First record of citrus canker, caused by *Xanthomonas citri* subsp. *citri* in Solomon Islands

R. I. Davis · R. K. Taylor · D. Rouse · M. Flack ·
D. Hailstones · L. M. Jones · J. B. Rossel · C. Fanai ·
F. Tsatsia · H. Tsatsia

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Abstract Citrus canker disease was detected for the first time in Solomon Islands on Guadalcanal Island. The bacterium was isolated from diseased citrus plants exhibiting distinct citrus canker symptoms consisting of crater-like lesions on the rind of fruit and corky yellow-brownish lesions on leaves. *Xanthomonas citri* subsp. *citri* was identified using biochemical and molecular tests and confirmed as the causal agent by fulfilling Koch's postulates.

Keywords Citrus canker · *Xanthomonas citri* subsp. *citri* · Solomon Islands

R. I. Davis (✉) · L. M. Jones
Department of Agriculture, Northern Australia Quarantine Strategy (NAQS), PO Box 96, Cairns International Airport, Qld 4870, Australia
e-mail: richard.davis@agriculture.gov.au

R. K. Taylor · D. Rouse
Plant Health and Environment Laboratory, Ministry for Primary Industries, PO Box 2095, Auckland 1140, New Zealand

M. Flack · D. Hailstones
Industry & Investment NSW, Elizabeth Macarthur Agricultural Institute, Private Bag 4008, Narellan, NSW 2567, Australia

J. B. Rossel
Department of Agriculture, International Plant Health Program, GPO Box 858, Canberra City, ACT 2601, Australia

C. Fanai · F. Tsatsia
Biosecurity Solomon Islands (BSI), Ministry of Agriculture and Livestock (MAL), PO Box G13, Honiara, Solomon Islands

H. Tsatsia
Agricultural Research Division (Plant Pathology), Ministry of Agriculture and Livestock (MAL), PO Box G13 Honiara, Solomon Islands

In May 2010, Biosecurity Solomon Islands (BSI) and the Australian Department of Agriculture conducted a plant health survey on the islands of Guadalcanal and Malaita. Symptoms of citrus canker disease, including raised and corky lesions on both leaf surfaces, with water-soaked margins and strong chlorotic haloes (Figs. 1, 2 and 3) were found at Visale, Guadalcanal. Affected trees included lemons, limes, oranges and mandarins. Citrus canker is caused by the bacterium *Xanthomonas citri* subsp. *citri*, previously known as *X. axonopodis* pv. *citri* and *X. campestris* pv. *citri* (Schaad *et al.* 2006). Citrus canker has not previously been reported from the Solomon Islands and its distribution in the Pacific is patchy, with records existing for only seven different countries /territories (Table 1).

Desiccated samples of young canker and leaf tissue from affected lemon (*Citrus x limon*) and orange (*Citrus x aurantium*) trees, were returned to Australia and subjected to mandatory gamma irradiation at 25 KGy. Nucleic acids were extracted using a Qiagen DNeasy Plant Mini Kit and screened by PCR using the primer pair J-pth 1/J-pth 2 (Cubero and Graham 2002) following the protocol from the Australian draft national standard. A PCR product of the expected size (197 bp) was produced. In confirmatory analyses in a second laboratory, this test was repeated successfully and a second assay, using the primer pair Xac01/Xac02 (Coletta-Filho *et al.* 2006), also gave a diagnostic fragment (581 bp). The amplified fragments from all three reactions (two J-pth 1/J-pth 2 products and one Xac01/Xac02 product) were purified and subjected to nucleotide sequence analysis, confirming that the bacterium was *X. citri* subsp. *citri*.

There are closely related bacteria that also cause similar, but less severe, canker diseases on a restricted range of citrus

Fig. 1 Canker disease symptoms found on lemon (*Citrus x limon*) foliage at Visale, Guadalcanal, Solomon Islands, May 2010

elsewhere in the world, and one of these is now named *X. fuscans* subsp. *aurantifolii* (Schaad *et al.* 2006). Whilst the PCR assay of Cubero and Graham (2002) is known to also detect this bacterium, the second PCR assay (Coletta-Filho *et al.* 2006) is specific only for *X. citri* subsp. *citri*. These tests do not exclude the less damaging restricted host range variants of *X. citri* subsp. *citri* known as the A* and A^w pathotypes. However, the severity and wide host range observed in the field suggests that this must be the highly virulent, broad host range variant formerly known as the ‘asiatic’ or pathotype ‘A’ form of *X. campestris* pv. *citri* (Stall and Civerolo 1993). These observations were further supported by later analysis of *Irp* gene sequences which showed the Solomon Islands isolates closely matched the A pathotype and were distinct from the A* and A^w pathotypes (Fig. 4).

