, UK) and direct sequenced in an ABI 310 automated sequencer using the sequencing service of Dundee University, Scotland (http://www.dnaseq.co.uk). Sequences obtained were compared with those of other phytoplasmas using BLAST. Results are presented in Table 1. The samples were also screened by transmission electron microscopy for the presence of virus particles and none were observed.

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Table 1. Phytoplasmas found on Futuna Island, territory of Wallis and Futuna

Database description	GenBank accession number	16S rDNA sequence similarity	Phytoplasma group[A]
Red tassel phyllody	AY787138	100% to alfalfa witches' broom (AY169323)	'*Ca.* Phytoplasma aurantifolia' (16SrII)
Cassava little leaf	AY787139	99% to aster yellows strain CHRY (AY180950)	'*Ca.* Phytoplasma asteris' (16SrI)
P. paniculata phyllody	AY787140	97% to sweet potato little leaf (AJ289193)	'*Ca.* Phytoplasma aurantifolia' (16SrII)
Indian mulberry yellows	AY787141	99% to clover phyllody (AF22206)	'*Ca.* Phytoplasma asteris' (16SrI)
Pineapple proliferation	AY787142	97% to strawberry phyllody (AY102275)	'*Ca.* Phytoplasma asteris' (16SrI)

[A]Groupings listed according to the IRPCM Phytoplasma/Spiroplasma Working Team (2004).

This is the first record of a phytoplasma infection of Indian mulberry and pineapple to be published worldwide. However, phytoplasmas have been found in both pineapple and Indian mulberry showing similar symptoms in surveys elsewhere in the Pacific (unpublished data, GenBank accession nos. AY377871, AY377866). These phytoplasmas, however, are from the '*Ca.* Phytoplasma solani' group (16SrXII). Multiple phytoplasmas associated with the same symptoms are known from a number of other plant species (Seemüller *et al*. 1998). These two tropical crops have received little attention from phytoplasmologists and it may be that other different phytoplamas are present in them in this or other regions. The finding of a phytoplasma in cassava is also significant as it is the first such record outside of South America where cassava frog skin disease is associated with a phytoplasma of the '*Ca.* Phytoplasma pruni' group (Alvarez 2002).

These new findings are also of practical significance to the region. Cassava and pineapple are both important food crops in the Pacific. Noni juice (a health drink prepared from fruits of *M. citrifolia*) is rapidly becoming a major export commodity for several Pacific Islands and Territories.

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