In later work, infected lemon and orange leaf and fruit material were sent to New Zealand, under relevant biosecurity protocols, and isolations were conducted in a biocontainment laboratory (Physical containment level 2 with additional controls). Saline suspensions of macerated diseased leaf and fruit tissues were streaked onto King’s B media (King *et al.* 1954)

Fig. 2 Canker lesions on lime (*Citrus x aurantiifolia*) fruit

Fig. 3 Close up of canker lesions on a lime (*Citrus x aurantiifolia*) leaf

and mucoid pale yellow bacterial colonies were consistently grown following 48 h incubation at 25 °C. All colonies were gram negative, oxidase negative, catalase positive and produced levan from sucrose and tested positive for *X. citri* subsp. *citri* by real-time PCR assays, developed by Cubero and Graham (2005) and Mavrodieva *et al.* (2004). The *gyrB* and *Irp* gene sequences were amplified from pure bacterial cultures and analysis was conducted according to the methodology of Parkinson *et al.* (2009) and Cubero and Graham (2004). BLASTn analysis of the *gyrB* and *Irp* amplicons showed that they were 100 % match to verified *X. citri* subsp. *citri* sequences in GenBank, for example, accession EU498950 for *gyrB* and AY227401 for *Irp*. *X. citri* subsp. *citri* was isolated consistently from lemon and orange tissue adjacent to leaf spots and fruit lesions. The cultures used in this study have been deposited in the International Collection of Microorganisms from Plants (ICMP), Landcare Research, Auckland, New Zealand. Pathogenicity tests with three isolates (ICMP 20576, 20577, 20578) were

Table 1 Summary of citrus canker records in the Pacific islands

PICT ^A	Identification method	Citation
CNMI	Biochemical testing ^B	Jenkins and Forsberg (1957)
Fiji	Biochemical testing ^B	Dingley <i>et al.</i> (1981)
FSM	Biochemical testing ^B	Jenkins and Forsberg (1957)
	Molecular testing (PCR)	Anon (2001)
Guam	Biochemical testing ^A	Jenkins and Forsberg (1957)
	Molecular testing (PCR)	Coletta-Filho <i>et al.</i> (2006)
Palau	Biochemical testing ^B	Jenkins and Forsberg (1957)
PNG	Biochemical testing ^B	Shaw (1984)
	Molecular testing (PCR)	Davis <i>et al.</i> (2000)
RMI	Molecular testing (PCR)	Anon (2001)

^A Pacific island country or territory: CNMI: Commonwealth of the Northern Mariana Islands, FSM: Federated States of Micronesia, PNG: Papua New Guinea, RMI: the Republic of the Marshall Islands

^B Biochemical testing presumed: the published listing states that plant specimens were collected and lodged in collections following laboratory identifications, but specific identification methods are not detailed

Fig. 4 Maximum likelihood tree illustrating the phylogenetic relationships between the Solomon Islands *Xanthomonas citri* subsp. *citri* (Xcc) strains (in bold type) and other Xcc strains. Phylogenetic analyses of the *Irp* gene were performed via the CIPRES Science Gateway (Miller et al. 2010), using the RAxML-HPC2 8.0.0 with the *-k* option for bootstrap analysis. Topology was rooted with *X. campestris* and the bootstrap values are given at the nodes; superscripted T indicates the type strain. Sequences produced in this study are deposited in GenBank under accession numbers KM678288 – KM678292

performed on young detached leaves of orange, alemow (*Citrus x macrophylla*), pomelo (*C. maxima*), and lime (*Citrus x aurantiifolia*). Each isolate was inoculated onto three leaves by placing 20 μ L of bacterial suspension (10^7 cfu/mL) on the surface and pricking through the drops using sterile needles. After inoculation, the leaves were kept in Petri dishes lined with moist filter paper, incubated at 25 °C and checked daily for symptoms. Controls were inoculated in the same way using sterile physiological saline (0.85 % NaCl). Lesions developed on the inoculated leaves for all varieties 7 days after inoculation (Fig. 5). The symptoms induced on leaves were similar to those caused by natural infections. Bacterial colonies were re-isolated from leaf lesions and identified as *X. citri* subsp. *citri*, fulfilling Koch's postulates. No symptoms developed in the negative controls. The pathogenicity tests were performed twice.

Following the 2010 survey, delimiting surveys on Guadalcanal found similar symptoms at several other locations at the west of the island and also in the capital, Honiara. Later citrus targeted surveys have covered much of the country and have

Fig. 5 Symptoms on detached *Citrus aurantiifolia* leaf inoculated with Solomon Island *Xanthomonas citri* subsp. *citri* isolate ICMP 20576

Table 2 Areas surveyed and found to be free of citrus canker symptoms by visual examination subsequent to initial identification of *X. citri* subsp. *citri* on Guadalcanal Island

Year	Locations
2010	Malaita Island, Malaita Province
2011	Ghizo, Kolombangara, and Parara islands, Western Province
2012	Taro, Mono, Poporang, Shortland, Maleai, Fauro islands, Choiseul Province
2012	Vella Lavella, Kolombangara islands, Western Province
2013	Ngella and Savo islands, Central Province
2013	Santa Isabel island, Isabel Province
2013	Malaita Island, Malaita Province
2014	San Cristobal Island, Makira Province
2014	Nendo Island, Temotu Province

failed to find canker-like symptoms on any other island (Table 2). These included islands closest to PNG, where the disease has been widespread for many years (Shaw 1984), and movement of infected fruits or leaves across the border is a likely threat. Effective survival of *X. citri* subsp. *citri* inoculum in cankers on leaves under tropical conditions has been demonstrated (Pruvost et al. 2002).

The effects of citrus canker on individual tree yield and fruit quality can be great in large monoculture orchards (Gottwald et al. 1992). In contrast to this, the impact observed on isolated trees in subsistence / domestic production situations on several other Pacific islands is minimal (R. Davis unpublished data). For this reason, a strategy of containment rather than the difficult and costly eradication (Gottwald et al. 2002), might be most suitable for Solomon Islands.

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